VG98004

Investigation of capsicum genetic resistance to tomato spotted wilt virus, tospovirus serotype IV and bacterial spot

D J McGrath
Queensland Horticulture Institute

Know-how for Horticulture™

VG98004

This report is published by the Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the vegetable industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the vegetable industry.

All expressions of opinion are not to be regarded as expressing the opinion of Horticulture Australia Ltd or any authority of the Australian Government.

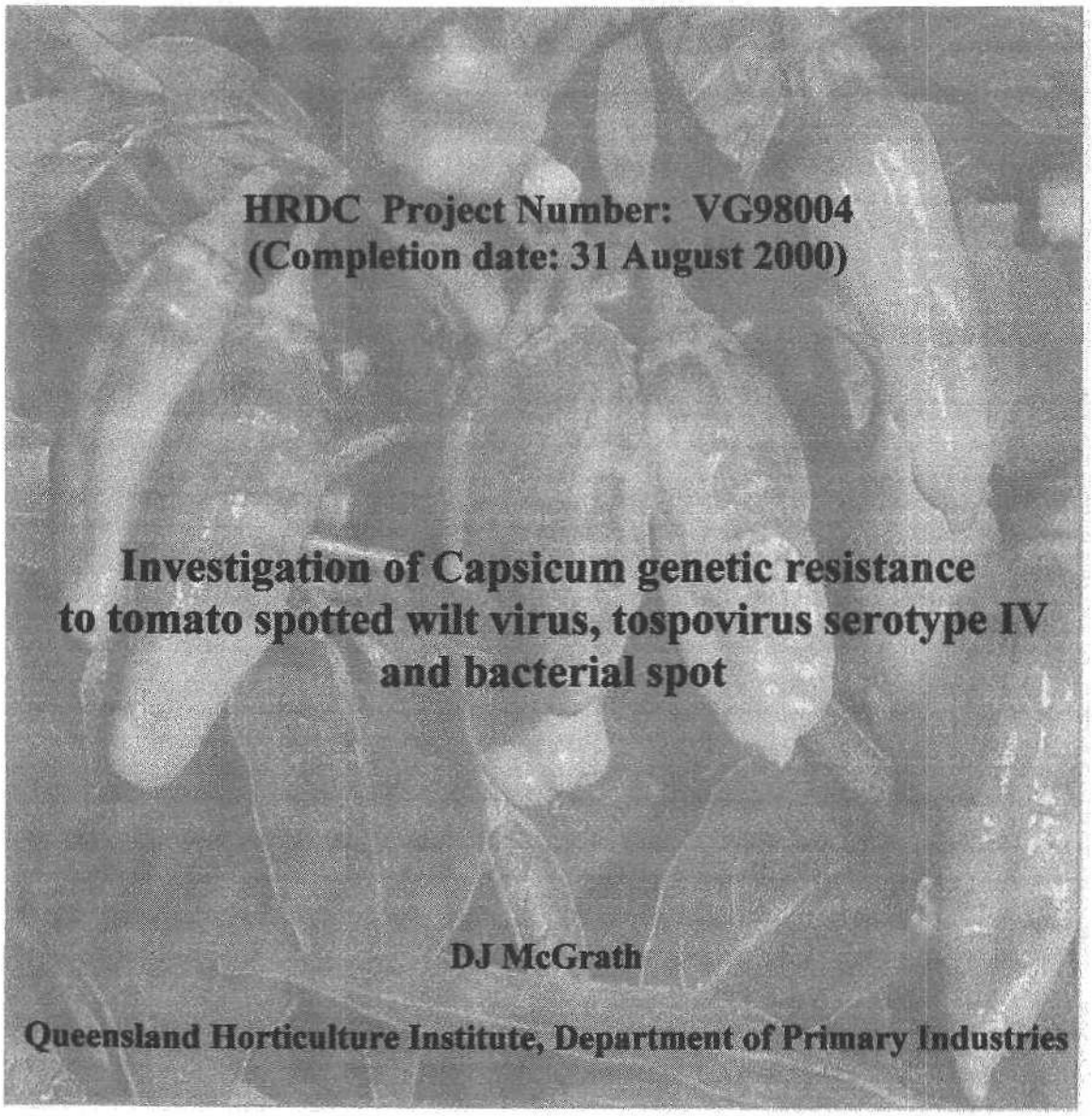
The Corporation and the Australian Government accept no responsibility for any of the opinions or the accuracy of the information contained in this report and readers should rely upon their own enquiries in making decisions concerning their own interests.

Cover price: $22.00 (GST Inclusive)
ISBN 0 7341 0228 3

Published and distributed by:
Horticultural Australia Ltd
Level 1
50 Carrington Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399
E-Mail: horticulture@horticulture.com.au

HRDC

FINAL REPORT



HRDC Project Number: VG98004
(Completion date: 31 August 2000)

Investigation of Capsicum genetic resistance to tomato spotted wilt virus, tospovirus serotype IV and bacterial spot

DJ McGrath

Queensland Horticulture Institute, Department of Primary Industries

Queensland Government
Department of Primary Industries

HRDC project number VG98004

Des McGrath
Centre for Dry Tropics Agriculture
PO Box 538
BOWEN QLD 4805
Ph: 07 47614031 Fax: 07 47852427
E-mail: mcgratdj@dpi.qld.gov.au

This publication is the final report for HRDC project number VG98004, Investigation of Capsicum genetic resistance to tomato spotted wilt virus, tospovirus serotype IV and bacterial spot.

The financial support of the following organisations is gratefully acknowledged:
Horticultural Research and Development Corporation
Australian vegetable industry
Queensland Horticulture Institute, Department of Primary Industries

December 2000

HRDC

Queensland Government
Department of Primary Industries

Table of Contents

1. Industry summary

Tomato spotted wilt virus (TSWV) is responsible for increasing losses in capsicum and other vegetable crops in most production areas of Australia. Although the virus has been active in a range of crops in southern Australia for many years, it has become significant in Queensland production centres only recently. Direct losses to capsicum crops in particular fields can vary from 5% to 60%, depending on vector activity. The damage occurs through stunting of plants, reduced yield and unmarketable fruit. It is estimated that production losses in Queensland alone account for up to $3M per year.

Bacterial spot (BS) of capsicum is a serious disease in warm humid conditions such as the early season environment of coastal Queensland. Typically losses are greatest in southern Queensland during periods of extended rainfall but early season damage can also be serious for producers in north Queensland. The disease can be particularly destructive with large economic losses during epidemics.

The work of this project was directed towards an understanding of the effectiveness of genetic resistances to both TSWV and BS in capsicum with a long-term objective of breeding high-quality, disease-resistant cultivars. A survey of TSWV isolates in Western Australia and Queensland revealed three Plant Introduction lines were highly resistant to all except two isolates in Western Australia. At Bundaberg in Queensland the first identification in Australia of a distinct tospovirus was made in a large number of chilli and capsicum samples. Although this virus produced symptoms similar to TSWV, it infected all three TSWV-resistant Plant Introduction lines. A survey of BS isolates from Queensland revealed they were all from one race for which resistance is available. A genetic analysis of TSWV resistance indicated that a major resistance gene was present in one of the resistant Plant Introduction lines. This was evident from both conventional analysis and studies with a DNA marker.

Excellent resistance to TSWV is available in all three imported Plant Introduction lines of capsicum. The major gene identified in one capsicum line is highly resistant to all field isolates of TSWV sampled in this study and can be incorporated into breeding lines for use throughout Australia. A DNA marker was found to be a very efficient means of identifying resistant lines in breeding populations. The new tospovirus, Capsicum chlorosis virus (CCV), is widespread in Bundaberg and now appears to be the major virus disease of capsicum there. TSWV-resistant varieties will therefore be necessary but additional resistance to CCV is required.

Bacterial spot isolates were all from one race and an effective resistance gene is available. Although commercial hybrids may possess three different resistance genes for protection against multiple races of BS, it appears that only one gene is necessary.

Further research and development should now produce breeding lines with multiple resistances to both TSWV and BS and excellent agronomic performance. A parallel program of screening germplasm for resistance to CCV and associated genetic analysis should also be implemented so resistance can be introduced to breeding populations as soon as possible.

2. Technical summary

Tomato spotted wilt virus (TSWV) is responsible for increasing levels of damage to capsicum crops in all states. Increased activity by the vector *Franklinella occidentalis*, Western Flower Thrips, is now responsible for widespread infection in southern states whereas other thrips species are responsible for damage in Queensland. In past years losses were sporadic and relatively minor in all areas, but much more significant and frequent damage occurs now.

In a survey of TSWV in Australia, three Plant Introduction (PI) accessions of *Capsicum chinense* were challenged by sap inoculation with 16 isolates from 4 states. All isolates were confirmed as TSWV by their serological reactions. The PI lines were reported to have strong TSWV resistance in overseas studies and this was confirmed here. Three isolates overcame the hypersensitive resistance in 4 plants of a total of 1650 inoculated. Two sets of TSWV-infected scions were grafted to stocks of 2 PI lines and induced a typical hypersensitive resistance reaction below the graft union; the second set of grafts differed in that one isolate induced a mottle symptom in one plant that later tested positive for TSWV with ELISA. The sap and graft inoculations demonstrated that the resistance in *C. chinense* is highly effective but can be broken on rare occasions.

A separate survey of Queensland isolates recorded the first observation of tospovirus serotype IV in Australia at Bundaberg in 1999. The new virus, named Capsicum chlorosis virus (CCV), was detected by serology from all of 19 chilli, capsicum and tomato samples tested in March 2000. TSWV was detected in only one sample, indicating that CCV was the dominant virus only one year after its discovery. The virus was virulent on all three TSWV-resistant PI lines of *C. chinense*. All TSWV isolates were avirulent on the PI lines.

An analysis of the inheritance of TSWV resistance in one PI line revealed good evidence for a major dominant gene, although there were some discrepancies in backcross segregation data which were not readily explained. A further analysis with DNA markers confirmed a useful cleaved amplified polymorphic sequence (CAPS) marker linked to resistance gene *Tsw*. The marker was robust and readily discriminated between resistant and susceptible genotypes of PI lines, commercial hybrids and segregating genotypes from various crosses. Polymorphisms were obtained which distinguished between the three resistant PI lines of *C. chinense*.

A survey of bacterial spot isolates in Queensland identified only one race. An effective resistance gene is available. Although commercial hybrids may possess as many as three different resistance genes for protection against multiple races of BS, it appears that only one gene will be necessary.

Further research and development should now produce breeding lines with multiple resistances to both TSWV and BS and excellent agronomic performance. A parallel program of screening germplasm for resistance to CCV and associated genetic analysis should also be implemented so resistance can be introduced to breeding populations as soon as possible.

3. Introduction

Tomato spotted wilt virus (TSWV) has a very wide host range of more than 800 plant species, and causes serious losses in vegetable crops and ornamentals worldwide. Although first identified in Victoria in 1915 (Brittlebank 1919), its significance for many years was limited to occasional damage of crops in eastern and south-western Australia. The recent introduction of *Frankliniella occidentalis* (western flower thrips), an efficient vector of TSWV, into Western Australia and parts of eastern Australia, has contributed to an increase in TSWV infection in southern States. At the same time, a similar increase in TSWV infection of tomato and capsicum crops, vectored by *Frankliniella schultzei* has been noticed in Queensland.

Control of TSWV is difficult because of several factors: its large host range, its transmission by several species of thrips, the nature of the interaction with thrips which allows it greater persistence in the vector, and the large number of adult thrips capable of infecting healthy plants in a crop. A further difficulty is the low efficiency of chemical control and the rapid development of resistance to insecticides by thrips. The development of resistant cultivars is one of the best alternatives in reducing crop losses from TSWV infection.

Genetic resistance to TSWV is best developed in tomato, where an extensive range of cultivars has been bred in most varietal types using the *Sw-5* gene. The only identified resistance gene in capsicum, *Tsw*, was identified and analysed in an uncultivated species, *Capsicum chinense*. The gene has been transferred to a small range of commercial cultivars, none of which appears to be entirely suitable for Australian production.

Genetic resistance to Tomato spotted wilt virus (TSWV) was reported in two accessions of *C. chinense* Jacquin, PI 152225 and PI 159236, by Black *et al.* (1991). Seven isolates of TSWV recovered from tomato, capsicum and weed species in Louisiana produced similar responses from the two PI lines. Progeny from selfed plants reacted the same as parent plants following inoculation and crosses between resistant plants and susceptible *C. annuum* lines developed local lesions, suggesting that the resistance was heritable.

PI 159236 was confirmed as resistant in Brazil by Boiteux *et al.* (1993) and both PI 159236 and PI 152225 were reported resistant in France by Palloix *et al.* (1993). Diez *et al.* (1993) also determined that these lines, as well as two other *C. chinense* lines, P 15 and P 18, *C. frutescens* 7204 and an unidentified accession of *C. pubescens* were also resistant to a Spanish isolate. Other work in Spain by Ortega and Arteaga (1994) demonstrated resistance in PI 159236 and PI 152225 of *C. chinense* and four accessions of *C. baccatum*; the latter accessions were as resistant as or more resistant than the *C. chinense* lines. In all cases of *C. chinense* and *C. baccatum*, the resistance was expressed as a hypersensitive reaction, although there were some individuals which showed systemic necrosis. The authors concluded that other factors such as plant age, climatic conditions and inoculum concentration could affect resistance.

Several studies have reported differences in reactions among TSWV isolates to resistant *C. chinense* lines. Hobbs *et al.* (1994) surveyed 61 isolates, of which 59 were avirulent on PI 152225 and PI 159236; the other two isolates produced chlorotic lesions and systemic symptoms. Progeny of a third line (AVRDC C00943) reacted similarly when inoculated with typical and atypical isolates. The TSWV-resistant cultivar PS 55289 was resistant to all

isolates. Boiteux and Nagata (1993) also reported a loss of resistance in PI 159236 following mechanical inoculation with three Brazilian isolates; the same breakdown was observed under field conditions. Immunodiffusion tests showed that virulent and avirulent isolates were related but serologically distinct. Latham and Jones (1997) inoculated three lines of *C. chinense* with 15 isolates from different locations in Australia and found that all isolates were avirulent on these lines. Some of the same 15 isolates had given mixed resistant and susceptible reactions on several TSWV-resistant tomato genotypes, and had given rise to resistance breaking isolates when subjected to repeated subculturing.

A collection of 70 cultivars and wild species of *Capsicum* was screened for field resistance to TSWV in Brazil with the objective of identifying new sources for breeding programs (Boiteux *et al.* 1993). The highest degree of field resistance was found in two *C. baccatum*, two *C. chinense* and three *C. annuum* lines. When these lines were mechanically inoculated with two distinct isolates, the *C. chinense* lines (CNPH 275 and PI 159236) were immune against one but susceptible to the other. There were no lines resistant to both isolates. The study suggested that in addition to vertical resistance gene(s) in the *C. chinense* accessions, there were also distinct field resistance mechanisms determining non-preference to vectors in *C. chinense* and *C. baccatum* lines.

The inheritance of TSWV resistance in three *C. chinense lines* (PI 152225, PI 159236 and Panca) was studied by Boiteux (1995) in crosses between a susceptible *C. annuum* cultivar and each of the PI lines, and in a second set of all crosses between the PI lines. The resistance response in all *C. chinense* accessions produced a typical localised, hypersensitive reaction leading to the formation of large local lesions and leaf abscission. The interspecific F1 generations all expressed resistance, indicating dominant gene action for all genes carried by the PI lines. No segregation was observed in any of the F2 generations of the crosses between PI lines, suggesting that resistance is determined by a single major gene or a group of tightly linked genes. Boiteux and de Avila (1994) previously identified a dominant major gene in PI 159236 which they designated *Tsw*, and it was considered likely that this same gene conferred resistance in the other two PI lines. However it was recognised that multiple genes for resistance could have been present in PI 152225 and Panca.

Moury *et al.* (2000) indicated five different *C. chinense* lines which possess a single dominant gene at the *Tsw* locus. They identified a DNA marker closely linked to *Tsw* which was useful for marker-assisted selection in a wide range of intercrosses among capsicum lines.

Bacterial spot (BS) of capsicum caused by *Xanthomonas campestris* pv. *vesicatoria* is one of the most destructive diseases of capsicum, particularly when crops are grown in warm humid conditions. Genetic resistance conferred by three genes, *Bs1, Bs2, and Bs3,* has been identified in three different plant introduction lines and each gene restricts a different set of races (Hibberd *et al. 1987).* Seven races of BS have been recognised world-wide and increasing reliance has been placed on genetic resistance as more races become established.

The most significant requirements for disease resistance in Australian commercial capsicum crops are combined TSWV and BS resistances. Although there are cultivars with adequate resistance to either disease, there is none with multiple resistances and good horticultural attributes suitable for commercial production in Australia. The purpose of this preliminary investigation was to establish the potential for breeding resistances to both TSWV and BS in excellent horticultural varieties of capsicum. Since the interactions between both pathogens

and their host plant are complex, a series of experiments was conducted to survey the distribution of virulent strains in different locations in Australia and therefore establish the usefulness of resistance genes reported elsewhere. An analysis of inheritance of TSWV resistance using conventional phenotypic segregation and a DNA marker was also conducted.

Black, L.L., Hobbs, H.A., and Gatti, J.M. (1991). Tomato Spotted Wilt Virus Resistance in Capsicum chinense PI 152225 and 159236. Plant Dis 75(8), 863.

Boiteux, L.S. (1995). Allelic relationships between genes for resistance to tomato spotted wilt tospovirus in *Capsicum chinense. Theor Appl Genet 90, 146-149.*

Boituex, L.S. and de Avila, A.C. (1994). Inheritance of a resistance specific to tomato spotted wilt tospovirus in Capsicum chinense PI 159236. Euphytica 75, 139-142.

Boiteux, L.S. and Nagata, T. (1993). Susceptibility of Capsicum chinense PI159236 to tomato spotted wilt virus isolates in Brazil. Plant Dis 77, 210.

Boiteux, L.S., Cupertino, F.P., and Reifschneider, F.J.B. (1993). Capsicum chinense 'PI 159236': a source of resistance to Phytophthora capsici and tomato spotted wilt virus (TSWV). Capsicum and Eggplant Newsletter 12, 76.

Brittlebank, C. C. (1919). Tomato diseases. Journal of Agriculture, Victoria 27, 231.

Diez, M.J., Nuez, F., Jorda, C., Juarez M., and Ortega, A. (1993). Busqueda de fuentes de resitencia al virus del bronceado (Tomato spotted wilt virus) para la mejora del tomate y pimiento. Actas de Horticultura 10, 1286-1291.

Hibberd, A.M., Stall, R. E., and Basset, M. J. (1987). Different phenotypes associated with incompatible races and resistant genes in bacterial spot disease of pepper. Plant Dis. 71, 1075-1078.

Hobbs, H.A., Black, L.L., Johnson, R.R., Valverde, R.A. (1994). Differences in reactions among tomato spotted wilt virus isolates to three resistant Capsicum chinense lines. Plant Dis 78(12), 1220.

Latham, L.J. and Jones, R.A.C. (1997). Occurrence of tomato spotted wilt tospovirus in native flora, weeds, and horticultural cops. Aust. J. Agric. Res. 48, 359-369.

Moury, B., Palloix, A., Gebre Selassie, K. and Marchoux, G. (1997). Hypersensitive resistance to tomato spotted wilt virus in three Capsicum chinense accessions is controlled by a single gene and is overcome by virulent strains. Euphytica 94, 45-52.

Moury, B., Pfieger, S., Blattes, A., Lefebvre, V., and Palloix, A., 2000. A CAPS marker to assist selection of tomato spotted wilt virus (TSWV) resistance in pepper. Genome. 43, 137-142.

4. Resistance to Tomato spotted wilt virus in *Capsicum chinense*. Research in Western Australia.

4.1 Introduction

An investigation of TSWV isolates, principally from Western Australia, was conducted to characterise the TSWV resistance in *Capsicum chinense* lines reported overseas. Although field resistance to TSWV was evident in resistant commercial cultivars, the nature and quality of resistance in glasshouse conditions to a range of Australian TSWV isolates was unknown. A number of glasshouse tests were undertaken in Western Australia to determine the effectiveness of resistance reported in PI lines overseas.

4.2 Materials and Methods

National survey of TSWV isolates, sap inoculation

In experiments in the glasshouse in WA, TSWV-resistant *C. chinense* accessions PI 152225, PI 159236 and AVRDC COO943, and QDPI crosses derived from them, were challenged by sap inoculation with a collection of 16 TSWV isolates from 4 different Australian states (Table 1).

Table 1. *Original and derived isolates of TSWV used: their origins and symptom reactions in susceptible tomato and capsicum cultivars*

Isolate Code[a]	Source Plant	*Original* source isolate	*Location*[a]	Date obtained	Symptom Reactions[b]	
					Tomato cv. Grosse Lisse	Capsicum cv. Rialto
An_{WA}-1	Anemone (*Anemone sp.*)	-	Yangebup, WA	iix. 98	P, LD, FA, S, TD	M, Y, LD
As_{WA}-2	Aster (*Callistephus chinesis*)	-	Yangebup, WA	xii.98	NLL, M, P	NLL, Y, LD, SM
Ca_{WA}-1 *	Capsicum (*Capsicum annuum*)	-	Banjup, WA	ix.94	M, P, LD, S, TD	VC, MM, MS
Ca_{WA}-3	Capsicum	-	Banjup, WA	xii.98	NLL, P, PSt	CR, NRS, SM, LD, FA, S
Ca_{WA}-4	Capsicum	-	Banjup, WA	xii.98	NLL, P, LD, PSt	NRS, M, FA
Ca_{WA}-5	Capsicum	-	Banjup, WA	xii.98	P, Y, NB, PSt	NLL, SM, LD, S
Ca_{QLD}-1	Capsicum	-	QLD		P, PSt, S	SM, S
Da_{WA}-1b *	Tomato cv. Stevens	Da_{WA}-1a	-	vii.96	M, P, LC, NB, PSt, S	VC, SM, SS
Da_{WA}-1d *	Tomato cv. Stevens	Da_{WA}-1c	-	i.97	M, P, Y, LC, NB, S	CR, SM, S
Le_{NSW}-1 *	Lettuce (*Lactuca sativa*)	-	Griffith, NSW	xi.95	Y, LC, NB, S, TD	M, S
Le_{QLD}-1	Lettuce	-	QLD		P, LD, S, TD	M, S
Li_{WA}-1	Lisianthus (*Eustoma sp.*)	-	Yangebup,WA	xii.98	M, P, LD,	NLL, Y, LD
Po_{WA}-1	Potato (*Solanum tuberosum*)	-	Ludlow,WA	xii.98	P, LC	MM, S
To_{WA}-2	Tomato (*Lycopersicum esculentum*)	-	Wellard, WA	xii.98	NLL, M, PSt, TD	NRS, Y
To_{WA}-3	Tomato	-	Gelor, WA	xii.98	M, P, LD	Y, SM, LD, FA
To_{TAS}-1d *	Tomato, 94RE17	To_{TAS}-1c	-	v.97	M, P, LC, NB, PSt, S	M, S
Derived isolates from TSWV resistant *C. chinense* plants maintained in original accession						
An_{WA}-1di	*C. chinense* PI 159236	An_{WA}-1ci		i.00	M, LD, S	M, LD, S
An_{WA}-1eii	*C. chinense* C00943	An_{WA}-1dii		ii.00	M, LD, S	M, TD
Le_{QLD}-1d	*C. chinense* PI 159236	Le_{QLD}-1c		i.00	P, M, LD	VC, M
To_{TAS}-1dc	*C. chinense* C00943-2-6 (open pollinated)	To_{TAS}-1db		i.00	M, LD	M

[a] NSW = New South Wales, QLD = Queensland, TAS = Tasmania, WA = Western Australia.

[b] Coded symptom descriptions: NLL = necrotic local lesions in inoculated leaves, CR = concentric chlorotic rings in inoculated leaves, NRS = necrotic ringspots in inoculated leaves, VC = vein clearing, MM = mild mottle, M = mottle, SM = severe mottle, P = purpling of leaves, Y = yellowing of leaves, LC = leaf down curling, LD = leaf deformation, LMN = Leaf margin necrosis, NB =systemic necrotic spotting or blotching in leaves, FA = flower abortion, PSt = purple stem streaking, MS = mild plant stunting, S = plant stunting, SS = severe plant stunting, TD = terminal shoot tip death then partial recovery of axillary shoots, SND = death. * Isolates from Latham and Jones (1998)

A total of 1650 plants were inoculated with the 16 isolates (at least 30/isolate and genotype combination). Initially they were scored visually for the numbers of hypersensitive necrotic local lesions that developed on inoculated leaves and then again for any apical leaf symptoms. If no such symptoms formed, plants without them were re-inoculated, a maximum of 3 times each if needed. Tip-leaf samples from each of the plants were tested for TSWV by ELISA, 6wks and 8wks after inoculation.

Graft inoculation

Two sets of graft inoculations with TSWV-infected scions were done to *C. chinense*. Tip leaves from all the plants were tested weekly by ELISA and symptom development was recorded. The first set of grafts using accessions PI 152225 and C00943 involved five original isolates of TSWV, AnWA-1, CaQLD-1, CaWA-1, DaWA-1 and ToTAS-1d (3-plants/isolate and accession combination).The second set of graft inoculations to PI 152225 involved the same five isolates (4 plants/isolate).

Resistance breaking TSWV isolates

As mixed infection with the original non resistance-breaking strain seemed to be present (indicated by necrosis) in some of the resistance breaking isolate cultures, attempts were made to purify the 5 resistant breaking isolates. This was done by repeated subculture in TSWV- resistant *C. chinense* plants using the genotypes from which they were first obtained. Five plants/isolate were used in consecutive cycles until necrosis was minimised. After several attempts, the Ca_{WA}-4 isolate failed to transfer by sap inoculation due its low virus concentration, so it was not maintained. Isolates An_{WA}-1i, Le_{QLD}-1 and To_{TAS}-1 were put through 3 further cycles of inoculations to the original corresponding *C. chinense* genotype to produce purer isolates.

The purified derived isolates An_{WA}-1id, An_{WA}-1iie, Le_{QLD}-1d and To_{TAS}-1dd were then inoculated to five plants each of TSWV resistant *C. chinense* accessions PI 159236, PI 152225 and C00943.

TSWV field experiment

A field experiment was done at a WFT infested site in South Perth. The experimental design was ten replications arranged in a radomised block design with single row plots consisting of seven plants per row. There were four treatments:- *C. chinense* accessions PI 159236, PI 152225 and C00943 and *Capsicum* cv. Rialto (control/susceptible), transplanted 8/10/99. To create a uniform virus source, at either end of each of the rows were TSWV-infected source plants (tomato plants sap inoculated with TSWV), transplanted 8/9/99. Marigolds were planted in between the source plants as a flower source to build up the WFT population. Leaf samples were collected fortnightly from each capsicum and *C. chinense* plant and tested by ELISA.

Survey of capsicum crops for TSWV and bacterial spot

A survey was done late November 1999 on horticultural properties in the Perth metropolitan area. The purpose was to determine the TSWV levels in capsicum and tomato crops and to look for presence of other tospoviruses and bacterial spot disease (*Xanthomonas campestris pv. vesicatoria).* For TSWV random tip leaf samples from 100 plants were taken per crop.

The leaf samples were tested by ELISA with polyclonal antiserum for TSWV (from Agdia) and tospovirus serogroup IV (from DSMZ). With bacterial spot, at least 100 plants were inspected for symptoms in each crop and any suspect leaf samples were collected.

4.3 Results

National survey of TSWV isolates, sap inoculation

In general, the hypersensitive resistance to TSWV held up very well in the 3 lines tested, but 2 Western Australian isolates (An_{WA}-1 from Anemone and Ca_{WA}-4 from *Capsicum*) and Queensland isolate (Le_{QLD}-1 from lettuce) overcame it on a few rare occasions in isolated plants.

Graft inoculation

The first set of grafts using accessions PI 152225 and C00943 involved five original isolates of TSWV, AnWA-1, CaQLD-1, CaWA-1, DaWA-1 and ToTAS-1d (3-plants/isolate and accession combination). Severe necrotic symptoms consisting of necrosis and death of axillary shoots or severe localised necrosis directly below the graft union resulted in death of the infected scion. The second set of graft inoculations to PI 152225 involved the same five isolates (4 plants/isolate). The same two types of necrotic reactions were seen, but there was one exception. One plant infected with isolate Ca_{WA}-1 initially caused axillary shoots to become necrotic but later they developed a mottle that was positive for TSWV when tested by ELISA. Thus by grafting a strain of the virus was selected that overcame the hypersensitive resistance in this plant. This additional resistance-breaking isolate was not maintained. The sap and graft inoculation tests in the glasshouse showed that the resistance in *C. chinense* was very effective but in some cases was overcome.

Resistance breaking TSWV isolates

The numbers of such plants in which resistance was broken out of the 1650 inoculated were only 2 of PI 159236 and 2 of AVRDC C00943. The AnWA-1 isolate actually broke resistance in both PI 159236 and C00943 and infected plants of each were kept separate throughout. They developed somewhat different symptoms. When isolate An_{WA}-1i in PI 195236 and Le_{QLD}-1 in PI 159236 broke resistance, there was systemic TSWV movement to the tip of the plant, resulting in apical necrosis and necrosis of upper axillary shoots but chlorotic spotting, mottle and leaf distortion subsequently developed in later formed axillary shoots. Isolate AnWA-1ii in C00943 caused the same reactions as isolate AnWA-1i but also induced stem streaking and was more severe. With isolate Ca_{WA}-4 in C00943 there was no systemic necrosis, only systemic movement with chlorotic ringspot symptoms. Hypersensitive necrotic spots always formed in the inoculated leaves regardless of isolate/genotype combination. Development of both systemic necrosis and mottle as occurs with An_{WA}-1i, An_{WA}-1ii and Le_{QLD}-1 suggested that the resistance breaking component and the original non-resistance breaking component might be present as a mixture.

Isolates An_{WA}-1i, Le_{QLD}-1 and To_{TAS}-1 were put through 3 further cycles of inoculations to the original corresponding *C. chinense* genotype to produce purer isolates. In PI 159236 Le_{QLD}-1d then caused symptoms consisting predominantly of mottle, vein clearing and leaf deformation. In PI 159236 An_{WA}-1id then caused predominantly mottle and chlorotic spots, while in C00943 To_{TAS}-1dd induced symptoms of strong mottle and chlorotic spotting with

slight leaf deformation. With An_{WA}-1iie, 4 cycles of inoculations to C00943 were less successful in decreasing necrosis. Here the symptoms were mottle, necrotic and chlorotic spotting, and occasional apical necrosis followed by death of the plant or partial recovery. With partial recovery, the symptoms in axillary shoots were mottle and necrotic/chlorotic spotting.

A commercial hybrid of capsicum, cultivar Yatasto, that is resistant to TSWV, was challenged in the glasshouse with 4 original TSWV isolates from WA and QLD, An_{WA}-1, Ca_{WA}-1, Le_{QLD}-1, Da_{WA}-1b. With the 3 WA isolates, local necrotic spots developed in inoculated leaves with no systemic infection. However with the Le_{QLD}-1 isolate, 2/10 plants also developed systemic infection resulting in systemic necrotic spotting followed by systemic necrosis and death, another form of the hypersensitive resistant reaction, the other 8 plants responding as with the WA isolates. In addition, 4 derived TSWV resistance breaking isolates were inoculated to Yatasto. These derived isolates An_{WA}-1di, An_{WA}-1eii, Le_{QLD}-1d and To_{TAS}-1dd, all overcame its resistance causing systemic mottle, chlorotic spotting and stunting. The symptom reactions are presented in Table 2.

Table 2. *TSWV resistant and susceptible genotypes of Capsicum, Capsicum chinense and Capsicum chinense backcrosses used*

Genotype	**Resistance Gene(s)**	***Supplier***
b) Pepper		
cv. Rialto	Susceptible	Henderson Seed Company, Australia
cv. Yatasto	*Tsw* ex PI 159236	Rijk Zwaan Company, Australia
DRP 3948 F1	*Tsw* ex PI 159236	Rijk Zwaan Company, Australia
c) *C. chinense*		
PI 152225	DHG[a]	H A Hobbs, USA
PI 159236	*Tsw*	H A Hobbs, USA
AVRDC C00943	?	H A Hobbs, USA
d) *C. chinense* Backcrosses		
PI 152225 1-1, 2, 3, 4, 5	DHG[a]	D McGrath, QLD
PI 152225 3-1, 2, 4, 5	DHG[a]	D McGrath, QLD
PI 159236 2 & 3	*Tsw*	D McGrath, QLD
C00943 2-3, 4, 5, 6, 7, 12	?	D McGrath, QLD

[a] DHG = dominant hypersensitivity gene

TSWV field experiment

TSWV was not detected in any of the plants in this trial. This was possibly due to the use of an old TSWV isolate which was no longer readily WFT transmissible and/or to late transplanting missing the peak spring population of thrips.

Survey of capsicum crops for TSWV and bacterial spot

In capsicum, 6/11 crops had TSWV with up to 40% infection detected. There were no tospovirus serogroup IV positives present in the samples.

No definite symptoms of bacterial spot were seen, but 3 suspect samples with bacterial spot symptoms were collected from different properties. Gram staining tests were revealed one sample was gram-negative and the sample was sent to QDPI Gatton for further analysis. *Xanthomonas campestris pv. vesicatoria* was not detected and therefore bacterial spot infection was not detected during this survey. In August 2000, a tomato sample was taken from a backyard in the southern suburbs of Perth containing possible bacterial spot symptoms. Gram staining results determined it was gram-negative. The BIOLOG system for bacterial disease diagnosis at South Perth was used to identify the species. However, the result was *Xanthamonas campestris pv. begoniae*. The isolate was sent to QDPI for further testing.

4.4 Discussion

The isolates of TSWV from four Australian states were generally avirulent on the three PI lines of *C. chinense* tested in this series of experiments; suggesting that all lines would be useful as sources of broadly-adapted genetic resistance. At least five different lines of *C. chinense*, including the three tested here, possess a single dominant gene at the *Tsw* locus when inoculated with a range of overseas isolates (Boiteaux 1995, Black *et al.*. 1996). It is possible that the same gene confers resistance to the Australian isolates, although these experiments do not test the hypothesis. However, Chapter 6 provides evidence of resistance to a Queensland isolate in one PI line co-segregating with a DNA marker linked to *Tsw* (Moury *et al.* 2000), suggesting the same gene is effective in Australia.

The resistance-breaking isolates identified here were obtained in the glasshouse following multiple cycles of inoculations to TSWV-resistant plants of *C. chinense*. There was no evidence that similar isolates were present in Western Australian field crops and that the resistance of commercial cultivars would readily break down in the field. The absence of multiple strains of TSWV in field crops may be due to the limited use of resistant cultivars. Increased areas of resistant cultivars may induce new virulent strains in the future, but the survey did not reveal such strains.

Although the Western Australian survey identified widespread TSWV infection, there was no evidence of tospovirus serogroup IV. The TSWV resistance identified in the PI lines, most likely *Tsw*, should therefore be transferred by conventional breeding to a range of adapted breeding lines. *Tsw* will provide complete protection against the current isolates of TSWV in Western Australia.

References

Black, L.L., Hobbs, H.A., and Kammerlohr, D.S. (1996) Resistance of Capsicum chinense lines to tomato spotted wilt virus isolates from Louisiana, U.S.A. and inheritance of resistance. In International symposium on Tospoviruses and Thrips of Floral and Vegetable Crops. Nov 7-10 1995, Taichung, Taiwan. Acta Hortic. 431, 393-401.

Boiteux, L.S. (1995) Allelic relationships between genes for resistance to tomato spotted wilt tospovirus in Capsicum chinense. Theor Appl Genet 90, 146-149.

Moury, B., Pfieger, S., Blattes, A., Lefebvre, V., and Palloix, A. (2000). A CAPS marker to assist selection of tomato spotted wilt virus (TSWV) resistance in pepper. Genome. 43, 137-142.

5. Capsicum Chlorosis Virus, a tospovirus serogroup IV species infecting capsicum and tomato in Queensland, Australia

by Lee McMichael, Denis Persley and John Thomas, Queensland Horticulture Institute, 80 Meiers Road, Indooroopilly 4068.

5.1 Summary

A tospovirus which failed to react with antibodies to serogroup 1(Tomato spotted wilt virus) was isolated from capsicum, chilli and tomato plants at Bundaberg in 1999 and 2000 and from capsicum in the Burdekin area of north Queensland in both years. The virus, tentatively named Capsicum chlorosis virus(CCV), did react with antibodies to tospovirus serogroup IV viruses and in polymerase chain reaction assays using primers specific for members of serogroup IV. Cloning of the N gene and subsequent sequence comparisons with other tospoviruses indicated a 80-85% identity of the amino acid and nucleotide sequences between CCV and three definitive members of serogroup IV(Watermelon silver mottle ,Peanut bud necrosis and Watermelon bud necrosis viruses). Identity with all other tospovirus serogroup members was less than 29% which places CCV as a probable new tospovirus species within serogroup IV. This is the first record of a tospovirus other than the type species, Tomato spotted wilt virus in Australia.

In surveys CCV was widespread in capsicum and chilli crops at Bundaberg and was the dominant tospovirus detected in these crops in March 2000. In September 2000 it was found in 10 of 12 samples collected from several plantings on a farm at Ayr. Symptoms are typically stunting of plants, interveinal chlorosis and leaf distortion. CCV was not detected in capsicum crops in the Bowen and Gumlu areas. TSWV was common with disease levels of up to 30% at Gumlu in September 2000. The experimental host range of TSWV and CCV were similar on a range of diagnostic species. Importantly, CCV infected three Capsicum chinense lines (PI 152225, PI 159236, AVRDC 00943) with resistance to TSWV and tomato carrying the Sw-5 resistance source to TSWV.

The thrips vector(s) transmitting either TSWV or CCV in Queensland has not been fully determined. The western flower thrips (Frankliniella occidentalis) is absent or has a restricted distribution in the major capsicum production areas. Tomato thrips (Frankliniella schultzei)is the dominant species in production areas while the possible role of Thrips palmi, a known vector of serogroup IV tospoviruses, has yet to be established.

5.2 Introduction

Viruses within the genus Tospovirus, family Bunyaviridae, are of increasing importance worldwide in crop and ornamental plants (Prins and Goldbach, 1998). The type species, Tomato spotted wilt virus (TSWV), was first found and investigated in Australia (Brittlebank, 1919; Samuel *et al.*., 1930). In recent years, considerable diversity has been found within the genus with at least ten distinct species being described on the basis of nucleotide and amino acid sequence, serological relationships, vector specificity and host range (Goldbach and Kuo, 1996; Bezerra *et al.*., 1999).

Tospoviruses are characterised by a genome comprising three separate, single stranded RNA molecules, termed S, M and L, tightly associated with nucleoprotein (N) and enveloped in a

lipid membrane carrying two types of glycoprotein (G1, G2). The nucleoprotein and the precursor to the glycoproteins are encoded on the viral complementary strand of the S and M RNAs, respectively. The L RNA is of negative polarity and encodes the viral RNA dependent polymerase (Prins and Goldbach, 1998).

All tospoviruses are transmitted in nature by thrips in a propagative and persistent manner (Mumford *et al.*., 1998) with at least seven species confirmed as vectors (Goldbach and Kuo, 1996). Established and tentative tospovirus species have been classified by serogroup based on serological characterisation using polyclonal antibodies to the nucleocapsid (N) protein and sequence homology (De Avila *et al.*., 1993, Goldbach and Kuo, 1996; Jain *et al.*., 1998). Ten serogroups are currently recognised within the genus (Jain *et al.*., 1998). The type species TSWV is a member of serogroup 1 and another well-characterised species, Impatiens necrotic spot virus (INSV), is a member of serogroup III (Goldbach and Kuo, 1996). Several species prevalent in Asia, eg Watermelon silver mottle virus (WSMV), Peanut bud necrosis (PBNV), are members of serogroup IV (Jain *et al.*., 1998, Yeh *et al.*., 1996).

Control of tospovirus has proven difficult across the wide range of crop plants affected. Control of the thrips vectors has been difficult because of the wide distribution and fecundity of vector species, particularly Frankliniella occidentalis and the rapid development of insecticide resistances within thrips populations (Mumford *et al.*., 1996). Some success has been achieved by cultural controls which minimise sources of infection and integrated disease management strategies (Cho *et al.*., 1989, Latham and Jones, 1996). Prospects for biological controls and integrated pest management for thrips are being investigated (Goldbach and Kuo, 1996). The development of resistant cultivars has been hampered by the lack of resistance to TSWV and other tospoviruses in many susceptible species and the instability of some resistance sources (Qiu and Moyer, 1999). Effective resistance against TSWV has been introgressed from Lycopersicon peruvianum into L. esculentum (tomato) cv. Stevens with resistance conferred by the single gene, Sw-5 (Stevens *et al.*., 1992). Several Capsicum chinense accessions have been used as sources of resistance to TSWV in pepper (C. annuum) breeding programs (Moury *et al.*. 1997).

TSWV has caused major losses in vegetable and flower crops in Australia in recent years with capsicum, potato, lettuce and tomato being particularly affected (Latham and Jones, 1996; Wilson, 1998; Jones and Cook, 2000). All tospovirus isolates assayed serologically in Australia from a wide range of crop, ornamental and weed species have reacted with antibodies to serogroup 1-TSWV (Latham and Jones, 1997; Latham and Jones, 1998; Wilson, 1998, Hill and Moran, 1996).

Capsicum and tomato production are major horticultural industries in the Bundaberg area of south Queensland and the Bowen/Burdekin region of north Queensland.

In 1999 samples of capsicum, chilli and tomato from the Bundaberg district of south-east Queensland had symptoms suggesting tospovirus infection but failed to react with serogroup 1 antibodies (McMichael *et al.*., 2000). The identification and characterisation of these isolates is described in this paper.

5.3 Materials and Methods

Virus isolates and plant inoculations

Virus isolates were stored in liquid nitrogen to maintain infectivity as frozen desiccated leaf tissue at –15°C. Cloning and sequencing experiments were conducted with capsicum tospovirus iv isolates #958 from capsicum at Bundaberg and #1043 from capsicum in the Burdekin district. Isolates were propagated in capsicum (Capsicum annuum) cv. Yolo Wonder and Nicotiana benthamiana. Sap inoculations were done by preparing leaf extracts in 0.1M phosphate buffer pH 7.0 containing 0.1% sodium sulphite and applying to carborundum-dusted leaves of plants. Plants were briefly rinsed following inoculation and maintained in a glasshouse. In host range and resistance screening experiments, plants were inoculated twice several days apart and inspected for symptoms for four weeks. Symptomless plants were assayed by ELISA.

Antisera and serological assay

The antisera used were to TSWV (Agdia Cat. No. 39300), TSWV/INSV (Sanofi Cat. No. 51225), combined antibodies to tospovirus serogroups I, II and III (Bioreba Cat. No. 1903315 and 190325), and antibodies to serogroup IV (DSMZ Cat. No. AS-0118).

ELISA assays were carried out in Nunc Maxisorb plates. Reaction volumes were 100 µL and all incubations were at room temperature.

Samples were extracted (1/10 dilution) in PBS-T-PVP. The ELISA tests were carried out according to suppliers' protocols.

Cloning and sequencing of the N gene

Total RNA was extracted from 100mg fresh capsicum tissue infected with capsicum chlorosis virus isolates #958 and #1043 using the Rneasy Plant Mini Kit (QIAGEN), according to manufacturers instructions.

Polymerase chain reaction (PCR) products were amplified from the 3' region of the SRNA (comprising the nucleoprotein gene and the intergenic region). Four primers were used; HRP37 and HRP38 (Jain *et al.*., 1998), TOS4R1 (CTA ATG TTG GTC CAG AGG AT based on primer HRP53, Jain *et al.*., 1998), and an oligo dT primer (GGA TCC CGG GTT TTT TTT TTT TTT TTT V, based on the potyvirdae 1 primer of Gibbs and McKenzie, (1997). cDNA was synthesised by reverse transcription using Superscript II (Gibco BRL)

Using Jain's primers, the products were amplified by incubating at 94°C for 1 min, followed by 25 cycles of 90°C for 30 sec, 54°C for 2 min and 72°C for 2 min, followed by 72°C for 5 min. When using the oligo dT primer in combination with TOS4R1 of Jain's primers, products were amplified by incubating at 94°C for 1 min, followed by 3 cycles of 90°C for 30 sec, 40°C for 2min and 72°C for 2 min, followed by 25 cycles of 90°C for 30 sec, 54°C for 2 min and 72°C for 2 min, followed by 72°C for 5 min.

PCR fragments were cloned into the PCR 2.1-TOPO vector using the TOPO TA cloning kit (Invitrogen) according to manufacturers' instructions. Clones were screened by PCR using T7 and M13R primers. The plasmid was purified using QIAprep Spin Minikit (QIAGEN) and sequenced using Applied Biosystems Inc. (ABI) automated sequencing system, using T7 and M13R primers and specific internal primers with the dideoxynucleotide chain termination method. Three clones of each PCR product were sequenced in both directions.

Sequence analysis was completed using the FASTA, CLUSTAL W and GCG programs available through Australian National Genomic Information Service (ANGIS), at the University of Sydney. The sequence of the putative viral N gene was compared with those of other tospoviruses obtained from Genebank. The accession numbers are as follows. Tomato spotted wilt virus (TSWV), D13926; Tomato chlorotic spot virus (TCSV), S54325; Groundnut ringspot virus (GRSV), S54327; Impatiens necrotic spot virus (INSV), D00914, Peanut bud necrosis virus (PBNV), Z46419; Watermelon silver mottle virus (WSMV), X78556; Watermelon bud necrosis virus (WBNV), AF045067; Peanut yellow spot virus (PYSV), AF013994; Iris yellow spot virus (IYSV), AF001387; Melon yellow spot virus (MYSV), AB024332; Zucchini lethal chlorosis virus (ZLCV), AF067069; Chrysanthemum stem necrosis virus (CSNV), AF067068.

5.4 Results

Virus identification

Twenty samples collected over a period of several weeks in March 1999 from capsicum, chilli and tomato crops growing at Bundaberg had symptoms of stunting, leaf chlorosis, leaf distortion and ringspots indicative of tospovirus infection. Samples from these species collected earlier in 1999 and in previous years from the same area had all reacted with TSWV serotype 1 antiserum (Persley and McMichael, unpublished data). However, these 20 samples all failed to react with antibodies to tospovirus serogroup 1 and to combined antibodies to tospovirus serotypes I, II and III. Negative results were also obtained in polymerase chain (PCR) assays using TSWV-specific primers and degenerate primers to serotypes I, II and III (Mumford *et al..*, 1996). Seven selected samples, however, from tomato, capsicum and chilli were positive when tested against antibodies to tospovirus serotype iv (DSMZ antiserum) by ELISA with absorbance values 5 to 12 times that of appropriate healthy controls. Absorbance values obtained with these isolates were not significantly different from those recorded for WSMV, a serotype iv positive control. The serogroup IV virus was given the tentative name capsicum chlorosis virus(CCV).

Cloning and sequencing of the N gene

The PCR primer pair designed by Jain *et al..*, (1998) for sequencing of the WBNV N gene were not successful for amplification of the N gene sequence of the CCV isolates. Thus the primers were either modified using known sequence information of WSMV and PBNV (primer TOS4R1) or used in combination with an oligo dT primer which bound to the intergenic region of the S-RNA. From isolate 958, two fragments were amplified, a 357 base fragment using Jain's HRP37/HRP38 primer set and a 746 base fragment using the oligo dT and TOS4R1 primers. From isolate 1043 a single 1174 base fragment was amplified using a combination of Jain's HRP38 and oligo dT primers.

The complete ORF on the viral complementary strand of the S-RNA is 825 nucleotides long potentially encoding for a protein of 275 amino acids with a predicted molecular weight of 30.7 kDa (Figure 1). Based on sequence homology with the N genes of PBNV, WSMV and WBNV, the ORF is predicted to encode for the N gene of CCV.

Comparison of N gene with other tospoviruses

There was a 97% identity between the N gene sequences of CCV isolates 958 and 1043 at the nucleotide level and 98% identity at the amino acid level. Comparisons of the nucleotide sequences of the CCV N gene with those of other tospoviruses (Table 1), showed an 80% - 85% identity with the three members of the tospovirus serogroup IV; WSMV, PBNV and WBNV. Similarly, amino acid sequence comparison showed a 80 to 85% identity with the N proteins of serogroup IV members, the highest identity of 85% for both isolates being with WSMV. The highest identity with other tospovirus serogroup members were with MYSV and IYSV with sequence identities of 57% and 40% respectively for both nucleotide and amino acid sequences. Identity with all other tospovirus serogroup members was less than 29%. The CCV N gene has a length similar to that of other serogroup IV members of around 830 nucleotides and 275 amino acids. MYSV with 839 nucleotides and 279 amino acids and IYSV with 810 nucleotides and 270 amino acids are more similar in length to CCV than other tospoviruses with nucleotide sequences ranging between 740-780 nucleotides and 250-260 amino acids in length.

Tospovirus surveys

Surveys in the Bundaberg district later in 1999 and in March 2000 confirmed the presence of a serogroup IV tospovirus (CCV) in capsicum, chilli and tomato crops. In March 2000, the virus was found at widely separated geographic areas within the Bundaberg district and was detected by ELISA in 41 capsicum and chilli samples and five tomato samples. TSWV was not detected in any samples.

Capsicum chlorosis virus was detected in one capsicum plant submitted for diagnosis in September 1999 from Ayr (isolate 1043). In September 2000, the virus was found in 10 of 12 samples collected from several crops on a farm at Ayr. TSWV was also detected in several samples at this location. Dual infection by TSWV and CCV was not found. In contrast, only TSWV was detected by ELISA in samples collected at Bowen and Gumlu on the same dates.

Host range and symptoms

The natural hosts found for CCV were capsicum, chilli and tomato. The symptoms on tomato were similar to those caused by TSWV with plants being stunted with necrotic flecks and spots on the leaves and petioles. Chlorosis, mottling and purple ringspots often developed on leaves.

Infected capsicum and chilli plants were stunted. Leaves had marginal and interveinal chlorosis and were often narrowed and curled. The chlorotic ringspots and line patterns consistently seen on capsicum plants infected by TSWV were uncommon on plants infected by CCV.

The experimental host ranges of an isolate each of CCV (958) and TSWV (873) are given in Table 2.

TSWV (isolate 873) and CCV (isolate 958) both caused symptoms typical of tospovirus infection in a range of diagnostic hosts, inducing necrotic or chlorotic local lesions and concentric rings on inoculated leaves. Systemic symptoms included mosaic, mottling, vein necrosis and leaf deformation. The two isolates had similar experimental host ranges. Some differences were the lack of systemic infection in Nicotiana glutinosa and Nicotiana rustica by CCV and the severe symptoms induced by TSWV in Datura stramonium.

Importantly, CCV systemically infected tomato with the Sw-5 TSWV resistance gene and three Capsicum chinense accessions (PI 152225, PI 159236 AVRDC 00943) resistant to TSWV. In contrast, these genotypes did not develop systemic systems with TSWV isolate 873 and virus was not detected by ELISA in new growth leaves.

The TSWV resistant varieties Yatasto, Novartis 636, 633 and 632 developed typical symptoms of tospovirus infection with all plants of the four varieties developing ringspots, vein-banding and chlorosis on new growth leaves. There was no evidence of hypersensitive, necrotic local reactions in inoculated leaves. When the four varieties were inoculated with TSWV isolate 873, necrotic ringspot lesions developed on the inoculated leaves followed by terminal necrosis and stunting. A proportion of plants then developed symptomless new growth leaves.

5.5 Discussion

Previous surveys for tospovirus in crop, ornamental and weed species in Australia have detected only TSWV, a member of serogroup 1 (Latham and Jones, 1997; Wilson, 1998; Hill and Moran, 1996). TSWV was detected in all plants with tospovirus symptoms so, although members of other serogroups may have been present, it would have been as mixed infections with TSWV.

In this work, a virus with symptoms suggesting tospovirus infection was isolated from capsicum, chilli and tomato. The isolates failed to react with antiserum to tospovirus serogroup 1 but did react with antibodies to WSMV, a member of serogroup IV. This was the first evidence of the existence of a serogroup other than serogroup 1 in Australia and of a tospovirus other than the type species, TSWV. These data were confirmed by PCR assays using known sequence information for members of serogroup IV viruses. Cloning and subsequent sequence analysis of the N protein gene revealed that, based on the sequence comparisons with other tospovirus N genes, the virus is a member of serogroup IV. The virus had a 80-85% nucleotide and amino acid sequence similarity with three members of this group, WSMV, PBNV and WBNV. The highest similarity with other tospoviruses was a nucleotide and amino acid similarity of 57% with MYSV, also a probable member of serogroup IV (Kato *et al.*. 2000). The data clearly indicates that the Queensland tospovirus is a member of serogroup IV and with an amino acid N gene sequence similarity of less than 90% with other known members of the group is most likely a new species (Goldbach and Kuo 1996) and has been given the tentative name Capsicum chlorosis virus.

The presence of a second tospovirus species (CCV) in capsicum, chilli and tomato crops in Queensland is of considerable significance. The incidence of TSWV in capsicum crops has increased in recent years in both southern and northern Queensland (Persley, McMichael and

McGrath, unpublished data) with levels of up to 30% in crops being recorded. Capsicum chlorosis virus has been found at Bundaberg and at Ayr in north Queensland. It was not detected in the Gumlu/Bowen region in 2000 although its presence in the Burdekin suggests that it will reach these production areas in the near future. In March 1999, CCV was widespread and the dominant virus found in capsicums at Bundaberg, although the incidence in crops was less than 5%. The thrips vector(s) transmitting either TSWV or CCV in Queensland has not been fully determined. The western flower thrips (Frankliniella occidentalis), the most efficient vector of TSWV, is absent or has a restricted distribution in the areas surveyed. Trapping data suggests that Frankliniella schultzei (tomato thrips) is the dominant species in Bundaberg and the Bowen/Burdekin region (Artlett and Abbott pers. comm.). The possible role of species such as Thrips palmi, a known vector of serogroup IV viruses (Goldbach and Kuo, 1996) has yet to be established.

Importantly, CCV overcame the resistance against TSWV in three Capsicum chinense accessions (Table 2) and the Sw-5 TSWV resistance gene in tomato. A collection of capsicum lines introduced from the University of Georgia Genetic Resources Unit is currently being screened for resistance to tospovirus iv (Persley and McGrath, unpublished data).

The experimental host ranges of TSWV (isolate 873) and CCV (isolate 958) were similar with both viruses causing local and/or systemic infection of several diagnostic hosts for tospoviruses. CCV caused systemic symptoms in one of eight peanut plants but did not systemically infect watermelon or other cucurbit species which are important hosts of WSMV, a tospovirus iv species common in parts of Asia (Yeh and Chang 1995). The origin of CCV is not known, although the large differences in sequence homology between the N gene of TSWV and CCV suggest that it is unlikely to have evolved by genomic reassortment within TSWV.

Acknowledgements

The assistance of Garry Artlett(CropTech,Bundaberg) and Dale Abbott (Bowen Crop Monitoring) in providing samples and information is gratefully acknowledged.

References

Bezerra, I.C., Resende, R. de O., Pozzer, L., Nagata, T., Kormeliknk, R. and De Avila, A.C. (1999). Increase in tospoviral diversity in Brazil with the Identification of two new tospovirus species, one from chrysanthemum and one from zucchini. Phytopathology 89, 823-830

Brittlebank, C.C. (1919). Tomato diseases. Journal of Agriculture, Victoria 27, 231.

Cho, J.J., Custer, D.M., Brommonschenkel, S.H. and Tanksley, S.D. (1996). Conventional breeding: host-plant resistance and the use of molecular markers to develop resistance to tomato spot wilt virus in vegetables. Acta Horticulture 431, 367-378.

Cho, J.L., Gonslaves, D. and Provvidenti, R. (1989). A multidisciplinary approach to management of tomato spotted wilt virus in Hawaii. Plant Disease 73, 375-83.

Gibbs, A. and McKenzie, A. 1997. A primer pair from amplifying part of the genome of all potyviroids by RT-PCR. Journal Virological Methods 63, 9-16.

Goldbach, R. and Kuo, G. (1996). Introduction. International symposium on tospoviruses and thrips of floral and vegetable crops. Proceedings. Taiwan Agricultural Research Institute, Taichung, Taiwan. Nov 7-10, 1995. Acta Horticulture 431, 21-26.

Hill, M.F. and Moran, J.R. (1996). The incidence of tomato spotted wilt tospovirus (TSWV) in Australian nursery plants. Australasian Plant Pathology 25, 114-19.

Jain, R.K., Pappu, S.S., Pappu, M., Reddy, K. and Vani, A. (1998). Watermelon bud necrosis tospovirus is a distinct virus species belonging to serogroup IV. Archives of Virology 143, 1637-1644.

Jones, R.A.C. and Cook, D. (2000). Proactive research and extension avoids damaging outbreak of WFT and TSWV in West Australian vegetable crops. WA Grower, June 2000, 2-3.

Kato, K. Hanada, K. and Kameya-Iwaki, M. (2000). Melon yellow spot virus: a distinct species of the genus tospovirus isolated from melon. Phytopathology 90, 422-426.

Latham, L. and Jones, R.A.C. (1997). Occurrence of tomato spotted wilt tospovirus in native flora, weeds and horticultural crops. Australian Journal of Agricultural Research 48, 359-369.

Latham, L.J. and Jones, R. A. C. (1996). Tomato spotted wilt virus and its management. W.A. Journal of Agriculture 37, 90-5.

Latham, L.J. and Jones, R.A.C. (1998). Selection of resistance breaking strains of tomaoto spotted wilt tospovirus. Annals of Applied Biology 133, 385-402.

McMichael, L.A., Persley, D.M. and Thomas, J.E. (2000). The first record of tospovirus serotype iv in Australia. Australasian Plant Pathology 29, 149.

Moury, B., Palloix, A., Gebre Selassie, K. and Marchoux, G. (1997). Hypersensitive resistance to tomato spotted wilt virus in three Capsicum chinense accessions is controlled by a single gene and is overcome by virulent strains. Euphytica 94, 45-52.

Mumford, R.A., Barker, I. And Wood, K.R. (1996). The biology of tospoviruses. Annals of Applied Biology 128, 59-83.

Prins, M. and Goldbach. (1998). The emerging problem of tospovirus infection and nonconventional methods of control. Trends in Microbiology 6, 31-35.

Qiu, W. and Moyer, J.W. (1999). Tomato spotted wilt tospovirus adapts to the TSWV N gene-derived resistance by genome reassortment. Phytopathology 89, 575-582.

Samuel, G. Bald, J.G. and Pittman, H. A. (1930). Investigations on "spotted wilt" of tomatoes. Australian Council Scientific and Industrial Research Bulletin. 44, 64.

Stevens, M.R., Scott, S.J. and Gergerich, R.C. (1992). Inheritance of a gene for resistance to tomato spotted wilt from Lycopersicon peruvianum. Euphytica 59, 9-17.

Wison, C.R. (1998). Incidence of weed reservoirs and vectors of tomato spotted wilt tospovirus on southern Tasmanian lettuce farms. Plant Pathology 47, 171-176.

Yeh, S.D. and Chang, T.F. (1995). Nucleotide sequence of the N gene of watermelon silver mottle virus, a proposed new member of the genus tospovirus. Phytopathology 85, 58-64.

Figure 1. Complete nucleotide and deduced amino acid sequence of the CCV nucleocapsid (N) gene.

TAAGTGTTTTGAATCTAAATTGTCTATAGATACATGGACATACATATCTTGATTATAATAGATATCTAT

GTTGTAAACACGCTTATACTTATTTGAGTAATTATATATATTTATATTTATGTGTATATAACTAATAAAC

ACAAACAATAAAATATTTATATATCTAATTTATAACATGATAATAGATTAATATGAATTGACTTATAAT

AAATATAACATACATTTATATATATATAAAAACATATATTCTAATATAAGTAATTACAAATATTTAAATT
*

ACACTTCTATAGAAGTACTAGGCTTTGAGCTCTTCTTTGAATGAACACCATAATCATCCACAGACAAAT
V E I S T S P K S S K K S H V G Y D D V S L N

GGCACTAAACGCTTTGTCCATATACTTAACTTGCTCATCATACTTCTTAAGTGAGATGGAACTAGCAGTA
A S F A K D M Y K V Q E D Y K K L S I S S A T

CCGGGGTTGCTTTCACTAAGCAACTTGACAGCCTGTTTGAACAATGTATTCAGATCTTCCTTAAATTCTA
G P N S E S L L K V A Q K F L T N L D E K F E

TTTGGGAAGCAGAAAGAACCTTAGCCACTTTGCAAACCTGTTCATAGGTAGAGAAGTTTTTGATGCCCAA
I Q S A S L V K A V K C V Q E Y T S F N K I G L

TTTCTCTTTTTTAACATTTTGGTAATAAGCTAATGGGAAAATAATTGGTGCAAGGCCCTTGATGCTGGAT
K E K K V N Q Y Y A L P F I I P A L G K I S S

AATAGTGGTAGAGGACCACCAATACACAACATCATCCTGAGAGCACATGAATCAAACAAAGCAGGTATGT
L L P L P G G I C L M M R L A C S D F L A P I

TCAATCCATATGCTGCCACTAAAGGTAATTCCATTACCTTTGCATACATCTCTTGTCTAGCACCTTCATT
N L G Y A A V L P L E M V K A Y M E Q R A G E N

CTTACTCCTTTCTACCATATTGATCATCTTAACTCCTATCAAAGCTTCTGTTCTCTTAAAAGTCCAATCC
K S R E V M N I M K V G I L A E T R K F T W D

TCTGGACCAACATTAGCATCCGTGGAAACAATTGTTTTTTCACAGAACTTATACTTTCCACTTTTGCAAG
E P G V N A D T S V I T K E C F K Y K G S K C

CAGCAAAAATCTGCTTTCTGCACTTCAAAATATTAAGACAGTTTGTGAAAGTCATTTCAACGTTTTTGTT
A A F I Q K R C K L I N L C N T F T M E V N K

ATTATCATAGAATGTCTTGAAACTGAATCCAGGAGTTGAATCCTCAGTTTCAATTTCAACATCTGCAGTT
N N D Y F T K F S F G P T S D E T E I E V D A T

CCACCAGCCAAGAGTTCTCTAATTTTCTTCTCGGTAAGTTGCCTAACGTTAGACATGGTGTTTACTATGG
G G A L L E R I K K E T L Q R V N S M

AAACGTCTGATATGATGACTTTAATCGGATTTTATTA

Table 1. Amino acid sequence homology (% identity) of the N gene of reported *Tospovirus* species

	TSWV	TCSV	GRSV	INSV	WSMV	PBNV	WBNV	PYSV	IYSV	MYSV	CSNV	ZLCV	CCV	CCV
							958	1043						
TSWV	100	77	78	53	28	27	26	16	31	26	76	72	27	28
TCSV		100	81	52	27	27	26	11	29	24	72	71	28	29
GRSV			100	52	27	26	26	18	28	27	73	75	28	28
INSV				100	26	26	26	17	25	24	53	51	26	27
WSMV					100	84	84	18	38	56	25	25	85	85
PBNV						100	82	16	42	58	25	26	82	83
WBNV							100	16	38	57	24	25	80	81
PYSV								100	17	17	13	13	19	19
IYSV									100	44	29	28	40	40
MYSV										100	26	24	57	57
CSNV											100	79	26	26
ZLCV												100	26	26
CCV 958													100	98
CCV 1043														100

Table 2. Comparative experimental host range of tomato spotted wilt virus (isolate 873) and capsicum serogroup IV tospovirus (isolate 958)

Host	TSWV-873		CCV-958	
	local	systemic	local	systemic
Amaranthaceae				
Gomphrena glabosa cv. Buddy Purple	NL*	NS, NR, GR	NL	—
Asteraceae				
Chrysanthemum paludosum	—	Mo	—	Mo
Latuca sativa	—	NS, Mo	—	NS, Mo
Chenopodiaceae				
Chenopodium quinoa	CL, NL	Mo, W	CL, NL	Mo, W
Chenopodium amaranticolor	CL, NL	—	CL, NL	—
Cucurbitaceae				
Citrullus lanatus cvv. Candy Red, Warpaint, Candy Sweet	NS	—	NS	—
Cucumis sativus cv. Green Gem				
Cucurbita pepo cv. Green Buttons	—	—	—	—
Leguminosae				
Arachis hypogaea cv. *Stretton*		Mo, GR, NS	—	Mo, GR, NS
Phaseolus vulgaris cv. Bountiful	NL, CL	—	NL, CL	—
Vigna unguiculata cv. Black-Eye	NL	Mo, CS	NL	—
Solanaceae				
Datura stranonium	RS	Mo, RS, LD	RS	NS, Mo
Lycopersicon esculentum				
cv. Gross Lisse	—	NS, Mo	—	NS, Mo
cv. Tolpot	—	NS, Mo		NS, Mo
cv. Stevens (*Sw-5*)	—	—	—	NS, Mo
Nicotiana benthaminana	CS	M, W	CS	M, W
Nicotiana glutinosa	NL	Mo, LD, GR, W	NL	—
Nicotiana rustica	RS	M, RS, LD	NL, CL	—
Nicotiana tabacum cv. *Xanthi*	NL	—	NL	—

Host	TSWV-873		CCV-958	
	local	systemic	local	systemic
Capsicum annuum cv. Yolo Wonder	CL	Mo, LD, RS		Mo, LD
Capsicum chinense				
PI152225	NL	—	—	Mo, LD, GR
PI159236	NL	—	—	Mo, LD, GR
AVRDC00943	NL	—	—	Mo, LD, GR
Petunia hybrida cv. Dreams White	NL	M	RS	Mo
Solanum melongena cvv. Epic, Snow Drop	—	Mo	—	Mo

* CL – chlorotic lesion, NL – necrotic lesion, NS = necrotic spot, NR = necrotic rings, GR = growth reduction, M = mosaic, Mo = mottle, LD = leaf deformation, W = wilt

6. Genetic analysis of TSWV resistance – DNA markers

by Jodie Guthrie, Plant Sciences Group, Central Queensland University, Rockhampton.

6.1 Introduction

Until recently selection for disease resistance has been by direct screening where a breeding population is exposed to natural or artificial inoculum and resistant lines are selected. Although this can be effective there are circumstances in which it is difficult to use this selection method successfully. Examples would include recessive genes that require progeny testing in later generations, multiple gene resistances and labour intensive screening regimes.

The recognition and development of linked DNA markers have provided a significant improvement in the efficiency of disease resistance breeding programs. Direct selection for a linked marker can bypass costly glasshouse bioassays and identify resistant individuals relatively easily in the laboratory, leading to cost-effective management of breeding progenies.

This research was undertaken initially to develop DNA markers for TSWV resistance in one breeding population and subsequently to confirm the presence of a marker identified in another population (Moury *et al.* 1999).

6.2 Materials and Methods

Populations generated for analysis. Test generations were constructed from an interspecific hybrid of a selection from the TSWV susceptible *Capsicum annuum* cv. Merlin (M 1-1) and TSWV-resistant selections from *Capsicum chinense* C00943 (2-3). An F1 of (M1-1 x 2-3) produced derived F2 and backcross generations to Merlin and C00943 (Table 1).

Management of plant material. The parent genotypes M1-1 and C00943 used to develop the generations were shown by bioassay to be uniformly susceptible and resistant respectively to TSWV. Numbered seedlings of parent lines and derived populations were grown in containerised trays in the glasshouse, sampled for DNA extraction and then inoculated twice with an isolate of TSWV for bioassay. Seedlings were rated initially for visual symptoms and then by ELISA to determine their status for genetic analysis. F2 and backcross seedlings were maintained in trays for up to eight weeks after inoculation to allow selection for resistance and later crossing.

Sample collection. One to two lower leaves were collected from each capsicum seedling and frozen at -80°C until DNA was extracted. Details of genetic populations sampled are given in Table 1.

Table 1: The number of individual capsicum seedlings sampled from each genetic population

Population	Date sampled	Number sampled
Capsicum annuum (F_4 Merlin 1-1-1)	March 1999	15
Capsicum chinense (2-3-1) 00943	March 1999	18
Capsicum chinense (2-3-2) 00943	March 1999	15
F_1 (M1-1 x 2-3)	March 1999	12
F_2 (M1-1 x 2-3)	March 1999	152
BC [M1-1-1 x F_1 (M1-1 x 2-3)-2]	March 1999	187
BC [F_1 (M1-1 x 2-3)-1 x 2-3-1]	March 1999	66
F_2 (BC [M1-1-1 x F1 (M1-1 x 2-3)-2]113)	October 2000	64
Merlin x (Merlin x Novartis CP633)	November 2000	26
Yatasto (Rijk Zwaan)	November 2000	5
M (Merlin) 1-2 x 2-3	November 2000	4
Novartis CP636 x 2-3-1	November 2000	1
Merlin x Novartis CP633	November 2000	5
M (merlin)1-2 x 1-3	November 2000	3
Novartis CP636	November 2000	2
Novartis CP632	November 2000	2
Novartis CP633	November 2000	1
Novartis Matrix	November 2000	1

DNA extraction and purification. DNA was extracted from a single leaf sample using the method of Doyle and Doyle (1990) and purified using the Wizard DNA Cleanup System (according to manufacturer's instructions using a vacuum manifold).

Bulked segregant analysis. Initially, the parents (*Capsicum annuum* (Merlin) and *C. chinense* 00943) and F_1 population were used for screening 220 primers by RAPD (random amplified polymorphic DNA) analysis for their suitability for further analysis of the F_2 population (i.e. present of polymorphisms between the two parents). RAPD analysis was performed as described by Paran *et al.*. (1991). Nine to eleven F_2 individuals were used to comprise both the TSWV-resistant bulk and the TSWV-susceptible bulk based on their response and ELISA results to TSWV inoculations. Those individuals that did not have correlating response and ELISA results were not used in the bulks. The bulk solutions were prepared from equimolar quantities of individual leaf DNA to a final concentration of 10 ng/μL. In total, 27 TSWV-susceptible F_2 individuals and 62 TSWV-resistant F_2 individuals were screened. Bands that were present in the resistant parent and bulk but absent in the susceptible parent and bulk (polymorphism) were preliminarily assumed to be associated with the TSWV-resistant gene (*Tsw*). The segregation of the polymorphism was verified in the F_2 individuals.

SCAR and CAPS markers. Recently published SCAR (sequence characterised amplified region) and CAPS (cleaved amplified polymorphic sequence) markers (Moury *et al.*, 2000) were analysed to determine their usefulness in identifying the presence of the *Tsw* gene in segregating populations detailed in Table 1.

6.3 Results and Discussion

Bulked segregant analysis. A total of 220 primers were screened for the suitability of producing markers associated with the resistance (*Tsw*) gene. Of these, 23 primers produced a polymorphism between the two parents. The resistant and susceptible F_2 bulks were screened with this primer subset. One primer was identified that produced a marker band that was present in the resistant parent and bulks but absent in the susceptible parent and bulks. To confirm the validity of this primer (i.e. segregation with resistance), the primer was tested against the individual F_2 population (27 susceptible; 62 resistant). Primer UBC1 amplified a marker band of approximately 2210 bp (designated $UBC1_{2210}$) which was present in 26% of the susceptible individuals and 56% of the resistant individuals. $UBC1_{2210}$ was not assessed in any further F_2 individuals or other populations due to the high percentage of susceptible individuals and the relatively low number of resistant individuals observed to have the marker. This marker therefore may only be loosely linked to the resistance gene and have questionable use in a breeding program. Linkage analysis of this marker with the *Tsw* locus would need to be undertaken to confirm linkage distance. More primers were not screened due to the publication of a CAPS marker tightly linked to *Tsw* (Moury *et al.*., 2000).

SCAR and CAPS Analysis. Using the SCAR primers and a slightly modified amplification program, the expected 568 bp amplification product was seen. As no difference between resistant and susceptible samples could be discerned, the amplification product was digested with the restriction enzyme *Xba*I to obtain the codominant CAPS marker (250 bp polymorphism). The CAPS marker was not observed in the *C. annuum* (susceptible parent) while the CAPS marker was observed in *C. chinense* (resistant parent). Of the F_2 population, 3/27 (11%) susceptible F_2 had an observable CAPS marker while the CAPS marker was observed in 59/60 (98%) resistant F_2 individuals (Figure 1). Further testing of F_2 individuals that had not been included in the original bulks due to inconsistent response and/or ELISA result to TSWV was undertaken. The CAPS marker was observed in all individuals displaying no symptoms after TSWV inoculation despite having a positive or inconsistent ELISA (18 F_2 individuals). In 11 F_2 individuals displaying symptoms after inoculation but a negative or inconsistent ELISA the CAPS marker was not observed. Of the 12 F_2 individuals that had a negative ELISA result and no symptoms, 11 had an observable CAPS marker. Five F_2 individuals displaying symptoms after inoculation and an inconsistent ELISA result had an observable CAPS marker. Generally, the CAPS marker is an efficient marker cosegregating with resistance as evidenced by these results. Those few individuals displaying symptoms and a CAPS marker may simply be individuals succumbing to TSWV infection after several concentrated inoculations with TSWV and hence may be resistant in a field situation.

The CAPS marker was reported to be present in both homozygous and heterozygous individuals (Moury *et al.*., 2000). Our results supported the codominance of the CAPS marker with both homozygous (*C. chinense* parent) and heterozygous (F_1 population) individuals producing an observable CAPS marker.

Novartis CP632, CP633, and CP636 and Yatasto (all known TSWV resistant cultivars) all had an observable CAPS marker, indicating the presence of the *Tsw* gene (Figure 2). The CAPS marker was not observed in Novartis Matrix, a known TSWV susceptible cultivar (Figure 2).

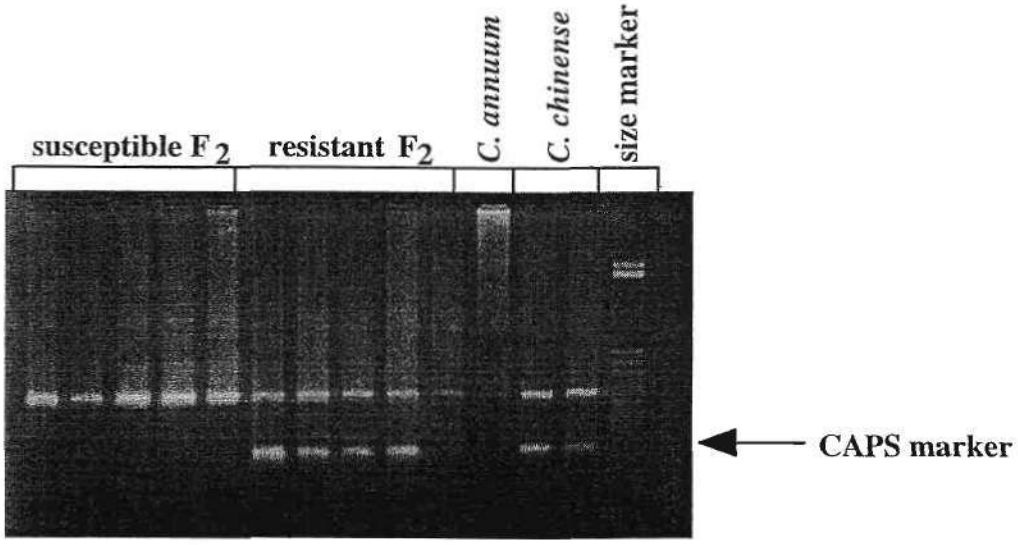


Figure 1. Segregation of the codominant CAPS $SCAC_{568}$ linked to the *Tsw* locus (resistance to TSWV).

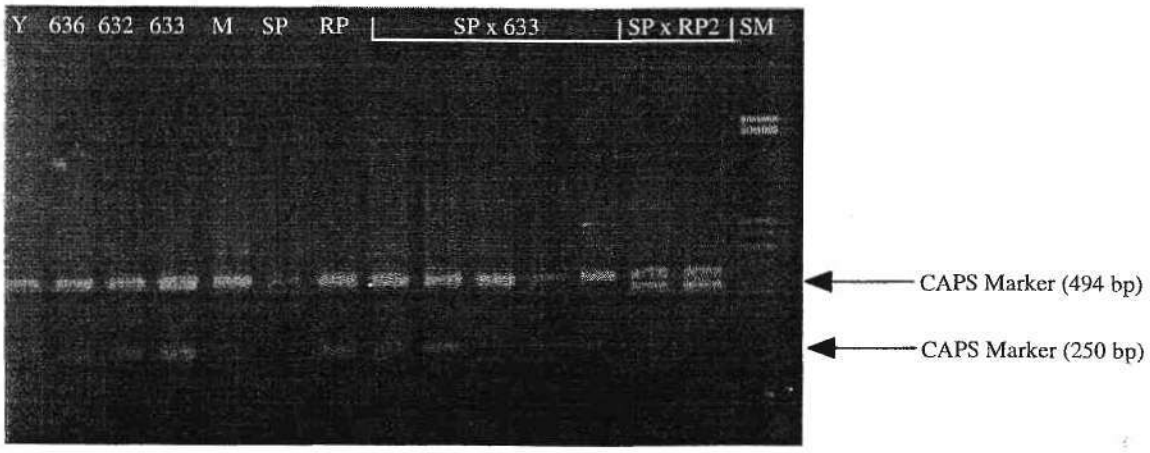


Figure 2. Segregation of the codominant CAPS $SCAC_{568}$ linked to the *Tsw* locus (resistance to TSWV). Y, Yatasto; 636, Novartis CP636; 632, Novartis CP632; 633, Novartis CP633; M, Novartis Matrix; SP, susceptible parent (*C. annuum*);

RP, resistant parent (*C. chinense* PI 00943); SP x 633, *C. annuum* x Novartis CP633; SP x RP2, *C. annuum* x *C. chinense* PI 152225; SM, size marker.

Cleavage of the 568 bp fragment amplified with the SCAR primers exhibits polymorphisms between the various resistant genotypes of capsicum. Cleavage produces 74 bp and 494 bp (easily visualised) products for the *C. chinense* lines PI 152225 and PI 159236 [Moury *et al..*, 2000; Figure 2] and 250 bp (easily visualised) and 318 bp products for *C. chinense* PI 00943 and the Novartis and Yatasto strains (Figure 2). Cleavage of the amplified fragment with different restriction enzymes (*Taq*I and *Hae*III) can differentiate between PI 152225 and PI 159236 (Moury *et al..*, 2000). These polymorphisms will have value in tracing the introgression of the *Tsw* gene in an agronomical *C. annuum* genetic background (Moury *et al..*, 2000).

In summary, a codominant CAPS marker tightly linked to the *Tsw* locus and cosegregating with resistance to TSWV has enormous potential for marker-assisted selection of TSWV resistance in capsicum crosses.

References

Doyle, J. J. and Doyle, J. L., 1990. Isolation of plant DNA from fresh tissue. Focus. 12, 12-15.

Moury, B., Pfieger, S., Blattes, A., Lefebvre, V., and Palloix, A., 2000. A CAPS marker to assist selection of tomato spotted wilt virus (TSWV) resistance in pepper. Genome. 43, 137-142.

Paran, I., Kesseli, R., and Michelmore, R., 1991. Identification of restriction fragment length polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce, using near-isogenic lines. Genome. 34, 1021-1027.

Publications

Guthrie, J.N., D. McGrath, D.M. Persley, and D.T. White. 2000. Capsicum breeding for resistance to tomato spotted wilt virus. *In* Proceedings 1st Asian Conference on Plant Pathology. August 25-28 2000, Beijing, China. China Agricultural Scientech Press, Beijing. p.123.

7. Genetic analysis of TSWV resistance - segregation data

by Des McGrath, Denis Persley and Lee McMichael, Queensland Horticulture Institute.

7.2 Introduction

At least five different lines of *C. chinense* possess a single dominant gene at the *Tsw* locus conferring a hypersensitive response to TSWV: PI 152225, PI 159236, CNPH 275 (Boiteux 1995), C00943 (Black *et al.* 1996) and 7204 (Moury *et al.*. 1997). Several analyses have either failed to distinguish resistance alleles at the *Tsw* locus (Boiteux 1995) or to identify additional resistance loci in *Capsicum* species (Boiteux *et al.*.1993; Boiteux 1995; Moury *et al.* 1997). In the F2 population generated for the current project, a DNA marker tightly linked to *Tsw* (Moury *et al.* 2000) co- segregated with resistance derived from *C. chinense* 00943 (Chapter 4, J. Guthrie), providing strong evidence for the presence of *Tsw*. Several inheritance studies have therefore identified *Tsw* as the only known gene conferring TSWV resistance in *Capscium* species.

A series of test crosses was developed from resistant PI line 00943 to study the inheritance of resistance to Australian isolates and to provide the foundation for an applied breeding program. The objective of this analysis was to study segregation for resistance and confirm the presence of a major gene as suggested in other research.

7.2 Materials and Methods

A series of test crosses was constructed from susceptible *C. annuum* F_3 line Merlin (M1-1) and resistant *C. chinense* line 00943 (2-3). The following F_1, F_2, and backcross generations were made in the planthouse as follows:

F_1 M1-1 x 2-3
F_2 (M1-1 x 2-3)-1, F_2 (M1-1 x 2-3)-2, F_2 (M1-1 x 2-3)-3
BC M1-1-1 x F_1 (M1-1 x 2-3)-2
BC F_1 (M1-1 x 2-3)-1 x 2-3-1

Seedlings of parent lines, F_1, F_2 and backcross generations were raised in containerised trays and numbered individually for later reference. Two sap inoculations with TSWV, approximately one month apart, were made by preparing leaf extracts in 0.1M phosphate buffer pH 7.0 containing 0.1% sodium sulphite and applying to carborundum-dusted leaves of plants. Plants were briefly rinsed following inoculation and maintained in a glasshouse.

Symptoms were scored visually as positive or negative approximately three months after final inoculation and assays by ELISA performed two to three months after final inoculation.

Individual plants were rated as resistant or susceptible according to their ELISA score where visual symptoms were absent or by visual symptoms only when strongly positive. F_2 and backcross generations were tested by χ^2 analysis for the presence of a single dominant gene, based on the final ratings.

7.3 Results

There were 79% susceptible plants (15 susceptible from19) in the parent line Merlin F_4 M1-1-1, according to visual symptoms. Line 2-3 was uniformly resistant when scored visually (26 plants resistant) and by ELISA (11 plants resistant). In the F_1 M1-1 x 2-3 there were 10 resistant plants in a total of twelve.

The segregations observed for the F_2 populations were as follows:

F_2 (M1-1 x 2-3)-1: resistant 54, susceptible 19, $\chi^2 = 0.0420$, not significant
F_2 (M1-1 x 2-3)-2: resistant 25, susceptible 7, $\chi^2 = 0.1666$, ns
F_2 (M1-1 x 2-3)-3: resistant 15, susceptible 8, $\chi^2 = 1.17$, ns

The distributions of all three F2 populations indicated that resistance was controlled by one dominant gene.

Backcross M1-1-1 x F_1 (M1-1 x 2-3)-2 produced 5 resistant and 160 susceptible individuals, a highly significant deviation ($\chi^2 = 145.6$) from the 1:1 segregation expected. However, subsequent testing of the resistant genotypes by ELISA and a DNA marker linked to *Tsw* determined that these were susceptible genotypes. The reciprocal backcross, (M1-1 x 2-3)-1 x 2-3-1, was resistant, with one exception, based on visual symptoms. When scored by ELISA however, individual plants segregated 32 resistant to 19 susceptible.

7.4 Discussion

The reactions of parent lines, F_1 and F_2 generations suggested that resistance was conditioned by a single dominant gene. The F_2 distributions for three separate F_1 plants agreed closely with this model. It is most likely that the resistant gene is *Tsw*, since the DNA marker linked to *Tsw* (Moury *et al.* 2000) was present in these F_2 progenies (Chapter 4). There are no other resistance genes known in 00943 or other PI lines of *C. chinense*.

Preferential elimination of resistance alleles from the F_1 pollen parent could have been responsible for the lack of resistant genotypes in the BC M1-1-1 x F_1 (M1-1 x 2)-2, and may have resulted from a specific interaction between *C. annuum* and *C. chinense*. In both backcross progenies it is possible that heterozygotes did not express high levels of resistance and were classified as susceptible. However, the same classification error would have been expected in the F_2 generation. Moury *et al.*. (1997) refer to problems of segregation distortion in similar interspecific progenies. Although the distortions in the backcross generations do not support the evidence of the F_2 generations, the evidence for a single gene is compelling in other studies.

Additional DNA markers linked to *Tsw* were identified by Jahn *et al.*. (2000). A series of framework markers was identified in a mapping population derived from a cross to PI 152225 of *C. chinense*. *Tsw* was located within a range of tightly linked markers established on a comparative genetic map.

It is therefore reasonable to develop breeding strategies based on a major dominant gene, using one or more DNA markers as a criterion to enhance the efficiency of selection for resistance. Although not all types of markers can be easily transferred from one mapping

population to another, it was apparent that at least one specified marker (Moury *et al.*. 2000) could be routinely applied to the F_2 generation studied here. The use of markers is a preferred means of selecting among large numbers of backcross progenies in an applied breeding program; marker-assisted selection could be used initially and selected resistant genotypes then screened by glasshouse bioassay to confirm their status.

References

Black, L.L., Hobbs, H.A., and Kammerlohr, D.S. (1996) Resistance of Capsicum chinense lines to tomato spotted wilt virus isolates from Louisiana, U.S.A. and inheritance of resistance. In International symposium on Tospoviruses and Thrips of Floral and Vegetable Crops. Nov 7-10 1995, Taichung, Taiwan. Acta Hortic.431, 93-401.

Boiteux, L.S. (1995) Allelic relationships between genes for resistance to tomato spotted wilt tospovirus in Capsicum chinense. Theor Appl Genet 90, 46-149.

Boiteux, L.S. and Nagata, T. (1993) Susceptibility of Capsicum chinense PI159236 to tomato spotted wilt virus isolates in Brazil. Plant Dis 77, 210.

Jahn, Molly, Paran, Ilan, Hoffman Katrin, Radwanski, Elaine R., Livingstone, Kevin D., Grube, Rebecca C., Aftergoot, Ester, Lapidot, Moshe, Moyer, James. (2000) Genetic mapping of the *Tsw* locus for resistance to the Tospovirus Tomato spotted wilt virus in Capsicum spp. and its relationship to the Sw-5 gene for resistance to the same pathogen in tomato. Molecular Plant-Microbe Interactions 13 (6), 673-682.

Moury, B., Palloix, A., Gebre Selassie, K., and Marchoux, G. (1997) Hypersensitive resistance to tomato spotted wilt virus in three Capsicum chinense accessions is controlled by a single gene and is overcome by virulent strains. Euphytica 94, 45-52.

Moury, B., Pfieger, S., Blattes, A., Lefebvre, V., and Palloix, A. (2000) A CAPS marker to assist selection of tomato spotted wilt virus (TSWV) resistance in pepper. Genome 43, 137-142.

Acknowledgements

I wish to acknowledge the dedicated work of the researchers who contributed to the project. They include Dr Roger Jones, Ms Lindrea Latham, and Ms Monica Thomas from Agriculture Western Australia; Mr Denis Persley, Ms Lee McMichael, Dr John Thomas , Dr Steve Akiew and Mr Ian Walker from Queensland Horticulture Institute; Ms Jodie Guthrie and Mr Dan White from Central Queensland University.

8. Survey of Bacterial Spot Races

by Steve Akiew, Queensland Horticulture Institute and Monica Thomas, Agriculture Western Australia.

8.1 Introduction

Bacterial spot (BS) (*Xanthomonas campestris pv. vesicatoria*) of *Capsicum* is a serious disease capable of significant damage to crops in Queensland and to a lesser extent in other states. Typically losses are greatest in southern Queensland during periods of extended rainfall but early season damage can also be serious for producers in the Burdekin, Gumlu and Bowen districts of north Queensland. In recent years, only race 1 has been identified in commercial production in Queensland. The race(s) present in Western Australia is unknown.

Genetic resistance to BS races 1, 2 and 3 is available in several commercial hybrid cultivars. In Queensland the resistance of most commercial varieties with multiple gene resistance has been poor in recent seasons, suggesting that high inoculum pressure may adversely affect expression of resistance or that virulent races have emerged.

8.2 Surveys of Bacterial Spot Races in Queensland and Western Australia

The results of the BS experiment in the glasshouse confirmed that cultivars Matrix, Rubix and CPS0117 were resistant to Race 4 of *Xanthomonas campestris pv vesicatoria.* This isolate was previously isolated from infected capsicum leaves from Gumlu, and classified according to the differential reaction of the bacterium on pepper cultigens.

The plants were sprayed at the fruiting stage with 10ml each of a 48 hour culture suspension and incubated for three days. Symptoms of the disease appeared after three weeks, and were typical of bacterial spot symptoms in the field. On a disease severity index of 1 to 4, the severity of BS on Giant Bell and Wonder Bell was 3.1 and 2.5, respectively. No BS symptoms were observed on Matrix, Rubix and CPS0117. Disease severity index of 1, 2, 3 and 4 correspond to; no BS, 1 to 10 % of leaf area with BS, less than 50% but more that 10% of leaf area with BS, and more than 50% of leaf area with BS respectively.

No symptoms of bacterial spot were identified in the Western Australian survey although three samples were taken for diagnostic purposes. One sample was sent to the laboratory at QDPI Gatton where it tested negative.

Reference

Jones, J. B. and Stall, R.E. (1998). Diversity among *Xanthomonas* pathogenic on Pepper and Tomato. Ann. Rev. Phytopathology 36, 41-58.

9. Recommendations

This preliminary survey of tospovirus isolates indicated that the *Tsw* gene is highly effective against all field isolates of Tomato spotted wilt virus in Western Australia and Queensland. The few occurrences of resistance-breaking isolates were confined to artificial conditions in the glasshouse. Because virulent races of TSWV were not detected in field crops it is likely that *Tsw* will be highly effective in commercial crops for some years, although no guarantee of durability can be made. A breeding program to transfer *Tsw* to acceptable breeding lines and hybrids for Australian production environments should therefore be extended and developed fully to take advantage of this useful gene.

The survey of Queensland tospovirus isolates identified a new virus, CCV, previously unknown in Australia, which is responsible for significant damage to capsicum crops. Since CCV may become widespread and as damaging as TSWV, it is recommended that *Capsicum* germplasm should be screened for genetic resistance and transferred to commercial cultivars by breeding. A combination of resistances to both TSWV and CCV is highly desirable.

Because new isolates of TSWV can emerge over time, it is recommended that regular surveys of isolates be undertaken in all capsicum production locations as the breeding program progresses. This strategy will ensure that the resistance developed in breeding lines is appropriate to the strains of virus present.

In addition to TSWV and CCV resistances, it is desirable that adequate resistance to Bacterial spot (BS) be incorporated into Australian cultivars. The survey of BS races identified only one race in Queensland, against which effective resistance is available. Although multiple-gene resistance is available in some cultivars, it is feasible to introduce only the gene conferring resistance to the race identified. Even if it were technically possible to implement the development of multiple BS resistant genes, the resources required would be prohibitive. As with virus resistance, it is essential to undertake regular surveys of races to ensure an appropriate breeding strategy is followed.

It is also recommended that elite breeding lines with excellent agronomic performance be developed in a parallel series of crosses with broad adaptation for southern and northern production centres in Queensland. As multiple-resistant breeding lines are advanced from appropriate crosses, they should be crossed to broadly-adapted breeding lines to enhance their overall quality and field performance. This work has already commenced in the current project and should be continued.