

## REVIEWS

# Control of diseases induced by tospoviruses in tomato: an update of the genetic approach

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**Summary.** Advances in the search for genetic resistance to tospoviruses affecting tomato crops are reviewed. The economic losses caused by *Tomato spotted wilt tospovirus* (TSWV), the great number of hosts it affects and its wide distribution around the world has made TSWV one of the ten most important plant viruses. Other viruses in or related to the same genus also cause severe damage, although their presence in the world is much more localized. Due to the limited effectiveness of physical, chemical and biological control methods, the use of genetic resistance for control is the best management strategy on a medium-long term basis. Given the relative ease with which new TSWV isolates that overcome existing genetic resistance are generated, it is of prime importance to continue the search for new sources of resistance, as well as to promote a better exploitation of available ones. A better understanding of the mechanisms causing resistance and of their genetic control, as well as the identification of molecular markers linked to resistance genes, would enable the pyramiding of different resistance genes. This would be a positive contribution to the development of a greater and more durable resistance. It is also necessary to further the study of genetic resistance to other viruses of the genus *Tospovirus*, as globalisation can speed up their distribution throughout the world.

**Key words:** plant breeding, hypersensitive response, apparent recovery, durability.

## Introduction

The genus *Tospovirus* is a limiting factor in the development of agriculture because of the losses that *Tomato spotted wilt tospovirus* (TSWV) has caused during the last two decades. This genus belongs to the family *Bunyaviridae*, and is the only member with viruses able to infect plants. Viruses belonging to this genus are transmitted exclusively by

thrips (Thysanoptera: Thripidae) in a persistent and propagative manner (Ullman *et al.*, 1997).

The disease caused by TSWV was first recorded in 1919 on tomato plants in Australia (Brittlebank, 1919). In infected plants the younger leaves turn violet, but the seriousness of the disease is in most cases due to stem necrosis (Fig. 1) which causes complete loss of production.

In the European and Mediterranean region, TSWV was virtually absent for more than 40 years after the Second World War, possibly due to the paucity of onion thrips, *Thrips tabaci* Lind., in these regions (EPPO/CABI, 1997). This thrips at the time

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was the most important insect vector of this virus. However, in the 80's a series of serious generalised epidemics occurred in Western Europe (Marchoux *et al.*, 1991; Vaira *et al.*, 1993), linked to the fast spread of another much more efficient vector: western flower thrips *Frankliniella occidentalis* Perg. (Ullman *et al.*, 1997). From that moment there has been an extraordinary increase in the number of plant species reported to be hosts of TSWV (German *et al.*, 1992).

By 1968 TSWV was considered to have one of the widest host ranges of all viruses, comprising 157 dicotyledons and 6 monocotyledons (Best, 1968). However, it is now known that TSWV infects more than 1000 species; including monocotyledons and dicotyledons belonging to more than 82 botanical families (Prins and Kormelink, 2000). Hosts include horticultural crops such as tomato, pepper, lettuce, celery, potato, tobacco, peanut and pea, and ornamental plants such as dahlia, chrysanthemum, gerbera, impatiens and iris. TSWV is now listed among the ten most serious plant viruses and causes annual losses valued around 1,000 million dollars (Goldbach and Peters, 1994). The disease is widespread in areas of horticultural production in the five continents, mainly those that are warmer. In milder climates the damage it causes is mostly to greenhouse crops.

Recently several tospoviruses infecting tomato have been detected, mainly in South America, but their financial impact has not yet been assessed. Although some of these viruses have not yet been found in the Mediterranean basin, their study in depth should not be deferred. The effect of globalisation with its increase in the exchange of plant material must be considered, since these factors are important in spreading of viruses beyond the areas where they were originally detected (Marchoux *et al.*, 2001).

### Structure of the genus *Tospovirus*

The criterion that is currently accepted for the inclusion of new species in the *Tospovirus* genus, and their classification in existing groups, is based on the percent sequence homology between the genes of the nucleocapsid N protein of the isolates (De Avila *et al.*, 1993). Initially however, serological criteria were used, classifying the species by means of specific polyclonal antisera both for G1 and G2 proteins from the pleomorphic membrane

and for the N protein. It is for this reason that the *Tospovirus* species are still classified in five serogroups (Table 1).

TSWV is the only member of serogroup I. *Tomato chlorotic spot tospovirus* (TCSV) and *Groundnut ringspot tospovirus* (GRSV), which belong to serogroup II, are differentiated by the manner in which monoclonal serum reacts to N protein.

*Impatiens necrotic spot tospovirus* (INSV) belongs to serogroup III. Based on the N protein gene sequence, the differences between serogroups I and III are greater, while serogroup II is somewhere in between (De Avila *et al.*, 1993). Protein G2 of these first three serogroups shows considerable homology, whereas protein G1 from serogroup I shows important differences in size when compared with those of serogroups II and III (Feldhoff *et al.*, 1997).

Serogroup IV comprises those species whose N protein is serologically different from serogroups I, II and III: *Watermelon silver mottle tospovirus* (WSMoV), *Groundnut bud necrosis tospovirus* (GBNV) and *Watermelon bud necrosis tospovirus* (WBNV).

*Groundnut yellow spot tospovirus* (GYSV), also known as *Peanut yellow spot tospovirus* (PYSV), belongs to the recently created serogroup V. This group was proposed because of its low serological relationships and its low nucleotide sequence homology with other serogroups (Satyanarayana *et al.*, 1996).

Lastly, *Iris yellow spot tospovirus* (IYSV) has been proposed as a new species that would form a new serogroup. The S protein of IYSV shows low sequence identity with other species, being more closely related (44%) to the species in serogroup IV (Cortes *et al.*, 1998).

Other viruses have also been included in this genus although they have not been assigned to any of the existing serogroups. Such viruses are *Chrysanthemum stem necrosis tospovirus* (CSNV), *Zucchini lethal chlorosis tospovirus* (ZLCV) and *Melon spotted wilt tospovirus* (MSWV). A final group consists of seven isolates without a species entity or which have been very recently reported and are candidates to be assigned to the *Tospovirus* genus (Table 1).

### Genetic resistance

Among the viruses currently included in the genus *Tospovirus*, four species, TSWV, TCSV, GRSV

Table 1. Classification of the genus *Tospovirus* in serogroups.

Virus	Crops affected	Countries where it is found	Reference
<b>Serogroup I</b>			
<i>Tomato spotted wilt virus</i> TSWV	Tomato, pepper, lettuce, celery, tobacco, potato, peanut, pea	All over the world except in cold climates	Murphy <i>et al.</i> , 1995
<b>Serogroup II</b>			
<i>Tomato chlorotic spot virus</i> TCSV	Tomato, pepper, escarole	Brazil, Argentina	De Avila <i>et al.</i> , 1993 Boiteux <i>et al.</i> , 1993 Colariccio <i>et al.</i> , 2001a Williams <i>et al.</i> , 2001
<i>Groundnut ringspot virus</i> GRSV	Peanut, tomato, pepper, lettuce	Argentina, Brazil South Africa, USA	De Avila <i>et al.</i> , 1993 Colariccio <i>et al.</i> , 2001b Chaves <i>et al.</i> , 2001 Lima <i>et al.</i> , 2000
<b>Serogroup III</b>			
<i>Impatiens necrotic spot virus</i> INSV	Impatiens, begonia, dahlia, gloxinia, tomato, cucumber, pepper, lettuce	Europe, USA, Israel	Law and Moyer, 1990 Gera <i>et al.</i> , 1999 Sialer and Gallitelli, 2000
<b>Serogroup IV</b>			
<i>Watermelon silver mottle virus</i> WSMoV	Watermelon and other cucurbits	Japan, Taiwan	Yeh and Chang, 1995
<i>Groundnut bud necrosis virus</i> GBNV	Groundnut	India, South-eastern Asia	Reddy <i>et al.</i> , 1992
<i>Watermelon bud necrosis virus</i> WBNV	Watermelon	India	Jain <i>et al.</i> , 1998
<b>Serogroup V</b>			
<i>Groundnut yellow spot virus</i> GYSV PYSV	Peanut	India, Thailand	Satyanarayana <i>et al.</i> , 1996
<b>Not classified</b>			
<i>Iris yellow spot virus</i> IYSV	Iris, onion	Brazil, Israel, Netherlands	Pozzer <i>et al.</i> , 1999 Cortes <i>et al.</i> , 1998 Gera <i>et al.</i> , 1998
<i>Zucchini lethal chlorosis virus</i> ZLCV	Courgette and cucumber	Brazil	Resende <i>et al.</i> , 1996 Bezerra <i>et al.</i> , 1999
<i>Melon spotted wilt virus</i> MSWV	Melon	Japan	Kato <i>et al.</i> , 2000
<i>Chrysanthemum stem necrosis virus</i> CSNV	Chrysanthemum and tomato	Brazil, Netherlands	Resende <i>et al.</i> , 1996 Bezerra <i>et al.</i> , 1999
<b>Pending inclusion</b>			
<i>Peanut chlorotic fan-spot virus</i> PCFV	Peanut	Taiwan	Prins and Kormelink, 2000
<i>Tospovirus</i> (onion)	Onion	USA	Hall <i>et al.</i> , 1993
<i>Tospovirus</i> ( <i>Verbesina alternifolia</i> )	<i>Verbesina alternifolia</i>	USA	Prins and Kormelink, 2000
Br-10 (onion)	Onion	Brazil	Resende <i>et al.</i> , 1996
Tospo-PD2	Peanut	Taiwan	Yeh <i>et al.</i> , 1996
TSWV-W	Watermelon	India	Singh and Krishnareddy, 1995
<i>Tospovirus</i> ( <i>Physalis minima</i> )	<i>Physalis minima</i>	Thailand	Cortez <i>et al.</i> , 2001

and CSNV cause serious losses to tomato crops in different parts of the world, and INSV has recently been reported to infect tomatoes as well.

All these viruses are readily transmitted by thrips. Controlling the vectors is difficult because physical, chemical and biological methods of control are not very effective. They can reduce thrips populations to economically viable direct damage levels, but not enough to prevent the spread of the disease. Consequently, the development of genetic resistance has become the best management strategy for these diseases in both the medium and the long term, especially in the case of TSWV (Roselló *et al.*, 1996).

#### **Tomato spotted wilt tospovirus (TSWV)**

TSWV is still causing serious losses in several continents: in North America (USA, particularly the State of Georgia), South America (Argentina and Brazil), Africa (South Africa) and Europe (Mediterranean countries). In this last area, the disease causes important losses in Turkey (Fidan, 1993), Spain (Roselló *et al.*, 1996), Italy (Siviero and Motton, 1997), France (Marchoux *et al.*, 2000) and Greece (Chatzivassiliou *et al.*, 2000).

The severity of TSWV symptoms, the difficulty of controlling its vector *F. occidentalis*, the wide range of reservoir weeds, and the extensive distribution of the virus worldwide have made the identification of sources of resistance to TSWV one of the main objectives of tomato breeders during the last decade.

In germplasm screenings for resistance to TSWV, the response of a genotype when inoculated depends largely on the isolate used. The actual method of inoculation also has a decisive influence on the response. Mechanical inoculation is the most commonly used method in the search for resistance, with natural field inoculation and thrips-mediated inoculation being less common. In inoculation programmes serological analysis is usually carried out to detect the virus, combined with observation of typical symptoms (Best, 1968; Paterson *et al.*, 1989).

Natural field inoculation provides useful information about the agronomical value of the assayed material under specific climatic conditions. It is the only method that detects genotypes with an agronomical value. Nevertheless, it entails the considerable disadvantages of depending on the severity

of the infection in each assay, and of not being repeatable.

Mechanical inoculation allows testing a high number of accessions in a fast and simple way. With this method resistance to the virus is detected, but a vector-host interaction does not take place. Thus, material that might otherwise be of good field performance may also be considered susceptible (Lacasa *et al.*, 1994; Diez *et al.*, 1995).

The artificial TSWV inoculation method that most closely resembles natural infection is transmission by thrips. However, this method does not reveal whether genotypes found to be resistant are resistant to the virus, resistant to transmission by the vector, or both. Furthermore, it can exclude mechanisms of agronomical utility such as the 'non-preference' of a vector for another specific host. The main inconvenience of this method is the need to 'manage' the insects; this is an important limitation to its use (Roselló *et al.*, 1999).

Using one of these methods does not exclude using the other two, as they provide complementary information that may be useful to develop material with different resistance mechanisms, and therefore with enhanced durability.

In a search for sources of resistance, Finlay (1953) described 5 resistant genes (two dominant and three recessive) in different *Lycopersicon esculentum* Mill. cultivars. These genes, known as *Swa1*, *Swb1*, *sw2*, *sw3* and *sw4*, turned out to provide isolate-specific resistance which was soon overcome (Stevens *et al.*, 1992; Boiteux and Giordano, 1993). In the *L. esculentum* line 'Rey de los tempranos' Maluf *et al.* (1991) also identified considerable levels of resistance, controlled by a recessive allele. The scarce success obtained with this species meant that the search for new resistance sources was widened to include other *Lycopersicon* species. In many *L. pimpinellifolium* (Jusl.) Mill. accessions variable percentages of resistant plants have been found. These accessions were used to develop the cultivars Pearl Harbor, with resistance only to certain isolates, and BPX-198-03D, which was tolerant under field conditions in Brazil (Maluf *et al.*, 1991). In this latter species Paterson *et al.* (1989) also found segregation for resistance in several accessions.

Other materials, derived from the resistant accessions *Lycopersicon hirsutum* Humb. & Bonpl. PI-127826 and *L. hirsutum* var. *glabratum* PI-

134417 are tolerant to TSWV under natural infection conditions (Maluf *et al.*, 1991). Kumar and Irulappan (1992) obtained resistance in hybrids derived from the accessions PI-127826 and *L. hirsutum* var. *glabratum* LA-1223, controlled by a few recessive genes. Accession UPV16910 from *L. hirsutum* has also been reported to display high levels of resistance when mechanically inoculated (Soler *et al.*, 2000). Within this species resistance to transmission by the vector has also been found, as *L. hirsutum* has been reported to be antixenic to *F. occidentalis* (Krishna-Kumar *et al.*, 1995).

*Lycopersicon chilense* Dun. accession LA-2931 was classified as immune to mechanical and thrips-mediated inoculation, although the behaviour of this resistance in the genetic background of *L. esculentum* is still unclear (Krishna-Kumar *et al.*, 1993). The breeding line Y118, derived from *L. chilense* and accession LA-1938 from the same species have shown field resistance (Canady *et al.*, 2001). Stevens *et al.* (1994) found a variable number of resistant plants in 8 accessions of this species when they were inoculated with three different isolates.

*Lycopersicon peruvianum* (L.) Mill. appears to be the best TSWV resistance source, with many accessions of this species having been reported as resistant or immune. Paterson *et al.* (1989) identified 8 accessions of *L. peruvianum* with field resistance to TSWV. The gene *Sw5*, identified in this species by Stevens *et al.* (1992), conferred dominant resistance on TSWV, and was not isolate-dependent. This gene also confers protection against other tospoviruses such as TCSV and GRSV (Boiteux and Giordano, 1993). Stevens *et al.* (1994) reported 8 accessions of *L. peruvianum* with a high proportion of plants resistant to 3 isolates of TSWV. Roselló *et al.* (1999) found high levels of resistance in six accessions of *L. peruvianum*: PI-126935, PI-126944, CIAPAN-16, CIAPAN-17 and PE-18, based on a hypersensitive response (Fig. 3). The resistance, which was found in a mechanical inoculation assay with two aggressive isolates, was maintained when the accessions were inoculated with viruliferous thrips.

Roselló *et al.* (1998) identified gene *Sw6* derived from *L. peruvianum*, which segregated independently from *Sw5*. Although this dominant resistance has incomplete penetration and a possible gene-dosage dependence, it can be accumulated with

other resistance genes in order to enhance the level and durability of the resistance.

The *Sw5* gene, which has been mapped near the telomere of the long arm of chromosome 9 (Stevens *et al.*, 1995), has been widely used in the development of tomato hybrid varieties. Its use has led to a considerable reduction in the financial losses caused by TSWV in the Mediterranean basin over the last few years. However, the resistance of these materials can be overcome in situations of high inoculum pressure (Díez *et al.*, 1995; Roselló *et al.*, 1997; 1998). Under these conditions, in Spain in some cultivars carrying the *Sw5* gene, 4% of plants showed symptoms of 'ring-spot' on their fruits, probably caused by feeding of viruliferous thrips, though the plants remained apparently healthy. In the same work, the authors also reported an unusually high number of systemically infected plants that carried the *Sw5* gene (Aramburu *et al.*, 2000).

The resistance conferred by the *Sw5* gene, which relies on the development of a hypersensitive response (Fig. 2) (Soler *et al.*, 1999a), no longer constitutes a durable resistance system against the disease. Cho *et al.* (1996), in Hawaii reported that certain TSWV isolates overcame this resistance. In 1999, new isolates that overcame this resistance were selected from natural populations of TSWV (Latham and Jones, 1998). For some time, it was feared that resistance-breaking isolates might appear in the Mediterranean basin. This fear has become a reality with the recent detection of a TSWV isolate infecting tomato plants carrying the *Sw5* gene in Spain (Aramburu *et al.*, 2002). The appearance of these isolates complicates the epidemiological situation of the disease, especially in areas where *Sw5* resistance was widely used.

Various researchers are of the opinion that TSWV is found in nature as a heterogeneous population of isolates (Best, 1968; Sherwood *et al.*, 1989; de Avila *et al.*, 1990, 1993). The heterogeneity of a natural virus population provides an important genetic potential for adaptation to a wide range of hosts (Qiu *et al.*, 1998). This heterogeneity, along with the adaptability of the virus to different environments, is characteristic of TSWV. Qiu and Moyer (1999) suggested that genomic reassortment was one of the mechanisms that could explain this characteristic. Genomic reassortment consists in genetic information being exchanged

between RNA fragments of TSWV from different isolates. This phenomenon can even lead to the development of new isolates capable of overcoming resistance to the virus, when the original isolates are not capable of doing so (Qiu and Moyer, 1999).

Resistance-breaking isolates can appear *in situ* through mutation and selection, or they can be introduced from other countries where they already exist. In the first case, the multiplication or prolonged contact of TSWV isolates with resistance-gene carrier plants can lead to the development of new, more virulent isolates that overcome the resistance (Moury *et al.*, 1997; Qiu and Moyer, 1999). In this sense, the widespread use of varieties with the *Sw5* resistance gene gives rise to very high selection pressures. Therefore, considering the relative ease with which new TSWV isolates are generated, as well as the monogenic character of this resistance, the probability of the appearance of isolates that overcome this resistance in the field is relatively high, as has been demonstrated.

On the other hand, measures should be considered to avoid the introduction of foreign TSWV isolates capable of overcoming the resistance of the *Sw5* gene, as well as material infected with the other tospoviruses capable of causing damage to tomato, and which are not at present found in the Mediterranean basin.

The situation created by the new more virulent TSWV isolates imposes the necessity to search continuously for new sources of resistance to this virus, as well as taking better advantage of existing sources.

The characterisation of these resistance mechanisms is indispensable prior to their use, so that different mechanisms can be joined in the same material. Several studies have shown that the resistance conferred by the *Sw6* gene from *L. peruvianum* responds to resistance mechanisms that are different from those conferred by the *Sw5* gene (Soler *et al.*, 1999a) and therefore the association of these two genes would prove promising to improve resistance to TSWV.

An alternative to the hypersensitive response is found in tomato *platense* varieties (Von der Pahlen, 1970; Soler *et al.*, 1999b) and in certain accessions of *L. hirsutum* (Soler *et al.*, 1998a, b), which show apparent recovery following inoculation with TSWV. Apparent recovery occurs when the plant

generates new tissue that is virus-free, after having suffered systemic infection (Fig. 4). This type of resistance has been used in Argentina fairly successfully for decades (Von der Pahlen, 1970). The results obtained by Canady *et al.* (2001) with the Y118 line seem to indicate that the apparent recovery phenomenon occurs also in *L. chilense*, as the plants became initially infected with high titres of the virus but later recovered showing no detectable levels of TSWV. The joint use of this type of resistance, together with that conferred by other genes such as *Sw5* and *Sw6* or others to be identified in the future, would allow us to obtain higher and more stable resistance levels.

#### Tomato chlorotic spot tospovirus (TCSV)

This virus was first reported in Brazil, infecting tomato plants, by De Ávila *et al.* (1990). It spread quickly through the whole country causing severe damage to tomato crops. It also affected other crops, but since the middle of the 90's its seriousness has dropped off (Bezerra *et al.*, 1999). In Argentina it has been found in the states of Mendoza and Buenos Aires, where it has caused serious losses (Gracia *et al.*, 1999).

Cambraia *et al.* (2000) identified two tomato breeding lines, TOM-556 and BPX320F-7902, tolerant and immune to TCSV respectively. These lines showed the same response when inoculated with a wide collection of TSWV isolates. Lourenço *et al.* (2001) identified several tomato cultivars, six *L. peruvianum* accessions, two lines derived from *L. peruvianum* and one *L. hirsutum* accession with different resistance levels to TCSV in field inoculation assays. The lowest infection levels detected in the *L. peruvianum* accessions confirmed the occurrence of good resistance sources to tospoviruses in this species. The *Sw5* gene has been related to the resistance of tomato breeding lines to this virus (Boiteux and Giordano, 1993).

#### Groundnut ring spot tospovirus (GRSV)

GRSV was first found infecting *Arachis hypogea* L. plants in South Africa. Since 1995 it has spread throughout the northeast of Brazil, and now constitutes the most important *Tospovirus* in this area, seriously affecting tomato crops (Resende *et al.*, 1996; Bezerra *et al.*, 1999). It has also been reported to cause important losses to tomato crops in Argentina (Dewey, 1995; Williams *et al.*, 2001).

The spread of the disease in that country has been associated with the diffusion of the vector *Frankliniella shultzei* Trybom (Williams *et al.*, 2001). Nevertheless, its importance remains lower than in Brazil. It has also been identified as the most widespread *tospovirus* in the San Francisco Valley in the USA (Lima *et al.*, 2000).

GRSV is not recognised by the International

Committee on Taxonomy of Viruses (ICTV). This virus has similarities with other *tospovirus* species and is considered to be a candidate species to become part of it. In the meantime, its classification within the TCSV serogroup system of the genus is allowed.

The *Sw5* gene has been shown to be effective in the control of this disease. It should be added that



Fig. 1. Tomato plant infected with TSWV showing necrosis in the main stem.



Fig. 2. Hypersensitive lesions in leaves mechanically inoculated with TSWV from a tomato plant with the *Sw5* gene.



Fig. 3. Local hypersensitive lesions in leaves of accession PI-126944 from *Lycopersicon peruvianum* resistant to TSWV.



Fig. 4. *Lycopersicon hirsutum* plant with apparent recovery. The arrow indicates the transition zone between the infected and the recuperated parts.

some breeding lines have been reported to be resistant to GRSV (Lourençao *et al.*, 2001).

#### **Chrysanthemum stem necrosis tospovirus (CSNV)**

In 1994 typical symptoms of a disease with viral aetiology were observed in chrysanthemum plants in the State of Sao Paulo (Brazil). The symptoms consisted of necrotic spots with chlorotic rings, that might worsen with necrosis on the stems and floral peduncles. The infectious agent was characterised as a *tospovirus* isolate known as Ch-1 (Nagata *et al.*, 1994). From then on this virus has been detected in different parts of Brazil where it has seriously infected tomato fields. Resende *et al.*, (1996) suggest the classification of this virus as a new species (CNSV) of *Tospovirus* based on serological analysis and nucleotide sequence comparisons with other viral species of this genus. Due to its wide range of hosts it is expected that this new virus will acquire the same or greater importance in Brazil than that of TSWV, TCSV and GRSV (Bezerra *et al.*, 1999). There are no known sources of resistance to CNSV.

#### **Impatiens necrotic spot tospovirus (INSV)**

This was the first *tospovirus* species to be recognised as different from TSWV (Law and Moyer, 1990). It has a range of hosts that mostly include ornamental plants such as impatiens, gerbera, alstroemeria and cineraria. It was first reported in the USA in 1987 (Law and Moyer, 1990) and in Europe (Holland) in 1990 (De Ávila, 1992). Afterwards it became the most important *tospovirus* infecting ornamental crops in the USA (German *et al.*, 1992), where is currently considered to be one of the main pathogens that affect flower production (Daughtrey *et al.*, 1997).

INSV has recently been detected infecting tomato plants, this is a further step in the progressive adaptation of this virus to vegetable crops (Sialer and Gallitelli, 2000). The same authors reported that INSV also infected cucumber, chicory, lettuce and pepper plants. There is no genetic resistance available for tomato improvement, as INSV had not been previously reported to infect this species.

#### **The role of genetic markers in the development of genetic resistance**

In order to develop commercial tomato material that accumulates different types of resistance

to TSWV, it is necessary to use biotechnological techniques such as molecular markers associated with each of the described resistance genes. With these markers it is possible to select plants that accumulate several resistance genes, as well as to speed up breeding programmes, with the financial advantages associated.

Chague *et al.* (1996) identified three markers tightly linked to the *Sw5* locus (within a distance of 10.5 cM), one of them in repulsion phase. One of the RAPD markers was used to develop a SCAR marker, enhancing reproducibility. The linkage of the marker was checked with 13 F3 families and 8 BC2 populations segregating for TSWV resistance. These markers are presently being used in marker-assisted plant breeding.

Stevens *et al.* (1996) found a RAPD primer (UBC421) linked to the *Sw5* locus at a shorter distance (1cM). The codominant SCAR developed from this marker (SCAR421) showed two bands, of 500 bp and 440 bp, for the homozygous resistant genotypes, and two, of 550 bp and 400 bp, for the homozygous susceptible hosts. All three bands are present for heterozygous genotypes (Masuelli *et al.*, 2000).

Masuelli *et al.* (2000) optimized the use of the SCAR421 marker in a multiplex PCR reaction that enabled simultaneous detection at the same time of the nematode resistance *Mi* gene and the *Sw5*.

#### **Genetic resistance introduced by genetic engineering**

In TSWV the first attempts to achieve resistance through genetic engineering were based on the introduction of the *N* gene that codifies the nucleocapsid protein in tobacco. The resistance obtained was detected at the protein level by Mackenzie and Ellis (1992), at RNA level through post-transcriptional silencing by De Haan *et al.* (1992) and at both protein and at RNA levels by Pang *et al.* (1993).

In tomato, Kim *et al.* (1994) introduced the *N* gene obtaining variable levels of resistance in transformed plants when mechanically inoculated in the greenhouse. Ultzen *et al.* (1994) and Gonçalves *et al.* (1995) also achieved resistance in tomato transgenic plants expressing the *N* gene.

The spectrum of resistance was widened by combining in the same material nucleocapsid protein genes from different viruses, obtaining resistance



to TSWV, TCSV and GRSV in transgenic tobacco plants (Prins *et al.*, 1995). Resistance to viruses from different genera has been obtained by combining the *N* gene from TSWV with the coat protein gene from *Cucumber mosaic cucumovirus* (CMV) (Gonsalves *et al.*, 1995). The combination of the full length coat protein of *Turnip mosaic potyvirus* with a segment of the *N* gene from TSWV in a single chimeric transgene resulted in resistance in transgenic *Nicotiana benthamiana* plants (Jan *et al.*, 2000).

Other sequences besides the *N* gene have also been used. Prins (1997) transformed tobacco with sequences based on different parts of the TSWV genome, although only the *N* gene and the *NSm* gene (movement protein) led to resistance. Although higher levels of NSm protein produced aberrations in the growth and appearance of transformed plants, the use of untranslatable or anti-sense sequences based on the same gene in tobacco resulted in high levels of resistance without side effects, as resistance took place at the RNA level (Prins *et al.*, 1997).

Another strategy consists in the expression of defective interfering (DI) RNAs. With TSWV, DI RNAs are originated from L RNA (encoding the viral polymerase) with large internal deletions that interfere with the replication of the full-length viral genome, leading to symptom attenuation (Nagata *et al.*, 2000). This strategy is successfully applied by supplying the DI cDNA sequence with various types of ribozymes, so that the molecule is tailored, resulting in accurately copied DI molecules after autocatalytic cleavage. Expression of these molecules in transgenic plants has led to increased tolerance to the virus due to coreplication of the transgenic DI copies (Cardol *et al.*, 2002).

The use of human single-chain antibodies in plants has also proved to be a successful strategy in the control of TSWV. Transformed *N. benthamiana* plants containing the single-chain variable fragments (scFv) N3 and N97 that translationally expressed the scFv transgene to high levels (reaching quantities between 0.5 and 0.8% of total soluble protein) were fully immune to TSWV (Prins *et al.*, 2000).

The combination of host resistance (*Sw5*) with the *N* gene has achieved broad resistance to various tospoviruses: to a Brazilian isolate of GRSV (*Sw5*), to a Brazilian TSWV isolate (*Sw5* and trans-

genic *N* gene) and to a TSWV isolate from Hawaii (transgenic *N* gene) (Gubba *et al.*, 2002),

Genetic engineering can also be used to introduce 'natural' host resistance. With this strategy there is no need for long backcrossing programmes, and the genetic background of breeding lines can be preserved whilst introducing the resistance character. It can also widen the spectrum of variation available. At the moment, the *Sw5* resistance gene from tomato has been successfully introduced into tobacco, resulting in resistance (Spasova *et al.*, 2001).

#### Outlook for the future

Although several sources of resistance to TSWV in different *Lycopersicon* species have been found, most tomato breeding programmes for resistance to TSWV have been based on a single gene: *Sw5* from *L. peruvianum*. The high selection pressure associated with this gene has probably contributed to the emergence of resistance-breaking isolates throughout the world. It is now essential to continue the search for new sources of resistance, as well as to confirm the genetic control of the resistance already identified in several *Lycopersicon* accessions. The development of genetic markers linked to alternative resistance genes would enable these genes to be pyramided in the same material, thus improving resistance durability.

Regarding other *tospovirus* species that cause serious losses in tomato crops (TCSV, GRSV and CSNV), the *Sw5* gene effectively controls the first two. How tomato with the *Sw6* gene reacts against these viruses is yet unclear. It is necessary to widen our knowledge of the genetic control of identified TCSV and GRSV resistances so that we can exploit them. In both cases the search for sources of resistance to these new tomato viruses must be continued.

Although resistance derived from the pathogen has been found to be an effective strategy for the management of viral diseases, TSWV has developed isolates that overcome this type of resistance (Qiu and Moyer, 1999). This clearly shows the need to continue conventional breeding methods in conjunction with the new biotechnologies. Likewise, molecular markers, agro-inoculation systems, etc., can speed up conventional improvement programmes. It is our opinion that obtaining transgenic plants resistant to TSWV is a complement to conventional plant breeding and not a substitute for it.

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