Capsicum breeding for tospo virus resistance

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QLD Department of Primary Industries & Fisheries

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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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CONTENTS

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 glycoproteins 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

uniformly resistant to both viruses and used to generate test populations for inheritance studies and breeding.

Development of F1 and backcross 1 generations

Two F1 hybrids, F1 A (variety 1 x 12G) and F1 B (variety 2 x 12G) were made. Varieties 1 and 2 were virus-susceptible commercial bell capsicums with excellent production characteristics; 12G was a virus resistant accession of C. chinense as described above. F1 A and F1 B were backcrossed to their respective parents to provide backcross 1 generations. The construction of hybrids and backcrosses is represented diagrammatically as follows:

Variety 1 x 12G →	F1 A x Variety 1	→	Backcross (BC) 1A
	F1 A x 12G	→	Backcross (BC) 1A*
Variety 2 x 12G →	F1 B x Variety 2	→	Backcross (BC) 1B
	F1 B x 12G	→	Backcross (BC) 1B*

* F2 seed was obtained from both F1 A and F1 B but was not used because of poor germination.

Development of backcross 2, 3 and 4 generations

Backcrosses BC 1A and BC 1B were screened for CaCV resistance and shown to be segregating, as expected. A large number of resistant genotypes were identified in both populations but two highly resistant lines from BC 1B, designated 7-1 and 23-2, were selected as donor parents to construct four BC2 populations. The hybridisation details are as follows:

Variety 2 x (BC 1B) 7-1, Variety 2 x (BC 1B) 23-2, 21814 x (BC 1B) 23-2, 16134 x (BC 1B) 23-2.

21814 and 16134 represent inbred lines derived from variety 1 in a parallel program of inbred line development. The resistant donor parents were screened in the glasshouse and laboratory as seedlings and selections sent to a winter planthouse nursery in Bowen for evaluation. The final choice of lines 7-1 and 23-2 as donor parents was made on the basis of agronomic merit, primarily plant habit, fruit shape and size.

Each BC2 population segregated with large numbers of highly resistant plants, four of which were selected as before to provide BC3 populations. The cycle of selection, evaluation and hybridization was repeated until BC4 resistant lines were produced with similar agronomic characteristics to the original parent varieties. Two generations of inbreeding, screening and selection in the BC4 population produced a range of BC4 lines which are either uniform or variable for resistance.

Development of resistance to Tomato spotted wilt virus

Genetic resistance to Tomato spotted wilt virus (TSWV) is conferred by *Tsw*. It has been used extensively in capsicum variety development and was introduced to this program in the subline 12G of PI 1290972. A DNA marker for *Tsw* (Moury *et al.* 2000) confirmed 12G was TSWV resistant and was subsequently applied to BC1 and BC2 generations for selection of resistant lines. Segregation patterns for resistance to both CaCV and TSWV

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 µL of chloroform/isoamyl alcohol (24:1), mixed, left on ice for at least half an hour, and centrifuged at 11200 rcf 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 µL 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 µL containing 1X Roche PCR Buffer, approximately 50 ng of genomic DNA and 188 µM dNTPs. The PCR reaction mix also included 150 nM of each primer (1 primer with a 5´ fluorescent label), 4 mM total $MgCl_2$, 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 20006. 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.

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 F_2 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.

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

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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.

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