

Capsicum breeding for tospo virus resistance

Des McGrath
QLD Department of Primary
Industries & Fisheries

Project Number: VG02035

VG02035

This report is published by 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 QLD Department of Primary Industries & Fisheries and the vegetable industry.

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ISBN 0 7341 1475 3

Published and distributed by:

Horticultural Australia Ltd

Level 1

50 Carrington Street

Sydney NSW 2000

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E-Mail: horticulture@horticulture.com.au

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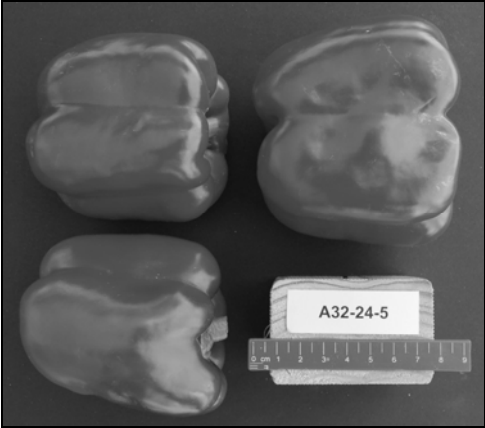
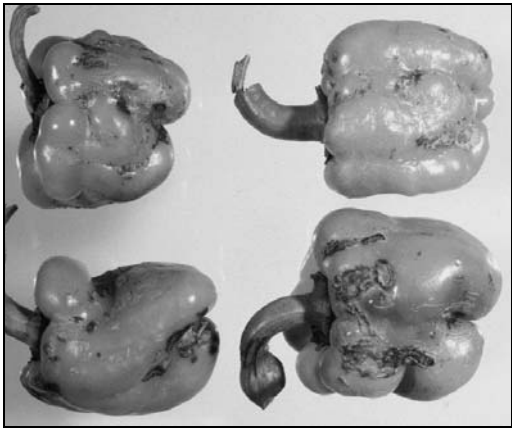
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Capsicum Breeding for Tospovirus Resistance

Project Number: VG02035 (completion date: 31/12/2006)

Des McGrath *et al.*

Queensland Department of Primary Industries and Fisheries



Horticulture Australia Project Number VG02035

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Purpose: This report provides details on the identification of resistance to tospoviruses affecting capsicums in Australia and the incorporation of this resistance into capsicum breeding material.

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Funding Sources: Horticulture Australia Limited
Department of Primary Industries and Fisheries, Queensland

Date of Report: 31/12/2006



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Media Summary

Capsicum chlorosis virus (CaCV) and Tomato spotted wilt virus (TSWV), two of the species of tospoviruses found in Australia, were studied in a project with two major objectives. The first was a better understanding of their distribution in horticultural crops and weed hosts; the second was the identification of germplasm resistant to CaCV and the transfer of resistance to suitable lines of bell capsicum by conventional breeding methods.

Surveys of CaCV revealed that it is endemic in all major capsicum production districts of Queensland, including Bundaberg, Bowen, Gumlu and Ayr. It has been the dominant tospovirus of capsicum at Bundaberg since 2000. CaCV was also recorded at Kununurra, WA, and in tomato at Coffs Harbour, NSW. The high incidence of CaCV in tomato and capsicum crops at Bundaberg is likely to be related to the presence of two thrips vector species, *Thrips palmi* and *Frankliniella schulezi* and the frequent infection of the common weed species *Ageratum conyzoides*. The control of this weed species will be a critical component of a broader strategy for the management of CaCV.

One Plant Introduction accession with high levels of resistance to both CaCV and Tomato spotted wilt virus was found in a screening of more than 100 accessions from several uncultivated *Capsicum* species. One major resistance gene was identified in this parent and transferred to a series of breeding lines in four cycles of hybridization and selection. In associated work, a DNA marker was developed to enable faster and more efficient selection of the resistance gene in breeding programs. The most advanced lines have good fruit quality and yield similar to commercial cultivars, but need a further cycle of breeding to improve fruit size.

Resistant breeding lines and populations from this project will be commercialised. They should be valuable either as parents in hybrid cultivars or as a source of resistance in new breeding populations. Given the extent of CaCV in Queensland and South East Asia, resistance may soon be an essential attribute of new cultivars for these areas.

Technical Summary

Capsicum chlorosis virus (CaCV) and *Tomato spotted wilt virus* (TSWV) were studied in a project with two major objectives. We first sought a better understanding of the epidemiology of these tospoviruses as a basis for their control in commercial crops and then identified germplasm resistant to CaCV which was used for breeding resistant lines of bell capsicum.

CaCV was detected in capsicum at Gatton, Gympie, Bundaberg, Rockhampton, Mackay, Bowen, Gumlu, Giru, Ayr, Mareeba and Stanthorpe in Queensland. The first record of CaCV outside of Queensland was from a capsicum plant from Kununurra, WA in August 2004. The virus was also detected in tomato from Coffs Harbour on the central coast of NSW. In the major production areas of Bundaberg, Bowen, Gumlu and Ayr, CaCV is now endemic. It is the dominant tospovirus at Bundaberg, most likely because of the presence of two thrips vector species, *Thrips palmi* and *Frankliniella schulezi* and the frequent infection of the common weed species *Ageratum conyzoides*. Recent work has established a consistent association between the high incidence of CaCV in capsicum and tomato crops in the Bundaberg area and the presence of *Ageratum* with a high rate of CaCV infection.

TSWV was found in all districts. In north Queensland the relative incidence of TSWV and CaCV fluctuated between and within a season, reflecting variations in the abundance of vector species and the distribution of virus inoculum in host species. TSWV was identified in glasshouse crops in South Australia and the Sydney basin. CaCV was not detected at these locations.

One Plant Introduction accession of *C. chinense* was identified with resistance to both CaCV and TSWV in a screening of 104 accessions of uncultivated *Capsicum* species. An analysis of segregation indicated one dominant gene conferred CaCV resistance and this was transferred to advanced breeding lines through four cycles of backcrossing and selection. A subline of this accession resistant to TSWV was derived by selection using a marker for *Tsw*. A DNA marker for CaCV resistance was developed in a parallel program and applied to selection of backcross progenies. Although it was clearly identified and useful, the marker is not sufficiently robust and needs to be cloned and sequenced for the development of locus specific primers.

A series of CaCV-resistant breeding lines was developed during the project. Segregating or resistant backcross 3 lines were identified and evaluated in field trials at Tatura, Victoria, and Gatton and Bowen, Queensland. The preferred resistant line, A32, produced marketable yields as good as or better than one or more of the commercial check cultivars in three field trials. Fruit shape and quality were acceptable although improvements can be expected with further breeding. Average marketable fruit size for the best backcross 3 lines was approximately 20% less than the standard cultivars. It is expected that fruit size will improve in the next backcross cycle. CaCV resistant backcross 3 lines and backcross 4 populations will be offered for commercialisation along with DNA marker technology.

Introduction

Fruit of the genus *Capsicum*, variously called capsicum, pepper, chilli, paprika or ajis, is a versatile and widely grown vegetable crop. Capsicum fruit is consumed worldwide as a fresh vegetable or dehydrated for use as a spice. Pungent and non-pungent red pepper products are one of the most important spice commodities in the world (Bosland and Votava 2000). Capsicum fruit are present in a wide range of fresh and processed food products and are an important source of vitamins and essential nutrients. Capsicum extracts are also used in pharmaceuticals and cosmetics (Bosland and Votava 2000).

There are approximately 25 species within the genus *Capsicum*, five of which have been domesticated. The most widely grown and economically important species is *Capsicum annuum* which has Mexico as the major centre of genetic diversity. The four other domesticated species are *C. baccatum*, *C. pubescens*, *C. chinense* and *C. frutescens*. All originated in the tropical Americas (Bosland and Votava 2000). Classification of capsicums below the species level is largely based on fruit types and uses. A wide diversity of fruit shapes exists both within and between species (de Wit and Bosland 1996). The sweet, bell capsicum type dominates Australian production.

Commercial capsicum production occurs in all mainland States of Australia. The total value of the crop in 2000 was \$ 60.2M (Australian Bureau of Statistics) with Queensland being by far the major producer with production in 2000 valued at \$49M. The majority of the Queensland crop is grown during the autumn / winter period with the major production areas centered around Bundaberg, Bowen, Gumlu and Ayr. Summer production occurs in Bundaberg and the Granite Belt, an elevated plateau with a moderate summer climate in south east Queensland.

Diseases caused by bacterial, fungal and viral pathogens cause considerable losses in capsicum crops worldwide (Pernezny *et al.* 2003). Capsicum crops in all Australian States have sustained major production losses from Tospoviruses over the last 12 or so years (Persley *et al.* 2006; Sharman and Persley 2006; Latham and Jones 1997). Tospoviruses belong to the virus family Bunyaviridae which is divided into five genera, four of which comprise viruses that infect vertebrates and insects with many transmitted by mosquitoes and other insects. All viruses within the family that infect plants are assigned to the Tospovirus genus (Nichol *et al.* 2005). The genus name is derived from the type species, *Tomato spotted wilt virus* (TSWV), which was first found and described from Australia around 1920 (Samuel *et al.* 1930). There are currently 16 recognised or proposed tospovirus species with three being recorded in Australia - TSWV, Capsicum chlorosis virus (CaCV) and Iris yellow spot virus (IYSV) (Persley *et al.* 2006).

Tospoviruses form pleomorphic, spherical particles within plant cells and are surrounded by a lipid envelope with two surface glycoprotein projections, enclosing three nucleocapsids. The nucleocapsids contain three single-stranded linear RNA segments which contain the genetic information essential for viral replication, movement and transmission. All tospoviruses are transmitted by thrips, sap-sucking insects within the

order Thysanoptera. Tospoviruses are not transmitted by aphids, leafhoppers, whiteflies or chewing insects.

The thrips/ tospovirus relationship is very specific and less than 20 thrips species worldwide are vectors of tospoviruses (Whitfield *et al.* 2005). Transmission can only occur if the viruses are acquired from infected plants by first instar larvae thrips. The larvae can acquire virus during feeding periods of less than 30 minutes. Once acquired by immature thrips, the viruses circulate and multiply within the insect and are transmitted to plants as the adult thrips pierce and suck the contents of plant cells. Thrips remain infective for life but do not pass the virus to their offspring through the egg. Thrips can transmit tospoviruses in feeding periods of from five to 10 minutes (Persley *et al.* 2006).

TSWV has a very wide host range among crop, ornamental and weed species with capsicum and tomato among the species most severely affected. The virus is transmitted by four thrips species present in Australia - *Frankliniella occidentalis* (western flower thrips), *F. schulzei* (tomato thrips), *Thrips palmi* (melon thrips) and *T. tabaci* (onion thrips) (Persley *et al.* 2006). Global spread of the efficient vector species *F. occidentalis* has been a major factor in the increased importance of TSWV in many countries since the early 1990s (Prins and Goldbach 1998). Several severe epidemics of TSWV in capsicum and tomato in South Australia and Western Australia have been linked to the abundance of *F. occidentalis* (Coutts and Jones 2002).

TSWV has been present in Australia since at least the early years of the 20th century. By contrast, Capsicum chlorosis virus was first detected from capsicum and tomato in 1999 in Queensland and has been present in the state since at least 1992 (McMichael *et al.* 2000). CaCV was found in capsicum from Kununurra, WA in 2004 (Jones and Sharman 2005) and has also been reported from Thailand, Taiwan and China (Knierim *et al.* 2006). The other known natural hosts of CaCV are peanut, Hoya and several weed species. The virus is a member of the Watermelon silver mottle virus or serogroup IV tospoviruses which are prevalent in Asia (McMichael *et al.* 2002).

The symptoms caused by CaCV on capsicum resemble those induced by TSWV, but have several distinct features. Marginal chlorosis and interveinal chlorosis develop on young leaves, which often become narrow and curled, with a strap-like appearance. Older leaves become chlorotic and ringspots and line patterns may develop. The fruit on infected plants is small, distorted and frequently marked with dark necrotic lesions and scarring over the surface.

Control of tospoviruses in capsicum and other crops is difficult. Reasons for this include the wide host range of the viruses, particularly TSWV, among weeds and other crop species. Several important vector species also have a broad host range which often overlaps with hosts of the tospoviruses, allowing vectors to both breed and acquire virus for spread into and within crops. Tospoviruses are persistent in their thrips vectors and only relatively short feeding periods are required for transmission which allows many plants to be infected as thrips migrate through a crop. The short feeding periods and the

capacity of thrips species to rapidly develop resistance to insecticides further complicates control efforts.

Although suitable sources of resistance are unavailable for many crop hosts of tospoviruses, genetic resistance has been utilised for TSWV control in capsicum and tomato. Resistance in tomato is largely based on the *Sw-5* gene introgressed from *Lycopersicon peruvianum* into the cultivar Stevens (Stevens *et al.* 1992). The resistance operates as a hypersensitive response preventing systemic movement of the virus within the host plant tissue.

Resistance to TSWV in capsicum, operating as a hypersensitive response and controlled by the single dominant gene *Tsw*, has been found in several *C. chinense* lines (Black *et al.* 1991; Mouray *et al.* 1997). This resistance has been used to develop virus resistant hybrids which are grown in several countries, including Australia. This resistance has generally proven to be durable, although field isolates of TSWV virulent towards the *Tsw* gene have been found (Roggero *et al.* 2002; Sharman and Persley 2006), emphasizing the importance of using resistant cultivars as part of an integrated management strategy to reduce virus inoculum and populations of thrips vectors (Jones 2004).

The increasing importance of tospoviruses in Australian capsicum crops was addressed in this project by seeking germplasm resistant to CaCV and, if found, incorporating this resistance into bell capsicum lines in combination with the *Tsw* gene to provide broad protection against tospoviruses.

Materials and Methods

Virology

Glasshouse bioassays

An efficient screening system was developed, allowing consistent detection of resistant genotypes or individuals in segregating populations following inoculation with either *Tomato spotted wilt virus* (TSWV) or Capsicum chlorosis virus (CaCV).

TSWV was propagated in tomato or capsicum and CaCV in *Nicotiana benthamiana*, *Datura stramonium* or capsicum. Symptomatic leaves were ground in a cold mortar and pestle with cold 0.1 M phosphate buffer to which 0.1% sodium sulphite has been added immediately prior to use. Diatomaceous earth and carborundum abrasives were added to the inoculum which was then applied with a finger or a pad dipped in the inoculum. Capsicum plants were inoculated when the first true leaves had expanded. Plants were rinsed with water after inoculation to remove excess inoculum and abrasive. Plants were usually inoculated a second time five days later to minimise the chances of escapes. Appropriate susceptible and resistant lines were included in each experiment. Plants were then maintained in a glasshouse and monitored for local and systemic symptom development over a period of three or four weeks.

Resistance was confirmed by selecting new growth tip leaves from plants and testing by Enzyme Linked Immuno Sorbant Assay (ELISA) using either TSWV antiserum or *Watermelon silver mottle virus* (serogroup IV) antiserum which detects CaCV. These tests were undertaken according to the suppliers' recommendations. Absorbance values of at least twice that of the appropriate healthy controls were ranked as positive. Further confirmation of resistance can be provided through PCR (polymerase chain reaction) assays using primers specific for either TSWV or CaCV.

Virus surveys

Surveys for virus diseases were undertaken in each year of the project. The major production areas at Bundaberg and Bowen / Gumlu were surveyed each year. Other areas were surveyed as opportunities arose.

The incidence of virus disease in each crop was estimated by counting the numbers of symptomatic plants at several random locations within a crop and assessing at least 50 plants at each location. At least ten samples were collected from each crop and stored at 5 C until assayed by ELISA for TSWV and CaCV. TSWV was detected using antibodies to this virus supplied by BioRad and CaCV by antibodies to the serogroup IV tospovirus *Watermelon silver mottle virus* supplied by Agdia or DSMZ. The ELISA tests were done according to the suppliers' directions. The identity of key virus isolates was confirmed by RT-PCR using virus specific primers (Jones and Sharman 2005; Sharman and Persley 2006). Representative isolates were maintained in long-term storage in liquid nitrogen or as desiccated leaf cultures. Surveys also targeted likely alternative weed hosts of tospoviruses.

Identification of resistance to CaCV and development of resistant breeding populations.

Capsicum germplasm

One hundred and four plant introduction (PI) accessions comprising 40 *Capsicum chinense*, 38 *C. frutescens*, 20 *C. pubescens*, 3 *C. baccatum* and 3 *C. chacoense* were obtained from the USDA/University of Georgia Plant Genetic Resources Conservation Unit, Griffin, Georgia. These PI lines, along with 25 commercially available *C. annuum* cultivars, were screened for resistance to CaCV in the glasshouse (see appendix 1). The numbers of seedlings available for each accession varied but were usually more than 20 and sufficient to sample the range of genetic variation in the line. After visual assessment individual seedlings without symptoms were tested by ELISA for the presence of CaCV. Plants which were free of infection after three weeks were selected as potential parents for resistance breeding.

Earlier testing of germplasm of several uncultivated species indicated PI 290972 of *C. chinense* was highly resistant to both CaCV and TSWV. All sub-lines of PI 290972 were uniformly resistant to CaCV although they varied in their reaction to TSWV, displaying either hypersensitive resistance, segregation or susceptibility. The determination of resistance to both viruses was based initially on hypersensitive reactions in glasshouse screening followed by ELISA. A subline of PI 290972 designated 12G was identified as

in backcross lines were studied to test for genetic linkage. Where appropriate, genotypes with both CaCV and TSWV resistances were selected.

Inheritance of resistance to CaCV and TSWV

The inheritance of resistance to CaCV was studied in the segregating backcross generations described earlier. Unselected backcross 1, 2 and 3 populations for each of variety A and B were screened in glasshouse assays and assessed for visual symptoms. Backcross 2 lines were also transplanted to the field where an independent evaluation of resistance was made. Individual seedlings were classified as resistant or susceptible according to symptom development in the glasshouse, supported by additional ELISA testing if required. A model of gene action was identified from the segregation and tested for goodness of fit.

TSWV resistance was confirmed initially in parent line 12G by glasshouse assay and later with ELISA. Progenies from a subset of CaCV-resistant BC 2 lines were tested for the resistance allele of *Tsw* by application of the published DNA marker to allow for selection of both resistances. Independence of segregation for CaCV was also tested.

Development of a DNA marker for CaCV resistance

Eleven microsatellite markers (AF244121, Hpms1-173, AA840692, AA840689, Hpms2-45, Hpms2-23, AA840721, Hpms1-227, HpmsCaSIG19, Hpms1-43, Hpms1-281) (Lee *et al.*, 2004) were identified as being near disease resistance genes based on the work of Grube *et al.* (2000). The microsatellite primer-pairs were used in PCR to amplify DNA fragments from 10 susceptible and 10 resistant individuals from a *C. annuum* (variety 1) / *C. chinense* (PI 290972) / *C. annuum* (variety 1), backcross 1 (BC 1A) population. DNA fragments suspected of being linked to CaCV resistance were assessed on a further 187 phenotyped-individuals from five segregating populations. Population details are provided in table x. The CAPS marker SCAC568, which is associated with TSWV resistance in capsicum (Moury *et al.*, 2000), was also assessed for linkage to resistance.

DNA extraction, PCR, and marker analysis

DNA was extracted from approximately 0.03g of fresh leaf tissue and ground with 1mL of extraction buffer (Edwards *et al.* 1991) at room temperature. The extract was added to 700 μ L of chloroform/isoamyl alcohol (24:1), mixed, left on ice for at least half an hour, and centrifuged at 11200 ref for 3 min. DNA was precipitated from the supernatant after adding 1 volume of isopropanol followed by 5 min centrifugation at full speed. The pellet was washed with 70% ethanol and resuspended in 100 μ L of Tris-EDTA (TE) buffer at pH 8.

PCR reactions were carried out on a Perkin Elmer, Gene Amp PCR System 9700. The reaction volume was 20 μ L containing 1X Roche PCR Buffer, approximately 50 ng of genomic DNA and 188 μ M dNTPs. The PCR reaction mix also included 150 nM of each primer (1 primer with a 5' fluorescent label), 4 mM total MgCl₂, and 0.5 units of Taq DNA Polymerase (Roche). The temperature cycling conditions were 3 min at 94°C;

followed by 35 cycles of 94°C for 30 sec, 50°C for 30 sec and 72°C for 1 min; with a final hold at 72°C for 1 min.

Fragments were sized using the Amersham MegaBACE 1000, capillary electrophoresis system, and the Amersham ET 400-R size standard, through the services of the Genetic Analysis Facility, Advanced Analytical Centre, James Cook University.

Field evaluation of CaCV- resistant backcross breeding lines

CaCV-resistant breeding lines selected from backcross cycles 2 (BC2) and 3 (BC3) were evaluated at several field sites for agronomic merit and to confirm disease resistance identified in glasshouse assays. The details of five field assessments in 2005 and 2006 are as follows:

1. BC2 F1 progenies of four backcrosses were planted at Gatton in January 2005 as single plant selections from CaCV screening in the glasshouse. Selections based on fruit size were made for hybridisation to construct BC3 populations and further evaluation in replicated trials. The four backcrosses comprising 133 plants were:
 - A - variety 2 x BC1 23-2; 41 plants
 - B – 16134 x BC1 23-2; 33 plants
 - C – variety 2 x BC1 7-1; 28 plants
 - D – 21814 x BC1 23-2. 31 plants
2. BC2 F2 progenies derived from selections at Gatton in May 2005 were assessed in a field trial at Bowen in October 2005. Twenty-one BC2 entries and two commercial check varieties were replicated twice in a randomised block design.
3. Selected BC3 F2 progenies were evaluated at Tatura in April 2006. Nineteen BC3 lines and three commercial check varieties were replicated twice in a randomised block design.
4. BC3 F2 progenies were evaluated at Gatton in May 2006. The entries comprised 11 BC3 lines and two commercial check varieties in two replications.
5. BC3 F2 progenies were evaluated at Bowen in October 2006. The entries comprised nine BC3 lines and three commercial check varieties in two replications. Single plant selections were made based on the resistance status of the lines in glasshouse screening.

Results

Virology

Glasshouse screening

The screening assay developed consistently resulted in the development of 100% infection in susceptible control lines and hybrids. The assay allowed clear discrimination between susceptible and resistant genotypes when screening germplasm and segregating populations. Resistant genotypes developed a hypersensitive necrotic reaction on inoculated leaves. Necrotic lesions developed on leaves usually within 4 to 5 days of inoculation with leaf abscission frequently occurring several days later. New growth leaves were free of symptoms and virus could not be detected in leaf samples by ELISA. Susceptible plants had few if any symptoms on inoculated leaves and developed systemic mottle, necrosis, leaf deformation, stem lesions and stunting. Many plants with these symptoms eventually died.

Virus surveys

The tospoviruses CaCV and TSWV were present in all capsicum production areas of Queensland. CaCV was detected in capsicum at Gatton, Gympie, Bundaberg, Rockhampton, Mackay, Bowen, Gumlu, Giru, Ayr, Mareeba and Stanthorpe. The first record of CaCV outside of Queensland was from a capsicum plant from Kununurra, WA in August 2004. The virus was also detected in tomato from Coffs Harbour on the central coast of NSW.

CaCV is endemic in the major capsicum production areas of Bundaberg, Bowen, Gumlu and Ayr. It has been the dominant tospovirus in capsicums at Bundaberg since 2000 with TSWV being detected in only a very few collections during the six year period. The dominance of CaCV at Bundaberg is likely to be related to the presence of two confirmed thrips vector species of CaCV, *Thrips palmi* and *Frankliniella schulezi* and the frequent infection of the common weed species *Ageratum conyzoides* by CaCV in the Bundaberg area. CaCV infection of this species was first found during surveys in 2005 and recent work has established a consistent association between a high incidence of CaCV in capsicum and tomato crops in the Bundaberg area and the presence of *Ageratum* with a high rate of CaCV infection. Infected *Ageratum* plants were symptomless but had high virus titre as determined by ELISA.

TSWV was found in all districts when surveys were made, although only at very low incidence at Bundaberg, as indicated above. In north Queensland (Bowen / Gumlu / Burdekin) the relative incidence of TSWV and CaCV fluctuates both between and within a season. On several survey dates, CaCV was detected in approximately 50% of samples from the region while at other times TSWV dominated. These differences between locations and times most likely reflected variations in vector species abundance and distribution and available virus inoculum in crop and weed hosts.

TSWV was found in a range of weed species covering several families during the survey period and several severe outbreaks of the virus in capsicum were associated with high

levels of virus infection in abundant weed populations near the crops. Weed hosts of TSWV are listed in Table 1.

TSWV was confirmed in several capsicum plants collected in the Sydney basin region in March 2006. Virus incidence was very low in the field and glasshouse grown crops inspected. CaCV was not detected in samples.

Table 1: Field hosts of Tomato spotted wilt virus found during project survey period

Plant family and species

Asteraceae

- Bidens pilosa* (Cobbler's pegs)
- Lactuca sativa* (lettuce)
- Tagetes minuta* (Stinking Roger)
- Sonchus oleraceus* (Common Sowthistle)

Chenopodiaceae

- Chenopodium album* (Fat hen)

Fabaceae

- Arachis hypogaea* (Peanut)

Malvaceae

- Hibiscus trionum* (Bladder kentia)

Solanaceae

- Capsicum annuum* (Capsicum)
- Datura* spp. (Thornapples)
- Lycopersicon esculentum* (Tomato)
- Nicandra physalodes* (Apple of Peru)
- Physalis* spp. (Wild gooseberries)
- Solanum nodiflorum* (Nightshade)
- Solanum melongena* (Eggplant)

Verbenaceae

- Stachytarpheta jamaicensis* (Snakeweed)
-

Inheritance of CaCV resistance

Screening and selecting CaCV resistant germplasm

From the 104 capsicum germplasm accessions and 25 commercially available capsicum cultivars screened for CaCV resistance by inoculation, five *C. chinense* (PI 290972, PI 315028, PI 257171, PI 159236 and ARVDC00943) and two *C. frutescens* (PI 159282 and PI 215728) displayed symptoms indicating a hypersensitive resistant response (appendix 1). Lines PI 290972, PI 159236 and AVRDC00943 also have TSWV resistance conferred by the *Tsw* gene. However, McMichael *et al.* (2002) reported that CaCV did

infect PI 159236 and AVRDC00943 indicating there may be some variability in their resistance to different CaCV isolates. The *Tsw* gene did not appear to provide any resistance to CaCV infection in the commercial cultivar which had this gene.

The sublimes and subsequent progenies of PI 290972 have been shown to be resistant to 24 CaCV isolates collected from the major capsicum production regions in north and south-east Queensland. One CaCV isolate from the Lockyer Valley was shown to at least partially overcome resistance in sublimes of PI 290972. The Lockyer Valley is a minor production area for capsicum and this isolate was the only sample of CaCV detected from several surveys in the same region.

While six sublimes of PI 290972 all displayed total resistance to the CaCV isolate used, there was a variable response to TSWV, some being totally susceptible and others apparently segregating. Only one subline, 12G, was totally resistant to both TSWV and CaCV and this subline was used for all further crosses.

Screening of BC1 F1 progenies for CaCV segregation

Two series of crosses derived from CaCV resistant line 12G to susceptible varieties 1 and 2 were screened and analysed for segregation in BC1, 2 and 3 generations. Each series comprised parent lines, F1 and backcrosses of the F1 to respective parent lines. Table 2 presents screening and segregation data for the BC1 series involving variety 1. Table 3 presents similar data for the series from variety 2.

Table 2: Segregation for CaCV resistance in BC1 derived from variety 1. X^2 tests fit to 1 resistant: 1 susceptible model of single gene segregation.

Backcross 1	No. CaCV Resistant	No. CaCV Susceptible	Total No.	X^2 (df = 1)
BC1 (F1 x V 1)	47	39	86	0.74
F1 (V1 x 12G)	6	0	6	NA
Variety 1	0	9	9	NA

Table 3: Segregation for CaCV resistance in BC1 derived from variety 2. X^2 tests fit to 1 resistant : 1 susceptible model of segregation.

Backcross 1	No. CaCV Resistant	No. CaCV Susceptible	Total No.	X^2 (df = 1)
BC1 (F1 x V2)	29	29	58	0
BC1 (F1 x 12G)	7	0	7	NA
F1 (V2 x 12G)	5	0	5	NA
Variety 2	0	20	20	NA
12G	10	0	10	NA

Both sets of data conformed to a 1 resistant: 1 susceptible model of segregation and provided strong evidence that resistance was conferred by one dominant gene derived from parent line 12G.

Screening of BC2 F1 progenies for CaCV segregation

Two resistant BC1 genotypes, 7-1 and 23-1, were selected for further hybridisation. It was presumed that both genotypes were heterozygous and after hybridisation with a susceptible parent the BC2 generation would be heterogeneous in a 1:1 ratio of resistant to susceptible plants. Segregation data are presented in Table 4 for the following BC2 populations: variety 2 x 7-1, variety 2 x 23-1, 16134 x 23-2 and 218184 x 7-1.

Table 4: Segregation for CaCV resistance in four BC2 populations. X^2 tests fit to 1 resistant :1 susceptible model.

Backcross 2	No. CaCV Resistant	No. CaCV Susceptible	Total No.	X^2 (df = 1)
Variety 2 x 7-1	50	50	100	0
Variety 2 x 23-2	63	46	109	2.65
16134 x 23-2	32	31	63	0.002
21814 x 7-1	49	61	110	1.31

Although two of the four populations fitted the single gene model less closely, all provided credible evidence of a 1:1 ratio to support this explanation of inheritance.

Screening of BC3 F1 progenies for CaCV segregation

Two resistant BC2F1 genotypes were selected for hybridization to generate BC3 populations. Segregation for three populations, variety 1 x (BC2F1) D4-10, variety 1 x (BC2F1) A3-4, and variety 3 x (BC2F1) A3-4 are presented in Table 5.

Table 5: Segregation for CaCV resistance in three BC3 populations. X^2 tests fit to 1 resistant :1 susceptible model.

Backcross 3	No. CaCV Resistant	No. CaCV Susceptible	Total No	X^2 (df = 1)
Variety 1 x D4-10	56	48	104	0.62
Variety 1 x A3-4	13	8	21	1.19
Variety 3x A3-4	11	21	32	3.13

The data from all three BC3 populations supported previous conclusions that one dominant gene conferred CaCV resistance. The first population (variety 1 x D4-10) fitted the model better than the other two, probably because of its larger sample size.

The screening data from BC1, BC2 and BC3 populations together provided consistent evidence for one major dominant gene for CaCV resistance, derived from *C. chinense* PI290972 line 12G, which was highly heritable and easy to manipulate in applied breeding.

Joint assessment of BC2 F1 progenies for CaCV and TSWV resistances

CaCV- resistant individuals from three different backcross 2 populations were screened for TSWV resistance with a DNA marker linked to gene *Tsw*. The results are presented in Table 6.

Table 6: Segregation for TSWV resistance conferred by gene *Tsw* in plants resistant to CaCV.

Backcross 2	Total No. CaCV resistant	No. TSWV Resistant	No. TSWV susceptible
16134 x BC1 23-2	10	5	5
21814 x BC1 23-2	10	5	5
Variety 2 x BC1 23-2	33	16	17

The 53 BC2 individuals were all CaCV- resistant but segregated for TSWV resistance, indicating independent genetic control for each attribute. Each BC2 produced a segregation ratio of 1 resistant: 1 susceptible genotype resulting from the dominant gene *Tsw*.

Development of a DNA marker for CaCV resistance

Linkage to CaCV resistance was identified for 1 fragment of the multilocus-marker Hpms2-23. No other putative markers were identified. The CAPS marker SCAC568, which is associated with TSWV resistance in capsicum (Moury *et al.*, 2000), was not linked to CaCV resistance.

The Hpms2-23 primer-pair produced a smear when analysed by agarose gel electrophoresis, with 2-3 noticeable bands between 70-300 bp. During the initial screening a fragment of 429 bp was identified in the 10 resistant BC1 individuals and absent from the 10 susceptible plants (Figure 1). The 429 bp product was not observed by agarose gel electrophoresis and staining with ethidium bromide. The assessment for linkage of the 429 bp product to CaCV resistance, in segregating-populations totaling 207 individuals, is presented in Table 7. Eight putative recombinants were identified which represents an approximate genetic distance of 4 cM from the resistance locus (% recombination).

Product from the Hpms2-23 primer pair was also analysed by a second genotyping service that used electrophoresis equipment from Applied Biosystems (AB). A 432 bp product from *C. annuum*, as determined by the MegaBACE 1000, was found to co-migrate with the 429 bp product from *C. chinense*. The marker could not be identified on the AB equipment.

Table 7: The assessment of linkage between a 429 bp fragment produced with the Hpms2-23 primer-pair and CaCV resistance.

Population	Phenotype	Number	Putative Recombinants
Backcross 1 ^A	Susceptible	38	0
Backcross 1 ^A	Resistant	46	0
Backcross 1 ^B	Susceptible	26	3
Backcross 1 ^B	Resistant	14	1
Backcross 2 ^C	Susceptible	10	0
Backcross 2 ^C	Resistant	33	1
Backcross 2 ^D	Susceptible	10	3
Backcross 2 ^D	Resistant	10	0
Backcross 2 ^E	Susceptible	10	0
Backcross 2 ^E	Resistant	10	0
		Total = 207	Total = 8

^A*C. annuum* (Merlin) / *C. chinense* / Merlin

^B*C. annuum* (Mazurka) / *C. chinense* / Mazurka

^C(*C. annuum* (Mazurka) / *C. chinense* / Mazurka) / Mazurka

^D(*C. annuum* (Mazurka) / *C. chinense* / Mazurka) / *C. annuum* (16134)

^E(*C. annuum* (Mazurka) / *C. chinense* / Mazurka) / *C. annuum* (21814)

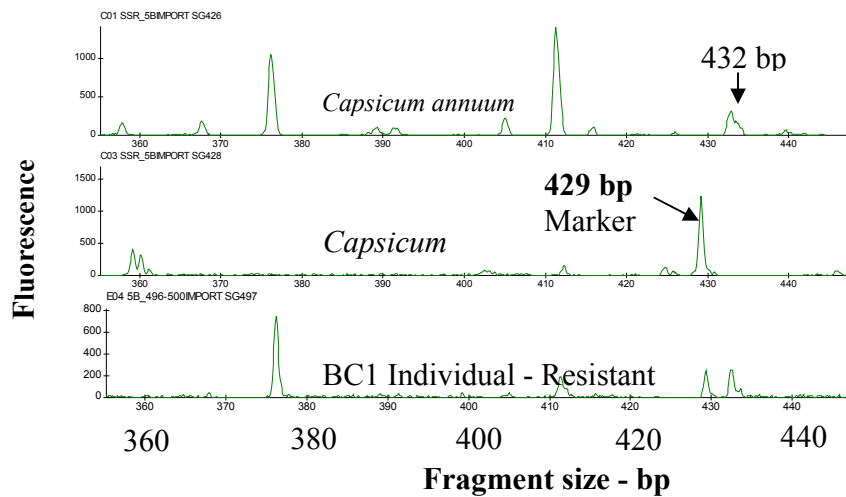


Figure 1: Electropherograms of PCR products, produced with the Hpms2-23 primer-pair, for *C. annuum*, *C. chinense* and a resistant BC1 individual. The 429 bp marker for CaCV resistance is absent from *C. annuum* and present in *C. chinense* and the resistant BC1 individual. Note the 432 bp fragment that maybe allelic to the 429 bp product.

Field evaluation of CaCV-resistant breeding lines

BC1 F1 progenies Gatton and BC2 F2 replicated progenies Bowen

From 133 resistant BC1F1 single plants field planted at Gatton, nine superior lines were advanced to replicated field evaluation in Bowen based primarily on fruit size and shape. Although increased fruit size was the major objective, smaller superior bell-shaped fruit were also selected. The agronomic performance of BC2F2 lines and two commercial check hybrid varieties at Bowen is presented in Table 8, along with a comparison of average fruit size for their parents at Gatton.

Table 8: Field performance of BC2F2 lines at Bowen, October 2005 and BC1F1 parents at Gatton May 2005. ¹ Mean marketable yield kg per 5 plant plot. ² Mean marketable fruit number per 5 plant plot. NA – LSD comparisons not applicable as F test was insignificant. LSD testing inappropriate. ³ Mean average marketable fruit size g. ⁴ Average marketable fruit size of BC1 parent lines sampled from single plants g. LSD testing inappropriate. Where LSD comparisons have been made, means sharing the same letter are not significantly different at $p < .05$.

BC2 Line/ Cultivar	Marketable Yield Bowen ¹	Marketable Fruit No. Bowen ²	Average Size Bowen ³	Average BC1F1/Cultivar Gatton ⁴
Warlock	6.45 a	30	215 a	144
Merlin	6.22 ab	29	218 a	-
D5-12	6.03 ab	47	130 c	98
A5-4	5.70 abc	44	130 c	111
D3-6	5.29 abc	44	122 c	91
B12-3	5.22 abc	32	168 b	78
D2-7	5.06 abc	41	126 c	68
B8-4	5.03 abc	37	135 c	136
B12-2	4.92 bc	40	124 c	62
D5-11	4.53 c	33	141 bc	91
C7-3	2.47 d	35	74 cd	54
LSD (p<.05)	1.48	NA	31	NA

Most of the BC2 F2 breeding lines produced similar marketable yields to the commercial standard cultivars but with significant reductions in fruit size of about 20% - 40%. As a group the breeding lines produced similar yields; seven were statistically equal to the check cultivar Merlin and six were equal to Warlock. There were no statistically valid differences among entries for fruit number although there were some associations of larger number and smaller size. The performance of their BC2F1 parents at Gatton for fruit size was not necessarily a good predictor of the BC2F2 lines' performance in the next generation at Bowen. However significant variation for fruit size in the BC2F2 lines allowed effective gains from selection.

BC3F2 Replicated Lines Tatura

Nineteen BC3F2 lines were evaluated in replicated field trial at Tatura, Victoria in April 2006. Means for three agronomic performance characters for the lines and two check cultivars are presented below in Table 9.

Table 9: Field performance of BC3F2 lines at Tatura, April 2006. ¹ Mean marketable yield, kg per 5 plant plot. ² Mean marketable fruit number per 5 plant plot. ³ Mean average marketable fruit size g. Where LSD comparisons have been made, means sharing the same letter are not significantly different at p<.05.

BC3F2 Line/ Cultivar	Marketable Yield¹	Marketable No.²	Average Size³
E4	6.52 a	39 a	168
Warlock	6.13 ab	24 bcd	254
A3	6.03 abc	33 ab	183
Merlin	5.66 abcd	24 bcd	240
Aries	4.45 bcde	23 bcd	212
A47	4.16 cdef	27 bc	158
A32	3.84 def	20 cde	193
A33	3.36 efg	18 cde	186
A13	3.32 efg	20 cde	166
A11	3.07 efg	27 bcd	122
A25	2.99 efg	18 cde	168
A26	2.99 efg	19 cde	157
A51	2.79 efg	17 cde	170
E16	2.75 efg	18 cde	160
A18	2.63 efg	19 cde	138
E7	2.62 efg	18 cde	149
A31	2.53 efg	15 de	164
A4	2.52 fg	23 bcd	118
A19	2.48 fg	21 cde	128
A20	2.46 fg	17 cde	145
A40	2.34 fg	17 cde	140
E10	2.34 g	10 cde	173
LSD(p<.05)	1.92	11	48

Data for a subset of four breeding lines and cultivar Warlock is presented in Figures 2, 3 and 4, indicating the range of breeding line performance at Tatura.

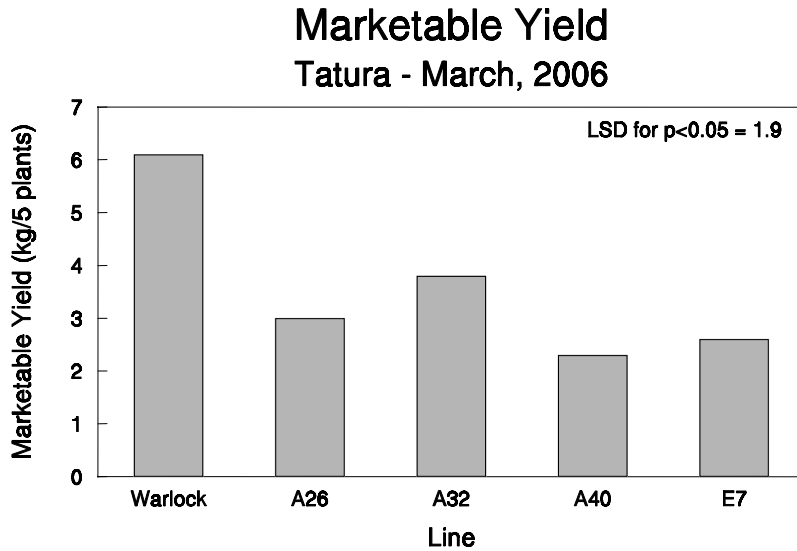


Figure 2 Marketable yield kg per 5 plant plot for BC3 breeding lines

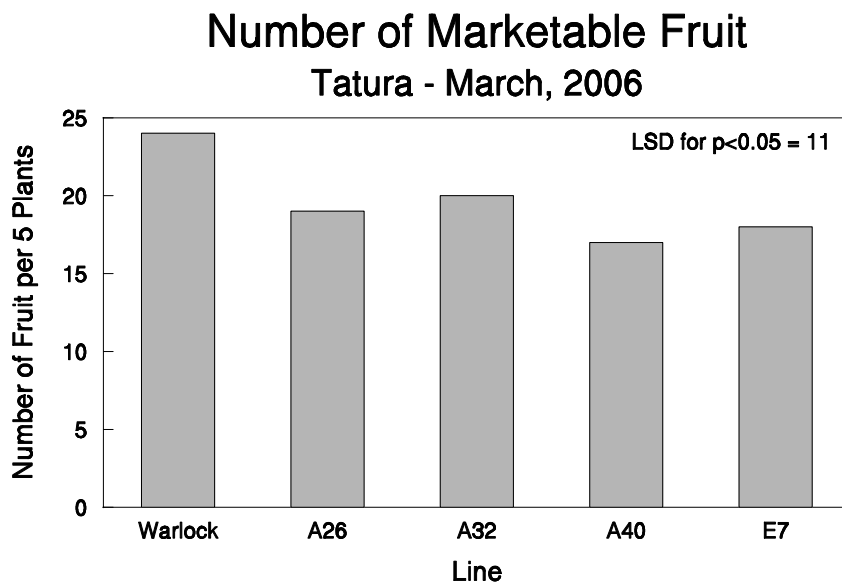


Figure 3 Number of marketable fruit per 5 plant plot for BC3 breeding lines

Average Fruit Size Tatura - March, 2006

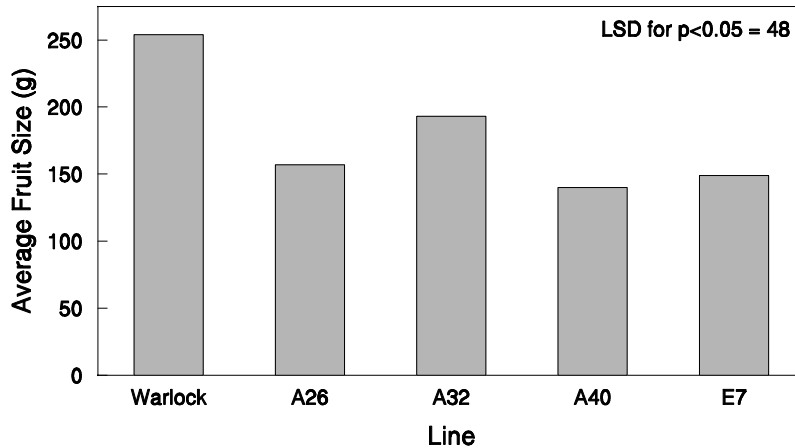


Figure 4 Average marketable fruit size g. for BC3 breeding lines

In this environment only four BC3 lines (E4, A3, A32 and A47) yielded as well as the best cultivars Warlock or Merlin. However even these best lines produced smaller fruit size than the standards; A3 had similar yield to Warlock but its fruit size was approximately 30% less. A32 was lower yielding than Warlock but similar to the other cultivars Merlin and Aries. Yields similar to the check cultivars were usually the result of larger fruit number combined with somewhat smaller size. Because the selection threshold for agronomic performance was lower for the BC3 lines, a larger number showed inferior performance relative to the standards. Nonetheless the best four lines indicated significant improvement in fruit size combined with satisfactory shape.

BC3F2 replicated lines Gatton

Eleven BC3F2 lines and two commercial cultivars were evaluated in a replicated trial at Gatton in May 2006. Line means for agronomic characters are presented in Table 10.

Table 10: Field performance of BC3F2 lines at Gatton, May 2006. ¹ Mean marketable yield, kg per 5 plant plot. ² Mean marketable fruit number per 5 plant plot. ³ Mean average marketable fruit size g. NA – LSD comparisons not applicable as F test was insignificant. Where LSD comparisons have been made, means sharing the same letter are not significantly different at p<.05.

	Marketable yield ¹	Marketable No. ²	Average size ³
A26	5.48	33	172 cd
A33	5.17	37	141 bc
A32	4.72	29	164 c
E4	4.61	33	141 bc
A19	4.20	33	127 ab
A51	4.19	34	121 ab
Warlock	4.02	20	201 d
A3	3.85	27	143 bc
Aries	3.53	18	201 d
A4	3.33	27	122 ab
E16	3.07	30	104 a
A40	2.99	31	99 a
E7	2.47	23	109 ab
LSD(p<.05)	NA	NA	35

Data for four breeding lines and cultivar Warlock are presented in Figures 5, 6 and 7. The lines represent a selection of superior and inferior genotypes.

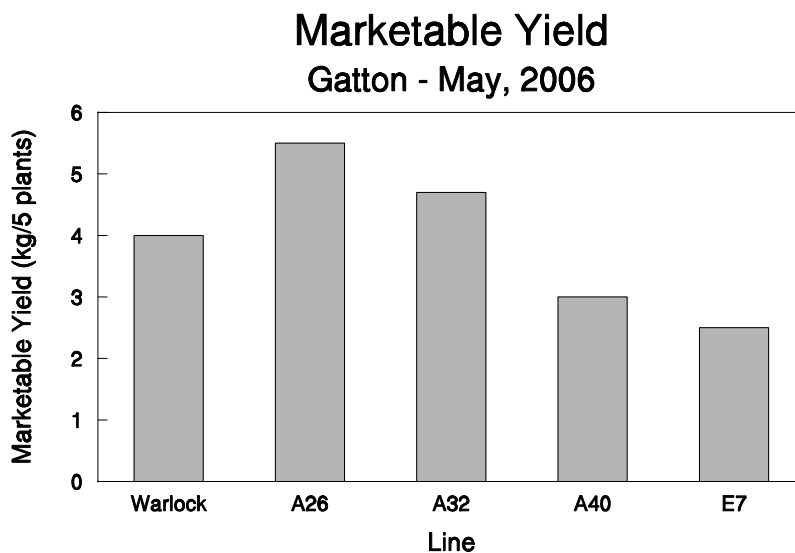


Figure 5 Marketable yield kg per 5 plant plot for BC3 breeding lines

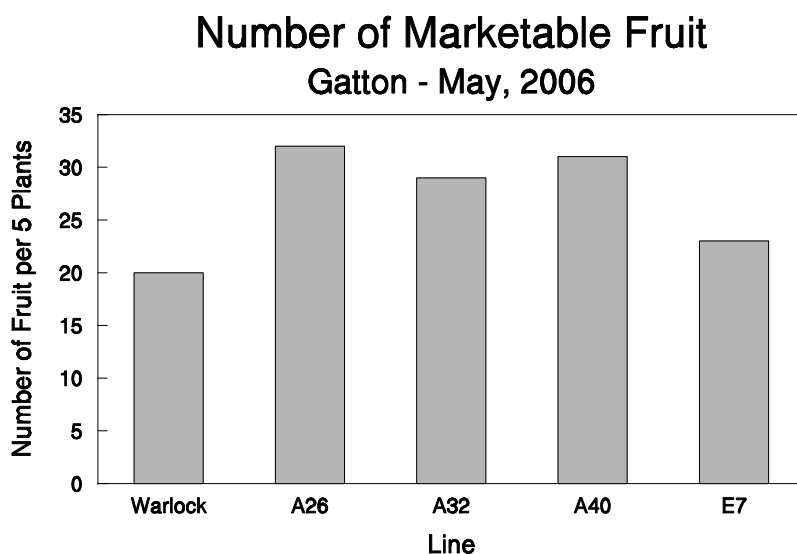


Figure 6 Number of marketable fruit per 5 plant plot for BC3 breeding lines

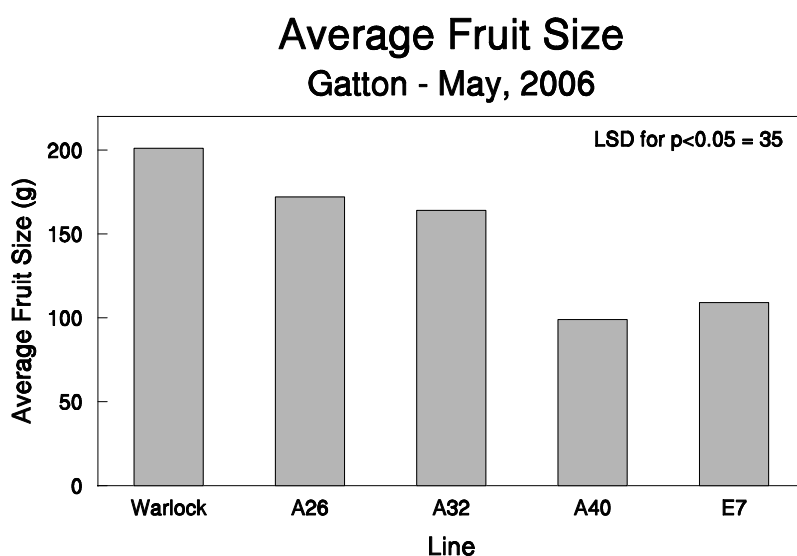


Figure 7 Average marketable fruit size g. for BC3 breeding lines

A26 and A32 produced well-shaped fruit and some of the best yields. There were no statistically significant differences among trial entries for yield or fruit number, although both A26 and A32 produced larger yields than the commercial cultivars. Fruit size for the breeding lines was inferior to the checks. Twenty-five selections from line A32 and nine from A26 were made based on fruit shape and size and screened for CaCV resistance. Additional single plants selections were made in A3, A4, A33, E4, E7 and E16.

BC3 replicated lines Bowen

A similar subset of nine BC3F2 lines and two check cultivars was evaluated in a replicated trial at Bowen in October 2006. Line means for agronomic data are presented in Table 11.

Table 11: Field performance of BC3F2 lines at Bowen, October 2006. ¹ Mean marketable yield, kg per 5 plant plot. ² Mean marketable fruit number per 5 plant plot. ³Mean average marketable fruit size g. Where LSD comparisons have been made, means sharing the same letter are not significantly different at p<.05.

BC3F2 Line/ Cultivar	Marketable Yield ¹	Marketable No. ²	Average size ³
E16	7.62 d	44 c	177 a
Merlin	7.40 d	27 ab	279 c
E7	7.27 d	44 c	167 a
A32	6.84 cd	32 abc	217 b
Aries	6.81 cd	22 a	310 c
Warlock	6.78 d	24 ab	283 c
E4	6.24 bcd	36 bc	178 a
A25	5.97 bd	34 abc	180 a
A31	5.58 bc	26 ab	216 b
A3	5.45 abc	30 ab	180 a
A26	4.67 ab	25 ab	194 ab
A40	3.80 a	24 ab	162 a
LSD(p<.05)	1.67	12	33

Figures 8, 9 and 10 illustrate performance data for four BC3 lines and Warlock.

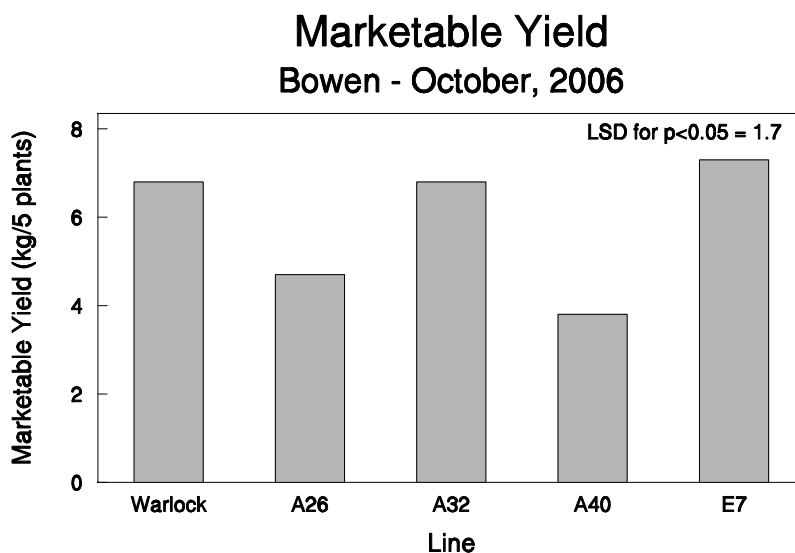


Figure 8 Marketable yield kg per 5 plant plot for BC3 breeding lines

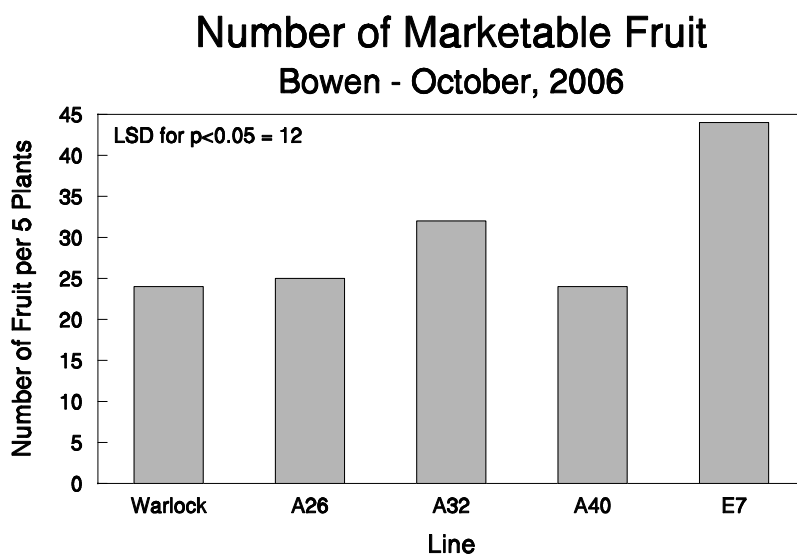


Figure 9 Number of marketable fruit per 5 plant plot for BC3 breeding lines

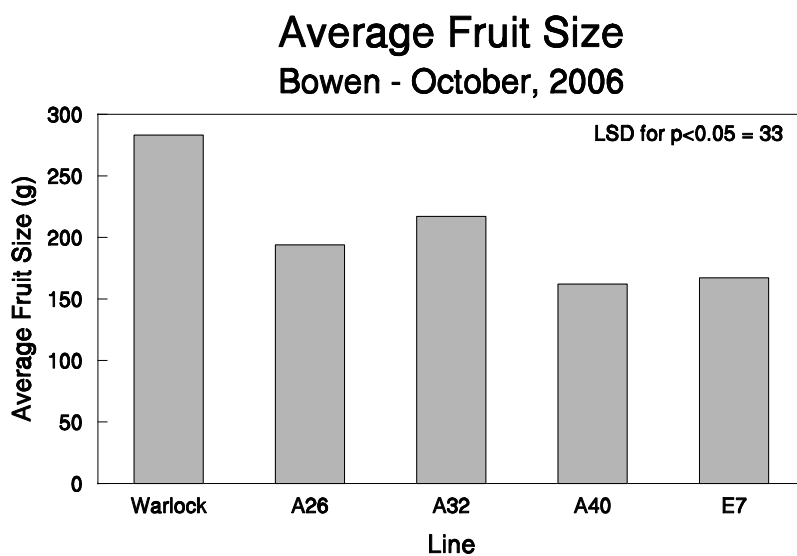


Figure 10 Average marketable fruit size g. for BC3 breeding lines

In this trial A32 was a preferred line based on yield and fruit size. Its performance here and in the other trials suggested good adaptation compared to the standards, Merlin, Warlock and Aries. A32 produced similar yield but smaller fruit size. It was however one of three breeding lines with the largest fruit size.

Breeding lines selected for commercialization

Because of its excellent performance sub-lines of A32 were also assessed in the field and further selected based on independent screening for CaCV resistance. A32 appeared to be the best adapted line with resistance. Of the derived sublines A32-24 was of most interest because it was uniformly resistant. Table 12 indicates the sublines selected from A32 and other lines with commercial potential and their CaCV resistance status. These lines form the collection of breeding material with potential for commercialisation.

Table 12: Selections of BC3F3 lines and their CaCV resistance status.

Selected Line	CaCV resistance status	No. Selections
A32-5	segregating	5
A32-6	unknown	3
A32-10	unknown	2
A32-17	segregating	10
A32-20	segregating	3
A32-24	resistant	6
E4-1	unknown	2
E4-2	segregating	4
E4-8	segregating	2
E7-9	segregating	2

Discussion

The significance of CaCV in Queensland capsicum and tomato crops has been increasing since its identification in 1999. Tospoviruses are often difficult to control because of their wide host range which may include the hosts of vector species. CaCV is now endemic in the major coastal production centres of Queensland and is the dominant virus of capsicum crops at Bundaberg. Survey work has demonstrated the importance of two thrips species, *Thrips palmi* and *Frankliniella schulezi*, as vectors and the commonly infected weed species, *Ageratum conyzoides*, in cropping areas at Bundaberg. High rates of infection in both *Ageratum* and nearby capsicum and tomato crops have identified the weed as a major host which should be controlled.

The control of TSWV is generally effective in tomato cultivars with genetic resistance conferred by the gene *Sw-5* and in capsicum cultivars with the gene *Tsw*. The importance of genetic resistance in a broader management program has been demonstrated by the successful use of these genes in diverse cultivars and locations around the world. A previous indication of good resistance to CaCV in an uncultivated species suggested a similar strategy of developing new cultivars of bell capsicum by conventional breeding. A primary objective of this project was to undertake a resistance breeding program based on an understanding of the inheritance of CaCV resistance.

The identification of CaCV resistance in *C. chinense* led to the discovery of a major dominant gene which is independent of *Tsw*, the gene for resistance to TSWV. This is the first identification of a gene for resistance and was the basis for the breeding work in this

project. The development of resistance was assisted by the robust nature of the gene, its strong expression and predictable segregation in breeding populations. Although the focus of the project was the resistance in one accession of *C. chinense*, additional accessions of several uncultivated species were also resistant. It is likely that they share the same resistance gene but possible they could also provide additional genes if resistance failed.

A useful PCR based marker for CaCV resistance was identified. The marker is a 429 bp fragment as sized by a MegaBASE 1000 capillary electrophoresis system. Assuming 100% penetrance of the resistance gene, and perfect inoculation conditions during phenotyping, the marker was determined to be 4 cM from the resistance locus. The genetic distance is close enough to be useful for marker-assisted-selection in the development of resistant capsicum lines. The marker is produced by a multi-locus PCR and requires sensitive equipment for detection. Although the marker was clearly identified and useful it may not be sufficiently robust for routine application under all conditions. To improve the marker the fragment should be cloned and sequenced for the development of locus specific primers and a more efficient detection system.

Although the primer pair was designed for a microsatellite locus the 429 bp fragment was not the target sequence. The 429 bp fragment may not contain a microsatellite. It is also interesting to speculate that the 432 bp product from *C. annuum* is allelic to the 429 bp product. The co-migration of the two fragments, when analysed with the equipment from Applied Biosystems, may support this hypothesis. If it is true the apparent size difference, as indicated by the MegaBACE system, may actually be due to sequence differences and subsequent conformational changes. Genetic mapping in an F₂ population should be performed to determine if the two fragments behave as a single locus.

The glasshouse screening procedure was developed for reliable detection of CaCV symptoms. Refinements in the biosassay procedure which led to 100% detection of positive controls and confirmation of resistance by ELISA provided accurate classification of genotypes. This was critical in the recognition of segregation patterns to test models of inheritance and the establishment of resistant and susceptible groups for marker identification.

The most advanced breeding material available from the project is an unselected backcross 4 population segregating for resistance and as yet not evaluated. However extensive field evaluation at three sites of backcross 3 lines identified several resistant or segregating lines with good agronomic performance similar to commercial hybrid cultivars. In particular, line A32 generated a series of excellent sublines, one of which, A32-24, is uniformly resistant and several others which are segregating. It will be a routine exercise to derive uniformly resistant lines from these. Field evaluations indicated A32 and several other lines produced marketable yields comparable to cultivars Warlock and Merlin at Gatton and Bowen. Fruit quality and shape were marginally inferior and average fruit size was about 20% less than the standard cultivars. This is reasonable for backcross 3 lines. It is expected they will provide excellent parent lines for immediate use

as hybrids or as an entry point for further breeding. The resistant breeding lines and marker technology will be offered for commercialization.

Technology Transfer

Publications/extension articles

Gosper, K. (2007) Capsicum breeding for tospovirus resistance. *Vegetables Australia* In Press.

Persley DM (2003) Capsicum chlorosis virus. **In:** Compendium of Pepper Diseases APS Press. K Pernezeny *et al.*,(Ed).

Persley, DM (2007) Thrips and Tospovirus – A Management Guide. Cooperative Research Centre for Tropical Plant Pathology.

Persley DM, Sharman M, McGrath D, Garland S (2005) Developing capsicum and tomato cultivars with resistance to Tospoviruses in Australia. VIII International Symposium of Thysanoptera and Tospoviruses, Asilomar, California, USA, September 2005, p 81

Persley DM, Sharman M, Thomas JE (2005) Capsicum virus diseases. VEGE note. Horticulture Australia

Persley DM, Thomas JE, Sharman M (2006) Tospoviruses - an Australian perspective *Australasian Plant Pathology* **35**,161-180.

Sharman M, Persley DM, McMichael L, Thomas JE (2005) Tospoviruses infecting capsicum and tomato in Australia VIII International Symposium of Thysanoptera and Tospoviruses, Asilomar, California, USA, September 2005, p 73.

Technology transfer activities

- Field trials were inspected by representatives from Rijk Zwaan Australia and Syngenta Seeds
- Project progress was discussed each year with the industry reference group formed at the commencement of the work.
- Crop consultants at Bundaberg and Bowen collaborated in disease surveys and developed an awareness of tospovirus management issues.

Recommendations

1. The CaCV-resistant backcross 3 lines and backcross 4 breeding populations should be commercialised along with the DNA marker technology developed in the project.
2. The performance of CaCV resistance should be monitored in field situations to ensure no virulent strains emerge to threaten its effectiveness.
3. The additional PI lines identified as resistant to CaCV in glasshouse assays should be screened using the DNA marker to determine if they have additional gene(s) not yet identified.

4. The DNA marker is not fully developed to the point where it is sufficiently robust for routine use in all situations. Further work to develop locus-specific primers and a more efficient detection system is recommended.
5. More work on the epidemiology of CaCV and its relationship to the weed host *Ageratum conyzoides* is recommended.
6. CaCV is widespread in several Asian countries, particularly Thailand and China. Collaboration with research partners in Asia would extend knowledge of the virus and encourage better control measures and germplasm development.
7. CaCV is becoming an increasing problem in tomato crops at Bundaberg and has caused crop failures in Thailand. A tomato PI line resistant to CaCV has been identified in this project and a study to determine the feasibility of developing resistant cultivars is warranted.

Acknowledgements

The advice and assistance of Dr John Thomas and Lee McMichael is gratefully acknowledged. Staff from Bowen Crop Monitoring and Crop Tech Bundaberg assisted with the virus disease surveys.

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Appendix 1

Reaction of lines of four *Capsicum* species to Capsicum chlorosis virus following manual inoculation

Capsicum species / Accession number	Origin	Reaction to CaCV**
<i>Capsicum baccatum</i>		
PI 199506	Guyana	3
PI 215741	Peru	3
PI 257130	Colombia	4
<i>C. chacoense</i>		
PI 260427	Argentina	3
PI 260429	Argentina	4
PI 260426	Argentina	4
<i>C. chinense</i>		
PI 435916	Mexico	3
PI 209028	Puerto Rico	3
PI 281402	Peru	3
PI 224443	Bolivia	4
PI 238047	Guyana	4
PI 257046	Colombia	4
PI 257136	Ecuador	4
PI 260610	Bolivia	4
PI 273426	United States of America	4
PI 215734	Peru	4
PI 290972	Colombia	1
PI 315021	Peru	3
PI 195299	Guatemala	3
PI 360725	Ecuador	4
PI 439474	Trinidad and Tobago	3
PI 438643	Mexico	3
PI 439424	Costa Rica	3
PI 441634	Brazil	4
PI 594139	Belize	4
PI 355822	Ecuador	3
PI 281423	Puerto Rico	4
PI 159236		1
PI 224448	Costa Rica	4
PI 238051	Peru	3
PI 257079	Colombia	3
PI 257171	Peru	1
PI 159241	United States of America	3
PI 260504	Peru	4

PI 152225		4
PI 281443	Venezuela	2
PI 439464	Peru	1-2
PI 260477	Peru	1-2
PI 315008	Peru	3
PI 439450	Peru	1-2
PI 438638	Mexico, Yucatan	4
PI 438535	Belize, Corozal	3
PI 406987	Panama	3
PI 315028	Peru	1
PI 315019	Peru	3
AVRDC00943		1
 <i>C. frutescens</i>		
PI 123474	India	3
PI 188477	Mexico	3
PI 194880	Guyana	3
PI 241676	Ecuador	4
PI 257104	Colombia	4
PI 368084	Malaysia	4
PI 215728	Peru	1
PI 188479	Vanuatu	4
PI 193470	Ethiopia	4
PI 194260	Ethiopia	3
PI 195296	Guatemala	4
PI 195770	Guatemala	4
PI 197406	Ethiopia	4 and 1
PI 281418	Philippines	5
PI 209109	Puerto Rico	3
PI 257181	Peru	3
PI 224426	Costa Rica	4 and 1
PI 224427	Ecuador	4
PI 260459	Brazil	4
PI 406847	Honduras	3
PI 439515	Nicaragua	3
PI 208738	Cuba	3
PI 439508	El Salvador	4
PI 224416	Costa Rica	4
PI 159282	United States of America	1
PI 370006	India	5
PI 439498	Costa Rica	4
PI 439512	Mexico	4
PI 439520	Philippines	3
PI 441644	Brazil	3
PI 159239	United States of America	3
PI 555643	Guatemala	4
PI 566812	Mexico	3
PI 585257	Ecuador	4
PI 586675	United States of America	4

PI 159238	United States of America	4
PI 441653	Brazil	3
PI 439488	Bolivia	4
<i>C. pubescens</i>		
PI 593618	Guatemala	1-2
PI 593619	Guatemala	1-2
PI 593625	Guatemala	1-2
PI 593628	Guatemala	2-3
PI 593629	Guatemala	2-3
PI 593630	Guatemala	2-3
PI 593639	Guatemala	3
PI 585277	Ecuador	1-2
PI 235048	Costa Rica	no germination
PI 593633	Guatemala	2-3
PI 585276	Ecuador	5
PI 585274	Ecuador	4
PI 585273	Ecuador	3
PI 585270	Ecuador	3
PI 585269	Ecuador	3
PI 585268	Ecuador	2-3
PI 585267	Ecuador	2-3
PI 585262	Ecuador	3
PI 614001	Bolivia	2-3
PI 585266	Ecuador	3

The reaction of inoculated plants to CaCV was ranked on a 0-4 scale with 0 = no symptoms on inoculated or new growth leaves; 1 = hypersensitive necrotic lesions on inoculated leaves and no systemic symptoms; 2 = necrotic and / or chlorotic lesions on inoculated leaves and limited systemic symptoms in at least some plants; 3 = no hypersensitive necrotic reaction and systemic mosaic, mottle and stunting; 4 = no hypersensitive necrotic reaction and severe systemic symptoms including mottle/mosaic/necrosis with death of all or most plants.

† The data on all lines tested are available from the authors.

* Plants were grown in pots in a glasshouse and inoculated with CaCV when the second set of true leaves had expanded.