The hypersensitivity resistance of european plum to the Plum pox virus and its potential impact on the epidemiology of the virus

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Abstract

Since the detection of the Plum pox virus (PPV) different strategies for Sharka containment were developed. One of the most important one is the breeding of resistant cultivars. Other than in *Prunus persica* and in *Prunus armeniaca*, in *Prunus domestica* a type of natural resistance was detected which seems to be able to prevent the spread of PPV both over long and short distances. Therefore, this type of Sharka resistance which is based on a hypersensitive response and has been stable for more than 20 years is suggested to have the most beneficial impact on the epidemiology of the virus compared to other mechanisms of resistance or tolerance.

Keywords: Sharka containment, *Prunus domestica*, plum breeding

Introduction

Already in 1935, Atanasoff stated that it should be possible to obtain cultivars of woody hosts of *Plum pox virus* (PPV) such as *Prunus domestica*, *P. persica* and *P. armeniaca* which are tolerant or resistant to the virus. In the meantime, great efforts were undertaken worldwide in developing strategies to reduce the impact of the Sharka disease on stone fruit production. Following the terminology introduced by Cooper and Jones (1983), both tolerance and resistance can reduce the economic effect of the virus disease.

During the second half of the 20th century, extensive breeding programs on European plum were established to obtain cultivars resistant and/or tolerant to the disease. The degree of resistance can only be determined by analyzing the virus titer in the plant tissue whereas the degree of tolerance can be defined by rating the visible symptoms and yield reduction of infected versus non-infected trees. Methods for determining the viral concentration have only been available since the development of ELISA tests but it took years after the first report (Dounin and Minoiu, 1968) until these and other methods (e. g. methods based on the detection of viral nucleotide sequence such as quantitative real time PCR (Olmos et al., 2006)) were made generally available. Therefore, during the first decades after detection of PPV, breeding strategies focused on developing tolerant varieties which only show mild symptoms but get infected by PPV. On the one hand, their use allowed the production of European plum to be maintained in eastern and central European countries which are strongly affected by PPV. On the other hand, their use contributed to a large extent to the spread of PPV over long distances via latently infected plant material which resulted in the current situation that PPV is present in all continents.

Breeding for resistance to PPV follows two strategies: Pathogen derived resistance by creation of genetically modified organisms and natural resistance obtained and transferred by cross pollination. Pathogen derived resistance uses viral genomic sequences which are introduced into the plum genome. Double-stranded (ds) replication structures of the viral genome, oder viral RNA transcribed by RDR6 to ds RNA triggers a defense mechanism called RNA interference which rersults in the sequence-specific degradation of viral RNA und thus in resistance for the respective virus. It was shown that the resistance of the transgenic 'C5'-hybrid is based on that phenomenon (Ravelonandro et al., 1993; Scorza et al., 1994; Ravelonandro et al., 1998; Hily et al., 2004b). Many working groups are trying to create transgenic PPV resistant *Prunus* genotypes based on the RNA-silencing mechanism. In recent years, PPV-specific Hairpin RNA constructs have been used for triggering the silencing mechanism in annual and woody PPV hosts (Pandolfini et al., 2003; Hily et al., 2007; Scorza, 2007; Tian et al., 2008). However, the degree of resistance of transgene plants varies. 'C5'-hybrid seems to withstand natural aphid inoculations in the field, but when inoculated with budsticks or in the case of natural infections of the non-transgenic rootstock, PPV symptoms could be detected in leaves, and the presence of the virus verified (Hily et al., 2004a; Malinowski et al., 2004; Malinowski et al., 2006). Thus, this kind of resistance allows to decrease the spread of PPV over short distances (by aphids) but not over long distances by latently infected plant material.

Natural Sharka resistance was found in *P. domestica* genotypes which show a hypersensitive response to PPV in the so-called 'K4'-hybrid (Kegler et al., 1983; Kegler et al., 1985; Bivol et al., 1987; Kegler et al., 1991; Kegler and Hartmann, 1998; Grüntzig et al., 2001; Kegler et al., 2001). In a twelve year long trial, trees of 'K4' remained free from PPV in the field (Kegler et al., 2002). However, the kind of hypersensitivity resistance found by these authors was shown to be overcome by a specific isolate called PPV-CG. Later, a second genetic source of hypersensitivity resistance was detected in the variety 'Jojo' (Hartmann, 1998). It was shown that this genotype elicited the hypersensitive response also against the PPV-GC isolate. Up to now, no isolate was found to break the hypersensitivity resistance of 'Jojo' and its hypersensitive relatives (Kegler et al., 2001; Neumüller et al., 2006). Its phenotypic and genetic background was described (Neumüller and Hartmann, 2008; Hartmann and Neumüller, 2009). This study deals with assessing the epidemiological impact of hypersensitivity resistance based on a long term experimental study in the field and on the synopsis of long term observations in plum orchards throughout Germany under natural inoculation conditions.

Material and methods

In 1989, eight one year old trees of *Prunus domestica* 'Jojo' grafted onto the rootstock 'GF 655/2' were planted into an experimental plot at Weil der Stadt, Germany. The trees were surrounded by PPV infected European plum trees of susceptible genotypes. Four of the trees were twice inoculated with a PPV-D isolate present in orchards in Southwest-Germany by chip budding (in 1990 and in 2003). Each time, four inoculation chips were grafted onto young shoots of the plants. The other four trees were not artificially inoculated. The natural infection pressure by aphids is high in the experimental orchard. Sensitive cultivars such as 'Common Prune' got infected not later than in the third year.

During the first five years annually, afterwards biannually, the presence of PPV was tested by ELISA (using 5B-IVIA antibodies) and RT-PCR (using the protocol given by Wetzel et al. (1991)) methods (EPPO, 2004) using leaves collected in June. Visual inspections for Sharka symptoms took place annually in June. In 2003, the 'GF 655/2'-rootstocks of the four 'Jojo' trees which were artificially inoculated with PPV in the second year were inoculated by chip budding by grafting suckers. Leaves of the rootstock suckers were taken in 2004 and 2006 for testing by ELISA and RT-PCR. In spring 2004, four budsticks of each 'Jojo' tree were taken for inoculation of indicator plants *Prunus tomentosa* which were observed for two years after grafting.

Results

No PPV symptoms could be detected on the leaves and fruits of 'Jojo' trees. No leaf sample tested positivel either with ELISA or RT-PCR-test during the whole period. In 80 % of the trees of PPV susceptible culitvars surrounding the 'Jojo' trees PPV could be detected. None of the *Prunus tomentosa* trees grafted with 'Jojo' budsticks developed PPV symptoms. PPV was detected in the susceptible rootstocks of all 'Jojo' trees growing at Weil der Stadt both in 2004 and 2006. This means that the rootstocks which were not artificially inoculated got infected by aphids landing on suckers.

During a period of 20 years, the 'Jojo' variety could neither naturally nor artificially be infected in the field by PPV. This is longer than today's plum orchards are cultivated. Trees with hypersensitivity resistance were the only trees which remained free from PPV. Quantitatively resistant genotypes such as 'Cacanska najbolja' got infected but displayed only a few symptoms.

Several hundred hectares of 'Jojo' orchards are grown in Europe the,oldest being 15 years old. In none of these orchards, could PPV infections of 'Jojo' trees be observed. Even if the rootstock got infected by PPV, the trees did not die off. The few tree losses which could be observed were usually due to *Pseudomonas* infections on the stem.

Discussion

The use of genotypes with a sufficiently high degree of hypersensitivity resistance against PPV such as 'Jojo' interrupts the cycle of PPV spread both over short and long distances. Hypersensitive plants do not get infected by aphid inoculation. As shown by Kegler (1994) and confirmed by Hartmann and Petruschke (2000), hypersensitive genotypes die off a few weeks after being grafted onto PPV infected rootstocks. Therefore, only trees free from PPV can leave the nursery. Up to now, the hypersensitivity resistance is the only resistance mechanism which is able to prevent the spread of PPV when the resistant genotype is grafted onto a latently infected rootstock.

Interestingly, there is a difference in the behavior of 'Jojo' trees growing on PPV infected rootstocks: If a budstick of 'Jojo' is grafted onto a PPV infected rootstock, the 'Jojo' scion will die off within a few days or weeks after budbreak. If the tree is older and the rootstock gets infected by inoculation of suckers the 'Jojo' trees grow normally without any symptoms of hypersensitivity. PPV cannot be detected neither by ELISA, RT-PCR nor biological indexing. At least for

young trees it is known that the transport of PPV through a hypersensitive interstem between the susceptible rootstock to the susceptible scion part is possible. This means that the virus is able to move through the vessel system (phloem and maybe xylem) of hypersensitive genotypes, but it is not replicating in the hypersensitive vessel tissue. We do not yet know whether this movement is able in full-grown trees as well. However, there is, up to now and with currently available detection methods, no proof of the presence of PPV in vessels of hypersensitive genotypes.

The different reaction of young and old trees of hypersensitive genotypes when growing on PPV infected rootstocks remains somehow mysterious. From the practical point of view it is a good situation: 'Jojo' trees are *per se* free from PPV when being planted in the field. If some years later, the rootstock of the 'Jojo' trees gets infected by aphids the tree remains unaffected and does not suffer from any kind of hypersensitive response. The grower does not lose any trees.

As already mentioned by Kegler et al. (2002) hypersensitive genotypes can stand the natural PPV inoculation pressure but not excessive PPV inoculation pressure after artificial inoculation. As shown by Hartmann and Petruschke (2002) the ratio between tree size and amount of inoculums can influence the testing results, e. g. if a young 'Jojo' tree gets inoculated by a high number of PPV infected chips or budsticks the 'Jojo' tree can die off partially or completely. With a full-grown tree, this is no longer possible. The influence of the inoculation method such as time of inoculation and amount of inoculums plays an important role in testing for PPV resistance as mentioned by Neumüller (2005) and Rubio et al. (2009).

Via latently infected plant material, PPV spreads over long distances. The use of scion and rootstock varieties which do not express clear PPV symptoms but which can get infected by PPV (even if the viral concentration is low) must be avoided in the future. This is not only true for *Prunus domestica* but for all other PPV hosts. If, in a species, no completely resistant genotype is available, it is better to use varieties which clearly show symptoms on the leaf because infected trees can easily be detected and removed. Only in regions where PPV is prevalent can the use of varieties, which show no clear symptoms but can contain the virus, be justified, but nurseries will have difficulties to keep their plantations free from PPV infections. The advantage of hypersensitivity resistance over all other known resistance mechanisms is that it withstands natural inoculation pressure (aphids) completely, i. e. the plant remains healthy. It dies off only in the case of high inoculation pressure which can just be the case under artificial inoculation conditions. The resistance has been stable for 20 years. Thus, the use of hypersensitive genotypes is the most effective method to prevent the spread of PPV over long and short distances and may become an important tool for influencing the epidemiology of PPV. In the future, the use of rootstocks with hypersensitivity resistance and interspecific hybridization could make the resistance trait available for other PPV hosts such as Japanese plum, apricot and peach (Neumüller et al., 2009).

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