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Genetic control of the response to a '*Candidatus* Phytoplasma trifolii' strain by *Solanum peruvianum*

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Abstract

A report describing the strong resistance and tolerance of the accession PI128655 of *Solanum peruvianum* to two BLTVA strains (beet leafhopper transmitted virescence agent; '*Candidatus* Phytoplasma trifolii') was published almost twenty years ago. This work was revisited using another BLTVA strain and the PI128655 plants. No resistance or tolerance was observed. Instead the plants showed either one of two different sets of symptoms. A genetic control of the response to the phytoplasma isolated by the PI128655 plants was demonstrated using clones of host plants. However we obtained experimental evidence that the BLTVA isolate lacked homogeneity or stability, which could also explain the occurrence of two sets of symptoms. A possible hypothesis is that the PI128655 plants, by interfering with the competition between the 'components' of the BLTVA isolate, can alter the final composition of the phytoplasma population and therefore influence the induced symptoms.

Key words: BLTVA, '*Candidatus* Phytoplasma trifolii', *Solanum peruvianum*, mixed infection, plasmid, branched inflorescence.

Introduction

Although a promising strategy for controlling diseases caused by phytoplasma infection, host resistance is still rarely applied and remains poorly understood. So far, one of the best documented cases of host plant resistance is the interaction between the apple proliferation phytoplasma ('*Candidatus* Phytoplasma mali') and hosts derived from *Malus sieboldii* stocks (Seemüller *et al*., 2009). In the resistant rootstocks, the phytoplasma concentration was lower than in the susceptible rootstocks M9 and M11 and the phloem did not show sieve tube necrosis and starch depletion. The molecular basis for this resistance is still unknown.

A report describing the complete resistance or tolerance of the PI128655 accession of *Solanum peruvianum* to two BLTVA (beet leafhopper transmitted virescence agent; 16SrVI group; '*Ca.* P. trifolii') strains was published almost twenty years ago (Thomas and Hassan, 1992) with no follow-up since then. Because these experiments demonstrated a case of resistance or tolerance of a herbaceous species relatively close to cultivated tomato, we decided to revisit the interaction between *S. peruvianum* accession PI128655 and BLTVA and assess whether this pathosystem could constitute a good starting point to investigate plant-phytoplasma interactions.

Materials and methods

The BLTVA strain used in this study was kindly provided by Jim Crosslin (USDA-ARS, USA). It originates from a potato tuber produced by a plant naturally infected in a field in Moxee, Washington (USA) and maintained by *in vitro* culture (J. Crosslin, personal communication). For convenience, we transferred the phytoplasma strain from potato to tomato (cv Ailsa Craig) by grafting. It was then further propagated in tomato by successive grafting. The BLTVA strain used in this study was confirmed to belong to the 16SrVI group ('*Ca.* P. trifolii') (data not shown) but is presumably not the same as the ones used in the experiments of Thomas and Hassan (1992). The *S. peruvianum* accession PI128655 was retrieved from the USDA-ARS Plant Genetic Resource Unit (USA).

Plants were cultivated in an insect-proof greenhouse under natural light. Healthy plants approximately one-month old were inoculated by side-grafting two pieces of stem from infected plants. Except when explicitly mentioned, infected tomato cv Ailsa Craig was used as the source of inoculum.

Cuttings were obtained by taking a piece of stem composed of 2-3 nodes from healthy plants. The cuttings were dipped into a commercial growth hormones rooting powder (Bayer or Rhizopon) and rooted in soil.

Results

Inoculation of the PI128655 plants with one BLTVA strain induced symptoms in all plants tested. However, based on the symptoms produced, the infected plants ranged within two types. Type I plants displayed a growth vigor and a leaf pigmentation similar to the healthy plants, but produced branched inflorescences bearing a greatly increased number of flowers or buds. In these plants, flowers could occasionally show partly attached sepals or inflated buds reminiscent of big bud symptoms (Shaw *et al*., 1993), or some signs of floral reversion. When the symptoms were more pronounced, buds were replaced by meristematic, cauliflower-like structures, corresponding to a continuous branching and a perpetually delayed flower development. Type II plants showed a reduction in growth vigor, chlorosis at the margin of the leaflets and/or paleness of the leaves, and an absence of flowers due to an early growth arrest of the buds (buds remain smaller than 1 mm). A few type II plants produced cauliflower-like structures after

some time. Clones of individual PI128655 plants were produced by taking cuttings (3 clones for 6 different individuals representing the two plant types). Inoculation of the cuttings with BLTVA resulted in plants displaying symptoms of the same type as the mother plant, thus showing that the symptoms induced upon infection were reproducible, and demonstrating that the factor controlling the formation of symptoms of type I or type II is the genotype of the plant, rather than random or culture conditions.

The formation of two symptoms sets might also originate from the BLTVA strain being a mixture of two strains with different virulence. The homogeneity of the BLTVA strain was assessed as follows. In a first step, PI128655 plants of type I and II were inoculated with BLTVA using infected stem pieces from the same Ailsa Craig plant. Next, healthy type I plants grown from cuttings were inoculated with stem pieces taken either from a type I or a type II infected plant. The type I stem pieces induced mild symptoms on the type I stock, consisting of branched inflorescences with many flowers. The infected type II stem pieces induced clearly stronger symptoms with some plants showing chlorosis, small leaves and cauliflower-like structures without flowers. This experiment therefore suggests that the BLTVA isolate may not be homogenous or may somehow vary in its virulence, since a stable, homogeneous phytoplasma strain should induce the same symptoms when infecting the same type I test plants, whatever the origin of the infected stem piece (type I or type II).

Discussion

This study was initiated with the idea to reproduce the results obtained by Thomas and Hassam (1992) and to investigate the *S. peruvianum*/BLTVA resistance/tolerance mechanism at work. However, we observed no resistance/tolerance as described by Thomas and Hassam (1992), most likely because the BLTVA strain that we used was different. All tested plants showed some symptoms: two types of plants were recognized based on the symptoms developed after inoculation with BLTVA. The occurrence of two symptom sets may be explained by some heterogeneity or lack of stability of the BLTVA strain after passaging in different host plants, as shown by the stronger symptoms displayed by type I plants when inoculated with infected pieces from type II donors compared to type I donors. Virus detection tests should be performed in order to eliminate the possibility that the BLTVA strain also contains a virus inducing differential symptoms between type I and type II plants. Apart from a virus contamination, the heterogeneity may come from the existence of two "components" i.e. from two phytoplasma strains with distinct virulence properties occurring in a mixed infection, or alternatively phytoplasma cells with variable contents of plasmids involved in virulence, as the BLTVA phytoplasma do possess plasmids (Liefting *et al*., 2004).

Moreover, the inoculation of clones obtained by cuttings revealed that the plant response to the BLTVA infection is genetically controlled by the host PI128655 plants, resulting in two distinct sets of symptoms.

The variation of symptoms thus appears associated with both some phytoplasma heterogeneity and a genetic control by the host plant. Our preferred hypothesis is that the genotype of the host plant differentially influences the multiplication of the "components" of the BLTVA strain, rather than directly controlling the plant response. The accumulation of one "component" in the host would simultaneously (i) prevent the other "component" to accumulate further and (ii) define the general trend of symptoms by imposing its own pathogenic properties. Such effects of the competition between phytoplasma strains of different virulence were already suggested for instance in the case of mixed infection of apple trees by '*Ca.* P. mali' (Seemüller *et al*., 2010).

The hypotheses of the above scenario will have to be experimentally tested. The perspectives for this work will therefore focus on the search for the putative "components" of the BLTVA strain and their molecular characterization. Each "component" should then be quantified in type I and type II plants. Other perspectives include infection challenges of PI128655 plants with other '*Ca.* P. trifolii' strains or phytoplasma other than '*Ca.* P. trifolii'. Finally, the herbaceous nature of the host may allow a genetic analysis of the plant genes involved in the response to the phytoplasma infection.

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