THE HYPERSENSITIVITY OF EUROPEAN PLUM AGAINST PLUM POX VIRUS (PPV) AS A PROMISING MECHANISM OF RESISTANCE

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Abstract

The phenotype of hypersensitivity of European plum (Prunus domestica) against Plum pox virus (PPV) was described based on the observation of more than 1150 seedlings derived from 25 different crossing combinations with at least one hypersensitive parent. Hypersensitivity was found to be a quantitative trait. Those genotypes classified as extremely hypersensitive cannot be naturally infected with PPV. They are of great pomological value. Histological investigations revealed the characteristics of hypersensitivity of European plum against PPV. These results are based on inoculation experiments with PPV-D- and -M-strain.

Key words: hypersensitivity, resistance, Plum pox virus, PPV, sharka disease, Prunus domestica

Introduction

In terms of economic damage, sharka is the most important virus disease in stone fruit crops in Europe. It is spreading rapidly all over the world. Quarantine measures have only decelerated this process. The causing agent of sharka disease, the Plum pox virus (PPV), threatens the cultivation of plum, apricot, peach and, recently, cherry. Up to now, no genotypes immune to the virus were found within the species Prunus domestica L. in spite of great efforts during the past decades (Hartmann 1998). Therefore, previous breeding programs in P. domestica aimed at producing new varieties with few or no symptoms on leaves and even more important, on fruits when infected with PPV. Observations made in recent years in different European regions indicate that this quantitative resistance is no longer
sufficient to prevent economic damage in plum production since varieties known to be quantitatively resistant suffer from sharka, probably due to increasing stress on trees. It is known that abiotic factors like temperature and light influence the degree of quantitative virus resistance in plants (Kegler and Schenk 1990). Against the background of this development, breeding of plum varieties hypersensitive against PPV becomes more and more important. Hypersensitive genotypes remain healthy in the orchard and are completely resistant to PPV.

Detailed knowledge on both histochemistry of defence response and inheritance of hypersensitivity of European plum against PPV is a prerequisite for the successful use of this resistance mechanism in breeding programs. The aim of the investigations at the University of Hohenheim is to gather information on histochemistry and inheritance of this trait and to develop molecular markers for use in breeding programs. This article focuses on the description of different types of symptoms characteristic for the hypersensitive defense response and on the characterisation of changes in the graft union of combinations of hypersensitive genotypes with virus infected budwood of susceptible cultivars.

Material and methods

Description of phenotypically visible symptoms

1171 genotypes originating from 25 crossing combinations with at least one parent hypersensitive to PPV (either the cultivar ‘Jojo’ or the clone ‘Ortenauer × Stanley 34’) were tested for their reaction on PPV-infection (method modified according to Kegler et al. (1994). Three plants per genotype were grafted on virus free Myrobalan rootstock with a PPV-infected interstem (double grafting). The scion wood used for interstems originated from plum trees heavily infected with PPV-D-strain. Double grafted plants were cultivated in 3-l-containers in the greenhouse starting in February 2003 and 2004 at temperature 15–25°C. The behaviour of the plants was monitored for at least four months. In spring 2004, some of the genotypes were also grafted on heavily sharka-infected trees in the experimental orchard in Weil der Stadt both on trees infected with D- or M-strain.

DAS-ELISA and IC-RT-PCR (Wetzel et al. 1992) were used to verify the viral infection.

Histological and histochemical investigations

To study the changes appearing in the tissue within and around the graft union after combining PPV-infected scion wood with scion wood of hypersensitive genotypes, three varieties (‘Jojo’ – hypersensitive, ‘K4’-hybrid – hypersensitive against PPV-isolate ‘CG’ (Kegler et al. 1994) and ‘Ortenauer’ – susceptible) were grafted on virus free Myrobalan rootstock and cultivated in the greenhouse as described above. Three weeks later, five chips of PPV-infected or healthy (control) budwood
of ‘Hanita’ were grafted onto the varieties. Two strains of PPV were used separately (D- and M-strain). Starting three weeks later, samples of each variant were collected twice weekly over a period of three months. Tissue was fixed in glutaraldehyde according to Karnovsky (1965) and embedded in resin (Ruddell 1967a, b, modified by Hermanns and Schulz 1981). Sections of 5 µm were stained with toluidin blue (0.5 g/l, 20 min) in order to localise phenolic compounds within the tissue.

Results

Description of phenotypically visible symptoms

Shoots of genotypes susceptible to PPV showed normal vegetative growth and developed typical sharka symptoms on the leaves (chlorotic or yellow rings, spots or bands). Starting 10 days after bud break (about five weeks after grafting), genotypes hypersensitive to PPV reacted with the sudden death of the tips of the young shoots (Fig. 1, Phot. 1). In many cases the whole shoot died within two to four days. In shoot tips undergoing rapid death, ELISA or IC-RT-PCR-tests could detect no PPV. Very often, the decay of shoot tips went along with necrosis on the bark of the young shoots. Genotypes showing this type of symptoms were classified to be extremely hypersensitive.

![Diagram showing hypersensitivity of European plum against PPV as a quantitative trait](image_url)

Fig. 1. Hypersensitivity of European plum against PPV as a quantitative trait. The figure gives the different traits characteristic for hypersensitivity. The more traits listed in the right column an individual genotype of *Prunus domestica* infected with PPV is showing the higher is its degree of hypersensitivity (and vice versa).
There was another group of genotypes showing no typical sharka symptoms on the leaves (Fig. 1, Phot. 1): the vegetative growth was reduced, there were no or abnormal sharka symptoms on the leaves, i.e. severely chlorotic rings appeared on the leaf blade with tendency to necrosis during the further development, the surface of the leaves was abnormally developed (curled leaves), and the leaves and/or the bark of young shoots showed necrosis. Some genotypes showed only one or few of these symptoms, some displayed all of them. In most cases, PPV could be detected in the leaves at least by IC-RT-PCR. This group is classified to be slightly or moderately hypersensitive.

Hypersensitive genotypes showed necrosis on the leaves and on the bark of newly grown shoots as well as tip necrosis. There was a broad range of symptoms, which can be used as criteria for hypersensitivity.

The PPV-strain (D or M) used for inoculation did not markedly affect the development of symptoms of hypersensitivity.

**Histological and histochemical investigations**

Within the first four weeks after grafting bark chips (healthy or infected with PPV-D- or -M-strain) on scion wood of different plum varieties there was no difference in histological structure of the graft union. There was a normal callus forma-
tion starting from both the bark chips and the rootstock. The callus filled the space between the two partners. Starting five weeks after inoculation, dramatic changes in combinations of ‘Jojo’ and PPV-infected chips appeared: phenolic compounds accumulated in cells marking the barrier between the callus of the two partners. Subsequently, those cells collapsed and died (Phot. 2). These dead cells formed a necrotic line within the graft union. In all the other grafting combinations none of these histological and histochemical changes could be detected.

Discussion

There is a broad range of different symptoms being correlated with hypersensitivity against PPV. Figure 1 and 2 give an overview of the observed symptoms. Using those parameters a distinction between hypersensitive and sensitive genotypes (using terms based on Cooper and Jones (1983) is possible. Only those genotypes reacting with the death of the tips of young shoots and with necrosis on the bark remain virus free in the field. Others showing only necrosis of the lamina or other mild symptoms are not able to localise the virus fast enough to prevent a systemic infection. The first group of hypersensitive genotypes are of high pomological value and useful as parents in breeding programs, the latter group of moderate hypersensitive genotypes are of special interest for research into the mechanism of PPV resistance mediated by hypersensitive response. All told, hypersensitivity of European plum against PPV has to be qualified as a quantitative trait.

The symptomatology of plants growing as double graftings in the greenhouse corresponds with that one of graftings on infected trees on the field. Therefore, the greenhouse test is considered to be suitable for screening large populations.

There are no substantial differences in the reactions of hypersensitive genotypes whether infected with a PPV-D-strain or a PPV-M-strain. Thus it appears that the resistance based on the hypersensitivity determined in the Hohenheim gene pool is effective with both PPV strains. This result corresponds with the findings of Hartmann and Petruschke (2002).
In the graft union of combinations of the hypersensitive cultivar ‘Jojo’ with budwood infected with PPV-D- or -M-strain cells collapsed three weeks after the callus tissues formed by the two partners came into contact and joined. This indicates the hypersensitive cell death at the barrier between the two partners. The accumulation of phenolic compounds preceded the cell death as it is known from many other plant species. Presumably the virus is not moving into the newly formed tissue until plasmodesmal microchannels as cell-to-cell-connections are formed in the young callus tissue. This would explain the gap in time between the joining of the callus tissues and the appearance of the necrotic line which goes along with the rapid death of the young shoot tip in double graftings as the transport of water into the upper scion is no longer ensured.

Conclusions

Hypersensitivity of European plum against the Plum pox potyvirus is an effective mechanism to prevent the infection of trees with PPV. Genotypes showing a strong hypersensitive response in double grafting system, e.g. cultivar ‘Jojo’, remain virus free in the field. They cannot get infected via aphid transmission. There is another important advantage of hypersensitive genotypes concerning the propagation of trees in the nursery: if they are grafted on rootstocks infected with PPV, the hypersensitive scion bud will die within a year. Consequently, using hypersensitive genotypes, sharka disease cannot be distributed into regions free of PPV via plant material.

As the degree of hypersensitivity against PPV is a quantitative trait, it is very important to use the appropriate selection methods in breeding programs in order to select only those genotypes which are able to isolate PPV after natural infection. Only those showing strong hypersensitive response are of great pomological value. Therefore, multiple testing methods are necessary.

Currently, the breeding of plums hypersensitive to PPV is one of the most promising ways to get cultivars ensuring the production of plums in sharka-infected regions. A series of new hypersensitive cultivars with excellent fruit quality ranging from early to late fruit ripening period are expected to be released by the University of Hohenheim during the next years.

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Literature


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