Agroinoculation of Tomato Yellow Leaf Curl Virus (TYLCV) Overcomes the Virus Resistance of Wild Lycopersicon Species

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With 4 figures

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Abstract

Accessions of the wild tomato species Lycopersicon chilense LA 1969 and L. hirsutum LA 1777 which are resistant to tomato yellow leaf curl virus (TYLCV) in field- and in whitefly-mediated transmission tests were agroinoculated with a tandem repeat of the TYLCV genome. Large amounts of viral DNA started to accumulate in the agroinoculated L. chilense and L. hirsutum plants about 10 days after the agroinoculation. Yellowing and narrowing of the upper leaves were observed in the L. chilense plants but no curling as in susceptible L. esculentum cultivars. The agroinoculated L. hirsutum plants showed typical yellowing and curling of young leaves. These findings indicate that TYLCV introduced by means of agroinoculation leads to the breakdown of natural resistance mechanisms which prevent the replication, spread and expression of symptoms in resistant tomato genotypes.

Key words: Lycopersicon spec. — wild tomato species — agroinoculation — geminivirus — virus resistance — whitefly

The tomato yellow leaf curl virus (TYLCV) causes severe damage to tomato crops in tropical and subtropical regions (Cohen and Har PAZ 1964; CZOSNEK et al. 1990). TYLCV is a whitefly-transmitted geminivirus with a monopartite genome (KHAYR-POUR et al. 1991, NAVOT et al. 1991). Breeding tomatoes resistant to TYLCV is based on introducing resistance genes from accessions of wild tomato species (Lycopersicon cheesmanii, L. chilense, L. hirsutum, L. peruvianum, L. pimpinellifolium) into the domesticated tomato (L. esculentum) (see references in ZAKAY et al. 1991). Progress in breeding for TYLCV resistance is slow due to the numerous backcrosses required to introduce resistance genes from wild Lycopersicon species into the cultivated tomato, and because of the lack of an accurate and easy selection system. One of the problems is to ensure 100% success with the inoculation of the plants tested, either in field tests, in whitefly-mediated inoculation in the laboratory or in grafting on infected plants.

Agroinfection offers an alternative method for the introduction of infectious viral nucleic acids into plants (GRIMSLEY et al. 1986). In this method a dimeric copy of a viral genome or of its cDNA is cloned in the T DNA of Agrobacterium tumefaciens Ti plasmid which is delivered by injection to plants. As a result, a genome-size copy of viral DNA is released (STENGER et al. 1991), replicates, is encapsidated, systemically spreads and expresses disease symptoms. Among other applications, this method showed that Agrobacterium can transfer viral DNA to plants which are not usually susceptible to Agrobacterium (e.g.
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maize, wheat) and produce viral disease symptoms (GRIMSMY et al. 1987, HAYES et al. 1988).

Here we demonstrate that delivery of cloned TYLCV genomic DNA by agroinoculation overcomes natural resistance of some wild tomato species in the TYLCV disease. Therefore the usefulness of agroinoculation as a virus delivery system in breeding programmes for TYLCV resistance is questionable.

Materials and Methods

TYLCV was maintained in tomato plants (Lycopersicon esculentum, cv. FA 144) by whitefly (Bemisia tabaci) — mediated inoculation.

The tomato cultivars, L. esculentum cv. ‘Monique’ and cv. ‘Mecline’, and the wild tomato species L. chilense LA 1969 and L. hirsutum LA 1777 were used for agroinoculation assays. A tandem repeat of the cloned genome of TYLCV isolated in Israel was cloned into the pCGN 1547 binary vector (Calgene) and introduced into the disarmed Agrobacterium tumefaciens LBA 4404 (At::pTY4, NAVOT et al. 1991). The bacteria were injected into tomato plants at their 4 to 6 leaf stage as described before (NAVOT et al. 1991). Agroinoculated plants were kept in an insect-proof growth chamber, using water treated with hypochlorite.

The presence of TYLCV DNA was detected by molecular hybridization. Total DNA extracts prepared from leaves were submitted to gel electrophoresis, blotted, hybridized with a full-length TYLCV DNA cloned probe and subjected to autoradiography (CZOSNEK et al. 1988).

Results

Accessions of the wild tomato species L. chilense LA 1969 and L. hirsutum LA 1777 showed high levels of resistance to TYLCV in fields infested with viruliferous whiteflies and in repeated whitefly-mediated transmission tests in the laboratories. Viral DNA was rarely detectable and no symptom appeared when all the cultivated tomato (L. esculentum) varieties were totally infected (ZAKAY et al. 1991).

Eight L. chilense and eight L. hirsutum plants were agroinoculated with the TYLCV dimer-containing Agrobacterium At::pTY4. The appearance of disease symptoms and of viral DNA was monitored following inoculation of the infectious bacteria. Ten days after agroinoculation, large amounts of viral DNA were detected in six of the eight L. chilense plants following hybridization of plant DNA blots with a virus-specific probe (Fig. 1). Fifteen days thereafter, viral DNA was detected in all agroinoculated plants. The single-stranded TYLCV genomic DNA and its double-stranded replicative form present in L. esculentum infected plants were detected in the L. chilense agroinoculated plants. Therefore a full-length copy of the TYLCV genome, released from the Agrobacterium Ti plasmid after agroinoculation of the L. chilense plants, has replicated and systemically spread in the plants. Residual high molecular weight Agrobacterium At::pTY4 DNA was undetectable using either the viral probe (Fig. 1) or a neomycin phosphotransferase gene probe (not
shown). The highest concentration of viral DNA was in the flowers and in the young leaves as demonstrated for the cultivated tomato (BER et al. 1991). Yellowing and narrowing of upper leaves was observed but no curling as in susceptible L. esculentum cultivars (Fig. 2).

All the L. hirsutum agroinoculated plants showed high concentrations of viral DNA about 10 days post inoculation (Fig. 3). The TYLCV-related DNA forms present in agroinoculated L. esculentum and L. chilense plants were also detected in the agroinoculated L. hirsutum plants. No residual Agrobacterium At::pTY4 DNA was detected. Typical yellowing and curling of young leaves appeared about 2 weeks thereafter (Fig. 4).

These results indicate that TYLCV introduced by means of agroinoculation into resistant plants leads to the breakdown of natural resistance mechanisms which prevent the replication, spread and expression of symptoms in field-resistant wild tomato genotypes.

Discussion

Certain accessions of the wild Lycopersicon species, such as L. chilense LA 1969 and L. hirsutum LA 1777, are resistant to the TYLCV disease in field conditions. In these immune Lycopersicon species, viral DNA is undetectable and no disease symptoms develop although viruliferous whiteflies feed on these plants (ZAKAY et al. 1991). This natural resistance does not collapse even when the number of inoculative whiteflies in tomato fields is extremely high as in the Jordan Valley, Israel, where 2,000 to 20,000 insects land weekly on each square meter, thus ensuring 100% TYLCV-infected L. esculentum plants within one week (COHEN 1990).
The genetic basis for the resistance exhibited by wild *Lycopersicon* species appears to range from a single incomplete dominant gene in the case of *L. pimpinellifolium* (KASRAWI 1988, PILOWSKI and COHEN 1974) to a polygenic pattern, recessive in *L. cheesmanii* (HASAN et al. 1984) and *L. peruvianum* (PILOWSKI and COHEN 1974), but dominant in *L. hirsutum* (HASAN et al. 1984). The target of the resistance gene(s) is unknown.

It is generally accepted (see reviews by HULL 1989, and MAULE 1991) that the establishment of viral infection in a susceptible host plant is dependent upon the virus spreading to the plant organs. Most plant viruses enter the initially infected cells by a biological vector or by mechanical abrasion. From the initially infected cell the virus spreads to the adjacent cells crossing cell walls via plasmodesmata (short distance spread) until it encounters the vascular tissues through which it spreads to the plant organs (long distance spread). Viral gene product(s) seem to be involved in the modification of plant subcellular structures, facilitating virus spread. In some situations the virus may replicate in individual cells (subliminal infection) but is unable to move to adjacent cells. In resistant plants, the resistance gene(s) product(s) may bring about functional changes that interfere with virus inoculation by the vector, uncoating, replication or translocation, or structural changes inducing physical barriers to virus spread (ATABEKOV and DOROKHOV 1984, FRAKER 1990).

*Agrobacterium tumefaciens* is not a natural virus vector but it is widely used to deliver viruses to plants. Following agroinoculation, large amounts of viral DNA cloned into the *Agrobacterium* Ti plasmid are introduced directly into the plant vascular system and spread quickly. A genomic size dsDNA copy of the viral DNA (replicating form, RF) arises after the transport of the T-DNA to the nucleus of the target cells, independently of T-DNA integration in the plant cell genome (GRIMSLEY et al. 1986). Viral genomes are synthesized on the RF template, are encapsidated, spread throughout the plants and can be acquired by
insects (NAVOT et al. 1991). As a result of agroinoculation, the early steps of the natural virus infection — namely insect-plant interaction, virus uncoating and synthesis of the RF — are bypassed.

In the wild Lycopersicon species immune to TYLCV, no virus accumulation could be detected in the inoculated leaf upon whitefly-mediated inoculation in the field and with leaf cages, even when as much as fifty viruliferous whiteflies per cage were used. Therefore there must be true inhibition of virus replication. The resistant gene(s) probably interfere with an early stage of infection, immediately after the virus has been inoculated by the insect vector and before viral DNA starts to accumulate in detectable amounts in the inoculated cells. Agrobacterium-mediated inoculation of TYLCV bypasses the early steps of virus infection mentioned above which may be the target sites of the host plant resistant gene(s). Once the virus finds its way into the plant vascular system, the host resistant gene product(s) become inoperative. It is also possible thatcircumvention of resistance is due to a virus dosage effect since agroinoculation involves the introduction of viral DNA amounts for greater than those introduced by insect vectors. The inoculation of Agrobacterium per se seems not to be the cause of the resistance breakdown. Agrobacterium can transfer viral DNA to plants which are usually not susceptible to Agrobacterium (e.g. maize, wheat) and establish viral infection (GRIMSLEY et al. 1987, HAYES et al. 1988). L. chilense and L. hirsutum are sensitive to Agrobacterium colonization; crown galls do form after inoculation of the Agrobacterium virulent strain C58 (unpublished observation). This sensitivity may facilitate the release of viral genomic DNA from the bacteria and its spread in the plant.

Agroinoculation cannot be a valuable test in breeding programmes for selecting TYLCV-resistant plants. As demonstrated here, accessions of L. chilense and L. hirsutum which are immune to TYLCV in natural whitefly-mediated inoculation tests can be infected by agroinoculation. L. chilense and L. hirsutum serve as a source of resistance genes in some breeding programmes for TYLCV resistance. These species are also resistant to other whitefly-transmitted geminiviruses affecting tomato, e.g. from Florida (SCOTT and SCHUSTER 1991) or from India (KALLOO and BANERJEE 1990). L. hirsutum has also been used in an attempt to introduce its ability to repel B. tabaci (BERLINGER and DAHAN 1987) into tomato cultivars.

Because delivery of TYLCV by agroinoculation overcomes some of the barriers which prevent the spread of the TYLCV disease, this technique needs to be used with great care.

Zusammenfassung
Agroinokulation des gelben Blattrollvirus' (TYLCV) der Tomaten überwindet die Virus-Resistenz von Lycopersicon-Wildarten


References
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