Resistance to tospoviruses in pepper

P. Roggero[1], S. Pennazio[1], V. Masenga[1], and L. Tavella[2]
[1]Istituto di Fitovirologia Applicata, CNR, Str delle Cacce 73, 10135 Torino, Italy; [2]Di.Va.P.R.A. Entomologia e Zoologia applicate all'Ambiente "Carlo Vidano", Università di Torino, Torino, Italy
E-mail: p.roggero@ifa.to.cnr.it

Abstract: Tospoviruses, mainly Tomato spotted wilt virus (TSWV), constitute a severe threat to *Capsicum* cultivation worldwide. Resistance to TSWV but not to other tospoviruses, based on a hypersensitive reaction, has been found only in accessions of *C. chinense* ‘PI152225’ and ‘PI159236’. The resistance, carried by a dominant gene called Tsw, is broken at high temperatures and depends on plant age, with young plants more susceptible. The Tsw gene has been introduced into several commercial sweet and hot pepper cultivars with good agronomic performance. Resistance-breaking strains of TSWV systemically infecting the resistant plants have been found under experimental conditions and in the field. Future directions for search on resistance in *Capsicum*, and management of resistance to tospoviruses in plants are discussed.

Introduction

Tospoviruses are a very dangerous threat to many crops. Major crops susceptible to Tomato spotted wilt virus (TSWV), the most important member and type species of the genus Tospovirus, family Bunyaviridae (Elliott et al., 2000), are tomato, pepper, lettuce, potato, papaya, peanut and tobacco (German et al., 1992). High infection rates have led to considerable economic losses in an increasing number of countries, so that TSWV is considered one of the ten most economically destructive plant viruses (Tomlinson, 1987). Severe damage to *Capsicum* spp. occur worldwide (Gitais et al., 1998; Lima et al., 2000; Momol et al., 2000). In Italy, for example, the *Capsicum annuum* crop is suffering increasing losses both in fields and glasshouses (Vaira et al., 1993; Tavella et al., 1997). Plants are highly susceptible at all stages of development, showing severe symptoms, usually necrotic, on leaves and fruits. The behaviour of the main vector *Frankliniella occidentalis*, feeding preferentially in flowers, complicates the picture by providing an alternative route to leaves for entry of the virus, and in mature plants sometimes fruits alone become infected.

The increasing problem of TSWV and other tospoviruses has stimulated a search for sources of genetic resistance, using either conventional or non-conventional methods (Cho et al., 1996; Prins and Goldbach, 1998). Here we review the results of a decade of work attempting to use Mendelian genes to obtain resistance to TSWV and other tospoviruses in *Capsicum* spp.

Resistance to TSWV

Since genes for TSWV resistance are not known in *C. annuum*, breeding experiments have been done utilising *C. chinense* Jacquin. In a pioneer paper, Cheng et al., (1989) described the development of *C. chinense* germoplasm from Amazonia. They suggested that this species was more suitable than *C. annuum* for cultivation in the tropics and that it was resistant to major fungal pathogens, the potyvirus Potato virus Y and also TSWV. Preliminary, successful crosses between a cultivar of *C. chinense* and *C. annuum* cv California Wonder were also described.

Black et al., (1991), later identified the accessions of *C. chinense* 'PI152225' and 'PI159236' as resistant to TSWV. Following mechanical inoculation of leaves, both accessions showed a hypersensitive reaction (HR), triggered by a mechanism under gene control (Fraser, 1990). Interestingly, some progeny derived from crosses between the 'PI' accessions and susceptible *C. annuum* lines were found to respond hypersensitively to this virus.

Inheritance of the resistance associated with both 'PI' accessions was confirmed by Black et al., (1996) and Boiteux (1995). The latter confirmed that resistance was conferred in both accessions by a single dominant gene, displayed resistance to a broad range of TSWV isolates. Boiteux concluded that the absence of segregation for resistance that was observed in all P2 generations from crosses between *C. chinense* 'PI' accessions indicated two possibilities: the occurrence of i) a tightly linked group of genes, or, ii) the same single gene (called ‘Tsw’) for both accessions. This question was solved by Black et al., (1996), who showed that resistance was governed by a single dominant gene, identical for both accessions.

It is known that at temperatures higher than 28°C viruses may overcome the HR and spread systemically (Pennazio, 1995). This possibility was tested in 'PI 15225' - TSWV system, but the breaking of HR depended only partially on temperature, because the developmental stage of the plants at virus inoculation was equally crucial. When young plants were inoculated at the 2 true leaf stage and then grown continuously at 33°C, 20% of the plants reacted hypersensitively, whereas with plants inoculated at the 4 true leaf stage the proportion reached 45 % (Roggero et al., 1996). This meant, in any case, that the resistance introduced via Tsw gene for HR from *C. chinense* might be limited by temperature in an uncontrolled environment. However, progeny from self-pollinated plants, which maintained resistance even at 33° C, displayed a remarkable increase in HR to about 80%; so it was proposed that selection of parent plants resistant at high temperature regimes could be useful for breeding for resistance (Roggero et al., 1996).

This finding was confirmed by Roselló et al., (1997), Moury et al., (1998) and Soler et al., (1998). Moury et al., (1998) showed that continuous high temperature destabilized the resistance in young but not in older plants; the most severe conditions (12 days at 32° C) resulted in up to 70 % of plants with systemic necrosis if these were inoculated at the cotyledon stage. Moury and co-workers observed a strong plant age effect on susceptibility to TSWV under high temperature regimes, and, in agreement with Roggero et al., (1996), they suggested the involvement of a mature plant and/or tissue resistance. Moury et al., (1998) also showed that it would be preferable to grow cultivars homozygous for the Tsw locus at high temperatures. Soler et al., (1998) found that all plants of both the 'PI' resistant accessions became systemically infected when maintained at 30/18 °C (day/night), independently of the stage of development. They also found that plants inoculated at the 2 leaf, but not at the 4 leaf, stage, became systemically infected when kept at 25/18 °C (day/night).

It can be concluded that the mechanism of this HR is strongly influenced by both temperature and physiological plant conditions. A temperature of 25°C is common in areas having a mild climate, so that precautions should be taken to avoid early infection of pepper seedlings.

Soler et al., (1999) studied the movement and distribution of TSWV in resistant and susceptible accessions of *Capsicum* spp. By serological assays, they confirmed that a small but significant number of plants of the 'PI152225' and 'PI159236' accessions became systemically infected if kept at 25/14 °C (day/night) just after TSWV inoculation. However, spread of the virus from the initial infection sites was slower in resistant than in the susceptible plants. They did not find any restriction of viral replication, as previously reported for other HRs (Otsuki et al., 1972; Devergne et al., 1984; Roggero and Pennazio, 1984). All these results suggest that resistance through HR may be due to an impairment of the mechanism of the long-distance transport of TSWV.

TSWV resistance-breaking strains

Good agronomic pepper cultivars showing resistance to TSWV conferred by Tsw are now commercially available. This, however, has not reassured agronomists and virologists because new TSWV strains can arise and be selected (Qiu et al., 1998). The ability of this virus to replicate in its thrips vector may increase the opportunity for genomic diversification of its population (Wijkamp, 1995). In addition, the complexity of TSWV architecture makes the recognition of new strains rather difficult (Adkins, 2000). A TSWV strain breaking the resistance of *C. chinense* was first noted by Boiteux et al., (1993). In the course of a wide screening programme in which a collection of seventy *Capsicum* cultivars was tested for resistance to the virus, they found that resistance displayed by two *C. chinense* lines (one of them 'PI 159236') against isolate BsB was not operative against isolate SF. Since a source of resistance against both isolates was not found, Boiteux et al., (1993) concluded that in *Capsicum* there might be different genetic mechanisms conferring resistance. Similar results were obtained by Hobbs et al., (1994); at least two strains isolated from tomato plants in Louisiana gave systemic symptoms in 'PI152225' and 'PI159236', which, as we know, were generally found to react hypersensitively against several TSWV isolates. Later, Moury et al., (1997) found that the resistance conferred on *C. chinense* accessions and controlled by the single dominant gene Tsw, was overcome by virulent strains. It should be noted that all these isolates overcoming resistance were not typed to assess if they were in fact TSWV or the closely related species Groundnut ringspot virus (GRSV) or Tomato chlorotic spot virus (TCSV) (see below).

All these findings disappointed expectations of controlling TSWV in pepper. Roggero et al., (1999a) found two field pathotypes of TSWV which overcame the HR of a *C. chinense* 'PI152225' X *C. annuum* resistant hybrid. Two plants of this hybrid, grown in Liguria region (Italy) with good agronomic performance, showed typical systemic TSWV symptoms on apical leaves and fruits. The viruses isolated from the plants were recognized as TSWV strains by serological assays and were transmitted by *F. occidentalis* with high efficiency. They gave severe systemic symptoms in susceptible pepper and a high proportion (40-60%) of systemic infection in 'PI152225' and 'PI159236'. Roggero et al., (1999) suggested that the absence of systemic infection in a portion of the parental plants may indicate that plants resistant to these pathotypes could be selected.

More recently, Roggero et al., (2001) reported again from Liguria that resistance to TSWV in commercial pepper cultivars grown under glass was seriously overcome. Two isolates (or pathotypes) were characterized and found similar to other isolates in infecting a wide number of plants species and being transmitted with high efficiency by *F. occidentalis*. The isolates survived in experimental mixed infections with a non-resistance breaking isolate in susceptible pepper and 'PI152225' when inoculated at the same time, but cross protection in susceptible pepper acted against them. Other resistance-breaking strains have also been found also one year later in other crops (tomato, pepper and artichoke) close to infected resistant pepper plants.

TSWV strains overcoming resistance in tomato carrying the SW-5 gene were reported by Latham and Jones (1998), but these strains did not overcome the resistance of the 'PI' accessions of *C. chinense*. However, Roggero et al., (2001) found that *C. chinense* 'PI152225' but not resistant tomato cultivars are susceptible to the TSWV resistance breaking strains isolated from pepper and artichoke crops. This confirms that the Tsw gene in pepper and Sw-5 gene in tomato have different characteristics, as also shown by molecular biology techniques (Jahn et al., 2000) and that resistant-breaking strains are highly specific.

Genetic resistance to other Tospoviruses

The genus Tospovirus includes seven confirmed and five tentative species (Elliot et al., 2000). The genetic bases of resistance to all these viruses are poorly known, and limited to some of them. Tsw is not effective against GRSV and TCSV (Boiteux and De Avila, 1994), nor against two isolates of Impatiens necrotic spot virus (INSV) (Roggero et al., 1999b); both these isolates gave about 50 % systemic infection in the 'PI' resistant accessions kept at 25/18 °C, day/night). In contrast to TSWV, systemic infection by the two INSV isolates was completely blocked at 33°C continuous temperature, but this does not actually help to solve the question of resistance related to Tsw. Green et al., (1996) evaluated the resistance of *C. annuum* cv 'Yolo Wonder' and *C. chinense* resistant 'PI' accessions to Watermelon silver mottle virus (WSMV) at relatively high temperatures ranging from 24 to 36°C. One line of 'PI152225' showed only local infection. No further search of resistance among Capsicum spp. has been reported for these viruses.

Conclusions

One can draw one major conclusion: further research is needed to reach the goal of genetic control of tospovirus diseases in pepper. This is expecially important for South America where GRSV and TCSV are prevalent, and Asia where WSMV is widespread. Adkins (2000) has written that:"The scarcity of host plant resistance genes and the large number of weed hosts providing between crop-virus reservoirs exacerbate the situation [of management of TSWV].

Livingstone et al., (1999) have published an improved genome map of pepper, and Jahn et al., (2000) have compared the map of the Tsw locus of *C. chinense* with that of the Sw-5 locus of tomato, while Grube et al., (2000) have analyzed in detail the loci of resistant genes for three members of the

Solanaceae. This research stressed the potential use of comparative genome mapping in order to rapidly identify other genes showing similar function and specificity against other pathogens.

Another genetic approach concerns pathogen-mediated resistance. Tomato and tobacco (Kim et al., 1994), chrysanthemum (Sherman et al., 1988), tobacco (Herrero et al., 2000) transgenic for the TSWV nucleocapsid (N) gene have been produced. Hoffman et al., (2001) have investigated the molecular basis of resistant breaking by TSWV. They demonstrated in vivo reassortment between two strains of the virus, and the occurrence of some new pathotypes with ability to overcome resistance in tomato. Unfortunately, these pathotypes could also overcome resistance conferred by the N gene in tobacco plants, a rather negative result for the possible use of such transgenic plants.

A last question is "mature plant resistance". Both susceptibility and hypersensitivity appear to be controlled in pepper within restricted stages of development. This type of resistance may be a general feature of plants (Pennazio and Roggero, 1996) but there appears to have been no discussion on its mechanism. Switching off one or more genes may induce RNA-mediated resistance analogous to that expressed by post-transcriptional gene silencing found in transgenic plants (Dougherty and Parks, 1995; Baulcombe, 1996; Dawson, 1996). Such a mechanism has been described, for example, by Jan et al., (2000), who showed that segments of the TSWV N gene were able to confer resistance against this virus via post-transcriptional gene silencing.

Acknowledgements
We thank R.G. Milne for critical revision of the manuscript.

References

Adkins S. 2000. Tomato spotted wilt virus - positive steps towards negative success. *Molecular Plant Pathology* **1**, 151-157.

Baulcombe DC. 1996. Mechanisms of pathogen-derived resistance to viruses in transgenic plants. *Plant Cell* **8**, 1833-1844.

Black LL, Hobbs HA and Gatti JM. 1991. Tomato spotted wilt virus resistance in *Capsicum chinense* PI152225 and 159236. *Plant Disease* **75**, 863.

Black LL, Hobbs HA and Kammerlohr DS. 1996. Resistance of Capsicum chinense lines to tomato spotted wilt virus from Louisiana, USA, and inheritance of resistance. *Acta Horticulturae* **431**, 393-401.

Boiteux LS. 1995. Allelic relationships between genes for resistance to tomato spotted wilt tospovirus in *Capsicum chinense*. *Theoretical and Applied Genetics* **90**, 146-149.

Boiteux LS and de Avila AC. 1994. Inheritance of a resistance specific to tomato spotted wilt tospovirus in *Capsicum chinense* 'PI 159236'. *Euphytica* **75**, 139-142.

Boiteux LS and Nagata T. 1993. Susceptibility of Capsicum chinense PI 159236 to tomato spotted wilt virus isolates in Brazil. *Plant Disease* **77**, 210.

Boiteux LS, Nagata T, Dutra WP and Fonseca MEN. 1993. Sources of resistance to tomato spotted wilt virus (TSWV) in cultivated and wild species of *Capsicum*. *Euphytica* **67**, 89-94.

Cheng SS, Green SK, Griggs TD and McLean BT. 1989. The use of *Capsicum chinense* as sweet pepper cultivars and sources for gene transfer. In: Tomato and Pepper Production in the Tropics. Proceedings of the international symposium on integrated management practices, Tainan, Taiwan, 21-26 March 1988. AVRDC Publication No. 89-317: 55-62.

Cho JJ, Custer DM, Brommonschenkel SH and Tanksley SD. 1996. Conventional breeding: host-plant resistance and the use of molecular markers to develop resistance to tomato spotted wilt virus in vegetables. *Acta Horticulturae* **431**, 367-378.

Dawson WO. 1996. Gene silencing and virus resistance: a common mechanism. *Trends in Plant Science*. **1**, 107-108.

Devergne JC, Cardin L, Pitrat M and Lecoq H. 1984. Dosage immunoenzimatique (ELISA) du virus de la mosaïque du concombre. II. Multiplication compareé du virus dans les melons sensibles et résistants. *Agronomie* **4**, 137-145.

Dougherty WG and Parks TD. 1995. Transgenes and gene suppression: telling us something more? *Current Opinion in Cell Biology* **7**, 399-405.

Elliott RN *et al.*, 2000. Family Bunyaviridae. In: van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR, and Wickner RB. - Virus Taxonomy: Classification and Nomenclature of Viruses. (7th Report ICTV) Academic Press, San Diego. pp. 599-621.

Fraser RSS. 1990. The genetics of resistance to plant viruses. *Annual Review of Phytopathology* **28**, 179-200.

German TL, Ullman DE and Moyer JW. 1992. Tospoviruses: diagnosis, molecular biology, phylogeny, and vector relationships. *Annual Review of Phytophatology* **30**, 315-348.

Gitaitis RD, Dowler CC and Chalfant RB. 1998. Epidemiology of tomato spotted wilt in pepper and tomato in Southern Georgia. *Plant Disease* **82**, 752-756.

Green SK, Hwang JT and Chou JC. 1996. Evaluation of selected *Lycopersicon* and *Capsicum* germ plasm for watermelon silver mottle tospovirus resistance. *Plant Disease* **80**, 824.

Grube RC, Radwanski ER and Jahn M. 2000. Comparative genetics of disease resistance within the Solanaceae. *Genetics* **155**, 873-887.

Herrero S, Cultbreath AK, Csinos AS, Pappu HR, Rufty RC and Daub ME. 2000. Nucleocapsid gene-mediated transgenic resistance provides protection against Tomato spotted wilt virus epidemics in the field. *Phytopathology* **90**, 139-147.

Hobbs HA, Black LL, Johnson RR and Valverde RA. 1994. Differences in reactions among tomato spotted wilt virus isolates to three resistant *Capsicum chinense* lines. *Plant Disease*. **78**, 1220.

Hoffmann K, Qiu WP and Moyer JW. 2001. Overcoming host- and pathogen-mediated resistance in tomato and tobacco maps to the M RNA of Tomato spotted wilt virus. *Molecular Plant Microbe Interactions*. **14**, 242-249.

Jan F, Fagoaga C, Pang SZ, Gonsalves D, Jan FJ and Pang SZ. 2000. A single chimeric transgene derived from two distinct viruses confers multi-virus resistance in transgenic plants through homology-dependent gene silencing. *Journal of General Virology* **81**, 2103-2109.

Jahn M, Paran I, Hoffmann K, Radwanski ER, Livingstone KD, Grube RC, Aftergoot E, Lapidot M and Moyer JW. 2000. Genetic mapping of the Tsw locus for resistance to the tospovirus tomato spotted wilt virus in *Capsicum* spp. and its relationship to the Sw-5 gene for resistance to the same pathogen in tomato. *Molecular Plant Microbe-Interactions*. **13**, 673-682.

Kim JW, Sun SSM and German TL. 1994. Disease resistance in tobacco and tomato plants transformed with tomato spotted wilt virus nucelocapsid gene. *Plant Disease* **78**, 615-624.

Latham LJ and Jones RAC. 1998. Selection of resistance breaking strains of tomato spotted wilt tospovirus strains. *Annals of Applied Biology* **133**, 385-402.

Lima MF, de Avila AC, de Resende R and NagataT. 2000. Survey and identification of Tospovirus species in tomato and pepper fields in the San Francisco Valley and Federal District. *Summa Phytopathologica* **26**, 205-210.

Livingstone HD, Lackney UK, Blauth JR, Wijk RV and Jahn MK. 1999. Genome mapping in *Capsicum* and the evolution of genome structure in the Solanaceae. *Genetics* **152**, 1183-1202.

Momol MT, Pappu HR, Dankers W, Rich JR and Olson SM. 2000. First report of tomato spotted wilt virus in habanero and tabasco peppers in Florida. *Plant-Disease* **84**, 1154.

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

Moury B, Selassie-Gebre K, Marchoux G, Daubeze AM and Palloix A. 1998. High temperature effects on hypersensitive resistance to tomato spotted wilt tospovirus (TSWV) in pepper (*Capsicum chinense* Jacq.). *European Journal of Plant Pathology* **104**, 489-498.

Otsuki J, Shimomura T and Takebe I. 1972. Tobacco mosaic virus multiplication and expression of the N gene in necrotic responding tobacco varieties. *Virology* **50**, 45-50.

Pennazio S. 1995. The hypersensitive reaction of higher plants to viruses: a molecular approach. *Microbiologica* **18**, 229-240.

Pennazio S and Roggero P. 1996. Mature plant resistance to virus infection. *Petria* **6**, 195-207.

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

Qui WP, Geske SM, Hickey CM and Moyer JW. 1998. Tomato spotted wilt tospovirus genome reassortment and genome segment specific adaptation. *Virology* **244**, 186-194.

Roggero P and Pennazio S. 1984. Quantitative determination by ELISA of tobacco necrosis virus from necrotic local lesions in tobacco. *Journal of Virological Methods* **8**, 283-291.

Roggero P, Lisa V, Nervo G and Pennazio S. 1996. Continuous high temperature can break the hypersensitivity of *Capsicum chinense* 'PI152225' to tomato spotted wilt tospovirus (TSWV). *Phytopathologia Mediterranea* **35**, 117-120.

Roggero P, Dellavalle G, Ciuffo M and Pennazio S. 1999a. Effects of temperature on infection in *Capsicum* spp. and *Nicotiana benthamiana* by impatiens necrotic spot tospovirus. *European Journal of Plant Pathology* **105**, 509-512.

Roggero P, Melani V, Ciuffo M, Tavella L, Tedeschi R and Stravato VM. 1999b. Two field isolates of tomato spotted wilt tospovirus overcome the hypersensitive response of a pepper (*Capsicum annuum*) hybrid with resistance introgressed from *C. chinense* PI152225. *Plant Disease* **83**, 965.

Roggero P, Masenga V and Tavella L. 2001. Field isolates of Tomato spotted wilt virus overcoming resistance in pepper and their spread to other hosts in Italy. Submitted

Rosellò S, Diez MJ and Nuez F. 1997. Utilization of *Capsicum* sp. resistance to TSWV in pepper breeding. *Capsicum and Eggplant Newsletters* **16**, 87-90.

Sherman JM, Moyer JW and Daub ME. 1998. Tomato spotted wilt virus resistance in chrysanthemum expressing the viral nucelocapsid gene. *Plant Disease* **82**, 407-414.

Soler S, Diez MJ and Nuez F. 1998. Effect of temperature regime and growth stage interaction on pattern of virus presence in TSWV-resistant accessions of *Capsicum chinense*. *Plant Disease* **82**, 1199-1204.

Soler S, Diez MJ, Rosello S and Nuez F. 1999. Movement and distribution of tomato spotted wilt virus in resistant and susceptible accessions of *Capsicum* spp. *Canadian Journal of Plant Pathology* **21**, 317-325.

Tavella L, Alma A, Conti A, Arzone A, Roggero P, Ramasso E, Dellavalle G and Lisa V. 1997. Tripidi e TSWV nelle serre di peperone in Liguria. *Colture Protette* **26**, 79-83.

Tomlinson JA. 1987. Epidemiology and control of virus diseases of vegetables. *Annals of Applied Biology* **110**, 661-681.

Vaira AM, Roggero P, Luisoni E, Masenga V, Milne RG and Lisa V. 1993. Characterization of two Tospoviruses in Italy: tomato spotted wilt and impatiens necrotic spot. *Plant Pathology* **42**, 530-542.

Wijkamp I. 1995. Virus-vector relationships in the transmission of tospoviruses. Doctoral Thesis Wageningen, 143 pages.