Ways of Increasing Resistance to Viruses into the Single Plant and in Populations

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Abstract

Viruses as phytopathogenes have been discovered at 1892, and 638 plant's viruses have been identified at 1989. Some of its may be epidemic and to cause significant yield losses of cultivated crops. Increasing resistance of the single plant and populations is the necessary condition for the control of viruses spread and damage. Our proposals for the increasing resistance to viruses: For the single plant the soft correction of plant's metabolism with pretreatment of the natural bio-antioxidants and immunizators – steroid glycosides. It results in lowering of virus infectivity, degree of plant's affection and increasing of the yield on 11–41% in fact (in ToMV-tomato pathosystem). For the plant's population– increasing to the necessary proportion the lot of tolerant and resistant forms into the plant's assortment; – selection of resistant and tolerant forms from populations have been selected earlier as resistant to other pathogens and obtaining of the basic material collection with complex resistance; – hybridization programs and developing of tolerant and resistant hybrids; – use molecular markers of resistance for the limitation of virus infection backgrounds in the breeding programs.

Keywords: induction of virus resistance; steroid glycosides; selection of tolerant and resistant forms; hybridization programs

INTRODUCTION

Viruses as phytopathogenes have been discovered at 1892, and more than 600 plant's viruses have been identified at 1989 (BOIKO 1989; WILSON 1989). Some of its may be epidemic: yield losses may to achieve about 70–88% of cereal – *Solanaceae* crops (BOIKO 1989) and 70–95% – of horticultural crops (LAKHMATOVA 1997). Following to VAVILOV (1918) and ROBINSON's (1980) concepts, we have been deepened into the study of virus-host pathosystem. Main violations of plant's metabolism have been connected with energetic systems of cells, and, in particular, of chloroplasts: activation of depended photophosphorylation, increasing of ATP-content, growth concentration of free peroxide radicals (LADYGINA *et al*. 1977). Virus-induced changes of susceptible plant's metabolism are similar with fast aging processes in many respects. Free peroxide radicals cause inactivation of protein's sulfhydrilic groups, activation of lipases, appearance of lipid drops into cells, deformation and, in some cases, destroy of mitochondria, chromosomes aberrations and autolysis (ZENKOV & DOSKOCH 1975; VASILIEV 1978; REUNOV & LAPSHINA 1979). Bioantioxidants remove the competitive ratio of free peroxidation and enzymic oxidation in the direction of enzymic oxidation, eliminating negative effect of free peroxidation. So, bioantioxidants may to posses of anti-viral potential (KALITCHAVA 1973).

Secondary plants metabolites named steroid glycosides (SG) are known of wide spectrum biological activities (KINTIA & LAZURIEVSKII 1979). SG show also the anti-oxidant activity, which can be explain of the high mobility of a hydrogen atom in the hemiketal hydroxyl group at the C-22 of aglycone (KINTIA *et al*. 1982).

Induction of virus resistance in host-plant with SG treatment

The anti-viral effect of steroid glycosides have been established at first against TMV in 1978. Two substances from SG class (asparagozide and pavs-tim) reduced infectivity of crude juice from tomato leaves infected earlier with TMV – on 7 and 70%, respectively. This effect was shown for the 10 other substances of SG class and for 6 of its – it was confirmed *in vitro* (on the clean TMV-preparation) and *in vivo* (on tomato seedlings, infected with the clean TMV-preparation) (BALASHOVA & KINTIA 1983;

Table 1. Mechanism of SG-action in general

No.	Criterion (KIRÁLY *et al*. 1970)	SG-action (inhibition of TMV infectivity, %)
1.	**Time**: inhibitor affects *in vitro*, if its effect increases as a function of time since the mixing of the inhibitor with a virus preparation	At the time of mixing – 35 1 h after mixing – 40 3 h after mixing – 45 24 h after mixing – 52 48 h after mixing – 53
2.	**Dilution**: inhibitor acts *in vitro*, if its effect is remained during dilution	**Dilutions**: not diluted – 36 1:1 – 42 1:2 – 38 1:4 – 43 1:8 – 41 1:16 – 37
3.	**Application**: inhibitor acts *in vitro*, if inhibition of virus infectivity results only from mixing with the inhibitor before plants inoculation. If a plant show inhibition of virus infectivity after treatment with inhibitor, the letter acts *in vivo*	• SG was mixed with clean TMV-preparation before test – 25 • Plants treated with SG-solution have been inoculated with clean TMV-preparation – 36

BALASHOVA *et al*. 1984). Mechanism of SG-action has been studied into pathosystem TMV-tomato on the model steroid glycoside with the clean anti-viral effect (pavstim 70%). Investigation has been carried out in three steps:

1. Mechanism of SG-action

It has been in general by means of a special criterion system for the evaluation of viral inhibitor action traits (KIRÁLY *et al*. 1970).

Conclusion: SG acts *in vitro* and *in vivo*.

2. SG effect on a plant metabolism

Protein metabolism. Virus infection is known to cause of new protein appearance in leaves of hypersensitive and resistant plants (Proceedings of the Workshop on Pathogenesis-Related (b) Proteins in Plants 1983). A similar effect is also observed under the influence of interferon inductors (GIANINAZZI & KASSANIS 1974). The polyacrylamide gel electrophoresis reveals that SG pavstim also induces of 3 new proteins formation in the leaves of susceptible tomato variety with Rf_1 = 0.24; Rf_2 = 0.40; Rf_3 = 0.44. These proteins were not isolated by authors and its molecular weight has not been determined, but its have been appeared every time after the treatment with SG pavstim.

Ribonuclease activity. Ribonuclease is a key cell's enzyme, which change of its activity in response to virus infection. A change of that kind might be conditioned by the necessity of protection, directed at suppression of the virus RNA replication (PATIUKHINA

Table 2. Change the RNA-ase activity and TMV infectivity after treatment with SG

No.	Variants	RNA-ase activity, units ($\bar{x}$)	Decrease of TMV infectivity (%)
1	Standard – "pure"* plants	0.30	–
2	Treatment with SG only	0.75	–
3	TMV inoculation only	0.71	–
4	SG-treatment 24 h before TMV inoculation	3.10	17
5	SG-treatment immediately after TMV inoculation	2.26	52
6	SG-treatment 1 h after TMV inoculation	1.93	21
7	SG-treatment 2 h after TMV inoculation	1.66	35
8	SG-treatment 3 h after TMV inoculation	0.50	42
9	SG-treatment 24 h after TMV inoculation	1.09	18

*non treated and non inoculated plants

et al. 1980). In this connection, we measured the activity of ribonuclease (by TATARSKAYA *et al*. 1966) in tomato leaves treated with pavstim and infected with TMV. Treatment and inoculation alterations were carried out so as to determine the stage of infection process, in which SG shows its inhibition effect. Plants treated with SG and inoculated with TMV were simultaneously tested for TMV infectivity (Table 2).

Increasing of RNA-ase activity in 2.5 time has been found results as in case of TMV- inoculation, as in case of SG-treatment. If the established effect is viewed as a cell-protective reaction, then SG may to induce it. Maximal protective effect (52%) can to show at the simultaneous SG-injection and TMV-inoculation, which seems to be caused by the SG-ability to act *in vitro*.

Conclusion: SG can to increase the level of plant's protective reactions from virus.

3. SG practical using to increase of plants resistance

Results of experiments by mechanism of SG-action gave us the evidence that SG were capable to induce protective reactions of tomato plant against TMV. On this base, we tested its as plant's resistance inductors (phytoimmunizators) in the field experiment. Tomato seeds have been treated with water solutions of pavstim, moldstim and ecostim during 24 h. After that its have been sown in the soil. Seedlings, grown from immunized seeds, have been more vigorous and resistant. Its flowered and bore fruits earlier then standard plants. According to phytopathological evaluation, degrees of infection with complex tomato diseases were considerably reduced (from 25% to 76%). It have been mentioned: reduction was not complete, but it has been observed for all diseases infected tomato plants these years (1982–1983). Its were not only mosaic caused with TMV, but diseases of bacterial (*Xanthomonas vesicatoria* D.) and fungal (*Alternaria solani* Sor., wilt diseases) nature. So, we obtained increasing of unspecific resistance. Plants yields increased from 11% to 41% (BALASHOVA *et al*. 1990).

Conclusion: SG pavstim, moldstim and ecostim may be used in farm management as phytoimmunizators.

Wide using of SG in agriculture has been started from 1986. Its have been applied for the increasing resistance and yield in 50 agricultural cooperatives in different regions of the former Soviet Union (Moldavia, Leningrad region, Baltic sea-region, Byelorussia, Far East-region). SG- immunizing effect was confirmed on cucumber, tobacco, pepper, egg-plant, potato, garlic, stock beet, rose and some cereals (KRISTIOGLO *et al*. 1989; LUTSENKO & DOROSHENKO 1989; MALINOVSKAIA *et al*. 1989; Proceeding of Workshop by Steroid Glycosides in Moldavian Academy of Sciences 1986–1988; FOMINYKH *et al*. 1989). It was very interesting, in connection with its immunizing effect, to clean function of endogenic SG in plant metabolism: is its connected with plant resistance to viruses?

SG as factors of plant resistance to viruses

Investigation have been carried out in *Plum pox virus* – plum pathosystem. Plum pox is the main and harmful virus disease of horticultural crops in Europe (NEMETH 1994). It has been found in South America, North America and Asia last years of 20th century (ACUNIA 1994; Phytopathology news, 2000; BHARDWAJ *et al*. 1994). So, the problem of resistance to PPV has the global mean. It has been known that plum (*Prunus domestica* L.) is the natural hybrid of *Prunus cerasifera* Ehrh. and *Prunus spinosa* L. It has 48 chromosomes and many of its traits is controlled with some genes: so named polygenic control (ERIOMIN & VITKOVSKY 1980). Plum resistance to PPV also have the polygenic base (BIVOL *et al*. 1987). It corresponds the quantitative type of resistance. Two questions follow from this conclusion:

1. How to evaluate of plants with this type of resistance?
2. How to select of resistant plants?

Genetic marks are known for the pathosystems with monogenic control of resistance (OHMORI *et al*. 1995). But its are not evident in pathosystems with polygenic control of resistance. Known about of SG immunizing effect, we proposed to use for this purpose the biochemical mark – the content of SG in plum buds.

1. SG participation in PPV-infection process

SG-presence in plum buds, leaves, fruits, stones and bark has been established by thin lay chromatography (silica gel G 50) at 1984. It was noted that the SG-content was higher in buds of resistant and tolerant varieties then the SG-content in buds of susceptible varieties (spots color was more intensive). But it was the qualitative analysis only. Spectrophotometry on Specord 40M has been used for obtaining of the quantitative information about of SG-content in extracts from plum buds, leaves, fruits and stones. Quantitative information has been analysed with statistical methods. It was shown the participation of SG in the PPV-pathogenesis. SG-content in plum's leaves was increased into infected plants, but SG-content in

plum's fruits was reduced into infected plants. These changes were significant at the 5% level of significance (LAKHMATOVA *et al*. 1995).

Conclusion: SG can to participate in PPV-pathogenesis.

2. SG-content in plum buds as biochemical mark of resistance to PPV

Connection between of the SG-content into plum buds and the degree if fruits infection has been investigated on 8 plum varieties contrasting by resistance to PPV with correlation analysis. The correlation between two above traits has been established with coefficient: $r = -0.75$. The higher of SG-content into plum buds, the smaller degree of fruits infection with PPV. Correlation was significant at the 5% level of significance. This result has been confirmed by analysis of 47 co-variation pairs in the field experiment. The correlation coefficient in the field experiment was: $r = -0.53$. Correlation was significant at the 0.1%-level of significance (LAHKMATOVA *et al*. 1997). Correlations have been established between 4 reliable parameters needed for evaluation of plum resistance to PPV (Figure 1). All its were significant at the 0.1% level of significance.

SG-content in buds ← $r = -0.53$ degree of fruits infection
↑
$r = 0.63$
↓
PPV-content (ELISA) ← $r = 0.69$ degree of leaves infection

Figure 1. System of traits for PPV resistance evaluation

Conclusion: SG-content in plum buds can be used as biochemical mark in the system of traits for PPV resistance evaluation.

3. Plum breeding for PPV resistance using system of traits (including SG-content)

All plum assortment of Republic Moldova (66 varieties and 59 hybrids) was estimated for PPV resistance, using system of traits. We used two standards in our experiments: standard of resistance (cv. Kirke) and standard of sensitivity (cv. Moldavskaya ranniaia). All data obtained have been treated by dispersion analysis. Forms with resistance traits statistically closed to characteristics of the resistant standard have been selected in our experiments. Estimation has been carried out in 1988–1995 in 2 steps:

- under natural infection with PPV (1988–1991)
- under artificial infection with PPV (1989–1995).

Ten varieties and 20 hybrids have been selected on the natural infection background. Its have been multiplied and planted as experimental plum plantation (3 hectare) at May 1989. We infected its with Moldavian isolate of PPV by chips at the August 1989. Resistance to PPV have been evaluated in 1991–1995. 13 varieties and 9 hybrids with different types of resistance have been selected after artificial inoculation (Table 3).

Table 3. Moldavian plum assortment tolerant and resistant to PPV (LAKHMATOVA *et al*. 1997a,b)

Field resistant	Tolerance	
	general	fruit
Udlinionnaya	Pamiaty Kostinoi	Pamiaty Vavilova
Opal	President	Stanley
Jelta boutilkovidna	Čačanska lepotica	Scoldus
Kirke	Čačanska Najbolja	Agen 707
	Berbank	Rene-Claude d'Oullins
	4/36	3-33-75
	22/72	3-32-119
	23/77	3-32-120
		3-23-112
		3/35
		4/50

Resistant and tolerant varieties and hybrids were multiplied in 1995. Next year its were planted as experimental plum plantation of resistant and tolerant forms. Some of its were used for crossings as maternal and paternal forms.

Conclusion: System of traits (including of SG-content in buds) may be used for PPV resistance evaluation in plum breeding.

Safe ways of virus resistance increasing in populations of plants

1. **Correction of protection measures, taking into account of plants resistance potential**

Evaluation of Modavian plum assortment resistance to PPV allowed us to divide it into 4 groups depended on resistance to PPV:

- high susceptible forms
- susceptible forms
- tolerant forms
- field resistant forms.

For the safe PPV-resistance increasing we proposed to plant its by blocks with its own measures of protection in every block.

Measures of PPV protection for high susceptible and susceptible forms:

1. use of virus free planting material
2. distant of plantation from the potential sources of infection (1 km et least)
3. phytosanitary control of young plantations with eradication of diseased trees every year
4. use of the treatment with insecticides against aphids and oil emulsion in autumn.

Measures of PPV protection for tolerant and field resistant forms:

1. use of virus free planting material
2. phytosanitary control of young plantations with eradication of diseased trees every year.

We recommend to apply of chemical measures for the tolerant and resistant forms by the necessity only.

Conclusion: protective measures against PPV have to account of plants resistance potential.

2. Aimed hybridization programs and use of hybrid's tolerance and resistance

Saturation of plants populations with tolerant and resistant forms may to carry out with help of tolerant and resistant hybrids. For successful hybridization tolerant and resistant varieties should be included in crossing programs. But how to select of parental forms for crossing to obtain the progeny with desirable traits? To answer of this question we analysed the inheritance of PPV resistance by plum's hybrids. Five plum varieties with different PPV-resistance traits have been used as maternal and paternal forms for the incomplete diallelic crossing scheme in 1989–1991:
Kirke – resistant to PPV
Stanley – tolerant to PPV
Renklod Hramovikh – tolerant to PPV
Kabardinskaya ranniaia – susceptible to PPV
Sopernitsa – susceptible to PPV.

More then 600 hybrid seedlings have been planted in the field at 1992. Analysis of PPV-resistance inheritance has been carried out in 1996–1997 under natural infection with PPV. As a basic parameter we used results evaluation of leaves degree affection. Analysis of PPV-resistance inheritance by two factorial dispersion complex show, that contribution of maternal forms in PPV-resistance transmission to progenies was:
h^2A = 20% (from the whole h^2 = 26%) in 1996
h^2A = 50% (from the whole h^2 = 57%) in 1997.

It was significant at the 5%- and 1%-level of significance (LAKHMATOVA *et al*. 1999).

Conclusion: PPV-resistant forms have been used in crossings as maternal forms for the transmission of PPV-resistance to progenies.

3. Selection forms with complex resistance

It is not a new or original idea for obtaining of resistant and tolerant forms. But the question is: "How to obtain the organism with complex resistance?" Genetic potential of living organism is limited, and we can not to include into it all desirable genes without of any limitation. But we can to choose the needed forms from genetic material selected earlier on resistance to other pathogens and abiotic factors.

Tomato is one from the most important vegetable crops for Russian Federation. There were 2 main problems for this culture in the Middle Russia: cold and *Phytophthora infestans* DB. Some early ripening, cold-resistant and tolerant to *Ph. infestance* DB varieties and hybrids have been bred in VNIISSOK after more then 20 years of selection. Unfortunately, its are proved to be subjects to virus pathogenes, such as well known TMV and potato viruses of potex- and carla-group. Degree of virus spreading in selective tomato populations has been changed from 0.60 to 100% for TMV, and from 7.31 to 41.8% for potato viruses. It was depended on the year's weather conditions and type of population (in open field, in polyethylen houses, in greenhouse). Virus infection reduced of the fruits mass (on 28% and higher), contents of dry substances and vitamin C into diseased fruits (LAKHMATOVA *et al*. 2001). So, we can to increase tomato complex resistance by selection forms with resistance and tolerance to viruses from genetic material cold-resistant and tolerant to *Ph. infestance* DB.

58 breeding forms without symptoms and viruses have been selected from different selection populations (530 breeding forms) with traditional methods (visual observation and DAS-ELISA). 4 samples from its had not any symptoms after artificial infection with TMV (strain U1) in the special box. Besides its, there were 5 samples from tolerant forms and 4 samples even from susceptible forms, which had not depression during seedlings phase. It is very important to know of its genetic base, by our mind. Time of breeding was 4 years and its effect was only 1.16%. We decided to use molecular analysis of tomato plants for:

- determination genetic bases of prospective forms
- increasing effect of selection on resistance and tolerance
- limitation of virus infection backgrounds in the breeding programs.

Conclusion: use of genetic material selected earlier as resistant to biotic and abiotic stresses is prospective for the breeding on virus resistance.

4. Use molecular markers of resistance for limitation of virus infection backgrounds in breeding programs

Our own experience show us, that the best way to maintain and increase resistance in populations of cultivated crops is limitation of virus infection backgrounds in breeding programs. Molecular markers of virus resistance can to help us. RAPD-markers are more rapidly and more easily detectable, then RFLP-markers (WILLIAMS *et al*. 1990) and RAPD-technique has been used in our laboratory for the identification of tomato genomes variability earlier (SUPRUNOVA 1997).

We used 10-base oligonucleotide primer $OPG_{700}09$ has been designed of T. Ohmori, M. Murata and F. Motoyoshi for the identification of polymorphic fragment of 700 bp, tightly linked with locus of *Tm-2* and *Tm-2a* genes (OHMORI *et al*. 1995, 2000) for the testing of our genetic tomato material. The presence of this fragment has been confirmed in 2 lines (with genes *Tm-2* and *Tm-2a*) used as resistant standards in our experiment, in 2 F_1 hybrids (without symptoms after artificial infection with ToMV, wild strain) and in 1 resistant variety (without symptoms after artificial infection with ToMV, wild strain). But it was absent in to susceptible variety used as standard of susceptibility.

Conclusion: molecular markers of virus resistance can be used in breeding programs.

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