MODESTUM
OPEN ACCESS
Eurasian Journal of Analytical Chemistry
ISSN: 1306-3057
2017 12(7b):1421-1427
DOI: 10.12973/ejac.2017.00269a

Physiological and Biochemical Characteristic of Elicitors Impact on the Vitis Vinifera Plants' Phylloxera-Resistance

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Received 22 April 2017 ▪ Revised 11 August 2017 ▪ Accepted 26 September 2017

ABSTRACT

The promising trend in the grape-vine cultivation technology is application of elicitors to improve the resistance of plants to disease and insect attacks, phylloxera particularly. The objective of work is a physiological and biochemical estimation of elicitors impact on phylloxera-resistance of the Vitis Vinifera plants. The target of research are the own-rooted plants of the grape-vine technical grade Bianca, interspecies hybrid, of Euro-American origin. The plant growth regulator preparation Furolan and amino acid methionine, and their composition served the function of elicitors. To determine the content of protein, pigments in the leaves, the spectral method of analysis was used, as for phenolcarbonic acids, amino acids, phytohormones, metal cations – the capillary electrophoresis method. The application of elicitors in the grape-vine cultivation technology permits to enhance the resistance of the Bianca grape-vine own-rooted plants to affection both by the root and leaf form of phylloxera and is highly competitive by its biological efficiency with a standard treatment variant, involving application of the multiple crop protection chemicals. The application of elicitors has an effect on metabolism of the grape-vine plants, increases the phenol compounds content, stabilizes protein synthesis, activates photosynthetic activity. It permits to activate the defense reaction of the grape-vine plants, to enhance the productivity, to yield ecologically clean production without sacrifice of the grape-vine quality.

Keywords: grape-vine, phylloxera, elicitors, metabolome, productivity

INTRODUCTION

The discovery of the grape-vine varieties, possessing the complex resistance to abiotic and biotic environment features prominently in the development of the highly productive ampelocenoses, which is critical in the context of the local changes in the weather and climatic conditions [1, 2]. The natural environment and climatic conditions of Krasnodar Territory favour the high yields of grape-vine, able to sustain competition at international market. At the same time the achievement of the steadily high yields is limited by the impact of such unfavourable environmental factors, as winter frosts, particularly after the long warm weather, and summer droughts. Therefore, only the varieties, combining the high quality and adaptedness to conditions of concerned region, may be successfully cultivated on a rather wide scale basis [3, 4]. The unfavourable climatic conditions may have a negative effect on the grape-vine plants' resistance to biotic stresses, and specifically the phytopathogenic and parasitic attacks. The climatic change makes particularly topical the study of physiological and biochemical regularities of forming both the productional and adaptive potential of the grape-vine varieties, differing in environmental and geographic origin, and detection of the key-value physiological and biochemical and anatomicomorphological parameters, characterizing the expressivity of the plants' genetic systems of adaptation to abiotic and biotic stresses [5, 6, 7, 8].

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One of the most expensive items in technology of the grape-vine cultivation is its protection against the hazardous organisms. The application of the chemical crop protection agents is highly efficient. However, their degree of utilization in the natural communities is low, they accumulate in the foodstuffs and feature high carcinogenecity [9].

The application of the chemical protection agents of different classes has an effect on the hazardous organisms, gradually adjusting to the pesticides. It negates the effectiveness of the latter and calls for creation of enormous mass of preparations, and also decimates the kinds of microorganisms "useful" in the farm ecosystem. Besides, the pesticides have a negative effect on the grape-vine plant metabolism, and their remnants get into the berries and the products of grape-vine processing, including wines, juices, etc. [3, 9]. One of this task solutions is the use of the low-toxic defense reaction inducers, applied in microdoses - abiogenic elicitors, including the synthetical low-molecular immunomodulating compounds, which are the analogues of natural substances [10, 11, 12, 13].

In conditions of the Russian Federation south region it is the most hazardous viticide – phylloxera (Phylloxera vastatrich Planch) – that is among the most commonly encountered ones, inflicting grave damage to the vineyards. The American varieties and hybrids of direct producers bear all the forms of phylloxera (root, leaf or gall, nymph, wing and fertile). The roots of European vine happened to be very convenient to feed the root form of phylloxera, whereas the leaves are hardly suitable to feed it and form the galls, therefore the leaf form is rarely harmful for European grape-vine. Lately the drop in resistance of such varieties to affection caused by the phylloxera leaf form is noted though. The root form of phylloxera opens a door to the in-soil pathogenic bacteria and fungi. Among the excitants, causing the decay of the grape-vine roots, are some species of fungi Gliocladium, Fusarium, Cylindrocarpon, as well as Pseudomonas, Bacillus bacteria [4].

To enhance the grape-vine plants' resistance to affection both by the phylloxera leaf and root form the use of elicitors – the pathogene-evolved substances in the tissue of a host plant – is promising as a biotechnological process. The specific selection of synthetical analogues of such compounds and their use in the doses harmless for a plant permits to attain the immunization effect in response to activation of metabolic processes, aimed at the detoxication of exogenous compound and makes the plants more resistant to affection [13]. In pathogenesis the plant accepts a signal of pathogene and starts the complicated program of chemical protection, including the synthesis of ethylene, abscisic, salicylic acid, the action of which involves the regulation of processes, running in a cell wall, apoptosis genes expression (lignification of affected tissue areas), synthesis of the stress proteins [14, 15].

It permits to realize more completely the potential of genotype through activation of defense reaction cascades for creation of intracellular medium, unfavourable for development of phytopathogens and feeding phylloxera, that may also create the preconditions to enhance the grape-vine plants' resistance to the abiotic stressors of a summer period. To enhance resistance of the own-rooted grape-vine plants of Euro-American origin to affection by the root-form phylloxera they use the composition of Furolan and methionine preparations, showing the properties of elicitors, but their influence on the grape-vine plants' resistance to the phylloxera leaf form was not studied [16, 17, 18, 19, 20].

The objective of work is a physiological and biochemical estimation of elicitors impact on phylloxera-resistance of the Vitis Vinifera plants.

MATERIALS AND METHODS

The target of research is the own-rooted plants of the grape-vine technical grade Bianca, interspecies hybrid, of Euro-American origin. It is relatively resistant to mildew, gray mold, phylloxera leaf form and low-resistant to affection by phylloxera root form [8]. The implantations were of year 2006. The Bianca grape-vine experiment was undertaken by "Primorskoye" Private Corporation of Temriuk district in Krasnodar Territory in 2014. 2015. 2016 [21]. The experimental design: 1 – control, 2 – standard treatment - reference, 3 –Furolan 10 g/ha + methionine 10 g/ha (1:1). Experiment replication - quadruple. 5 vine places per replication. As a control there were taken the plants untreated by elicitors. The elicitor-treatment (composition of Furolan and methionine preparations in 10 g/ha dosage for each of them) was carried out three times (May 20, June 8 and June 22) in the form of aqueous solutions by means of spraying with the use of CHAMPION PS 257 shoulder sprayer. The fluid consumption – 870–950 cub.dm/ha [10]. The benchmark standard (standard treatment variant) – the plants, cultivated with the use of chemical protection agents. In May of 2015 the grape-vine plants were treated against the complex of diseases (anthracnose, Alternaria leaf spot, blackspot) by Cuprosat KS and Polyram DF, WDG preparations. The insecticides were not applied, since no output of phylloxera leaf form was observed after the great drop in air temperature in the third decade of April down to 0.4°C. In 2016 the treatment of the grape-vine plants in late April in all variants of experiment was carried out for mildew- (Cuprosat KS), blackspot- (Cuprosat KS, Rapid Gold SP, Cabrio Top WDG, Polyram WDG, Tanos WDG) protection, oidium development prevention (four times Microtiol special WDG, one time Cabrio TOP WDG and Talendo KE), protection against phylloxera leaf form (Bi-58 new KE, Fastac KE) against grape moth and cotton budworm (Pirinex KE, Avant KE). The field experiment was held following the program and methodology of the fruit and berry crops and nucicultures variety study [21]. The modern high-

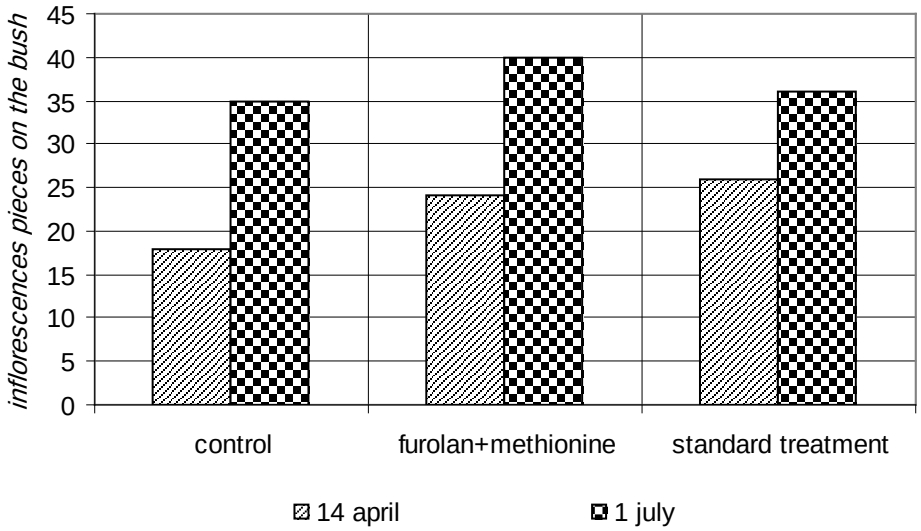

Figure 1. Number of the grape-vine inflorescences, pcs./vine

precision physiological and biochemical methods to research the content of chlorophyll (a+b), carotenoids, amino acids, phenolcarbonic, organic, abscisic, indoleacetic acid, proline, glycine, saccharose, glucose, fructose were used by the capillary electrophoresis method with application of the high-performance Kapel 105 M analytical equipment on the basis of "instrumental & analytical" common use center and Physiology and biochemistry of plants laboratory of FSBSI NCZSRIHV [22].

The experimental data obtained in 2014-2016 were processed through the use of variation statistics conventional procedures [23].

RESULTS AND DISCUSSION

The prolonged effect of the 2014 abiogenic-elicitor treatments on the plants overwintering was shown in 2015 at the start of the grape-vine vegetation. As on April 10, 2015 the number of swollen eyes varied over all experiment variants from 92 percent in a control variant up to 99 - in elicitor variant and 98 percent - in the standard treatment variant. But after the great drop in air temperature on April 14 down to -0.7°C and on April 22 down to 0.4°C the number of shoots, developing from the central buds, was no more than 30-40 percent, which reflected on the number of initially forming inflorescences (see **Figure 1**).

The early July of 2015 as compared with April saw the larger number of inflorescences in elicitors variant. In elicitors variant it increased by 81.8 percent, in a control one – by 94.4 and in standard treatment variant – by 37.1 percent. It is worth pointing out the increase in the number of inflorescences as a result of the later (by 2 weeks) budding and shoot growth from the reserve buds.

In 2015 the unfavourable conditions came about for development of the major pathogenes. The leaf form of phylloxera was absent in all experiment variants for all the vegetation period. The roots were unearthed in layers to the depth of 30-35 cm, i.e. down to the heel roots to forecast the possibility of phylloxera nymphs emergence on the soil surface. See **Figure 2** for the results of determining the grape-vine roots colonization by phylloxera root form.

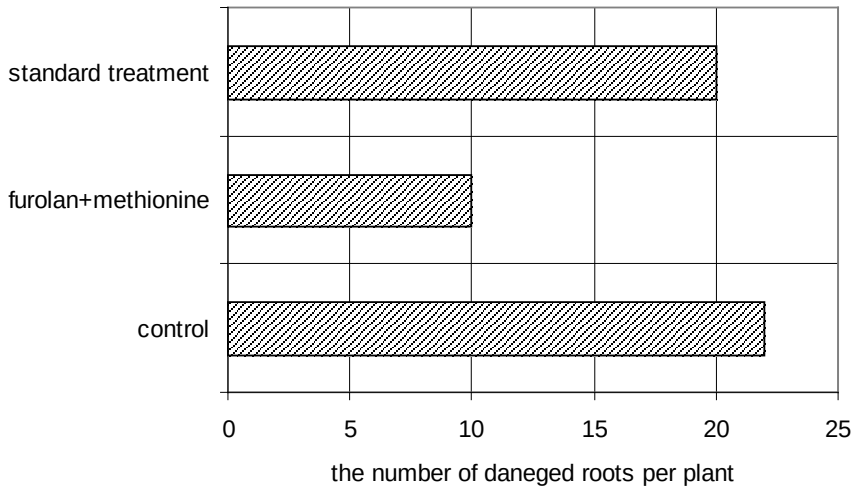

Figure 2. Colonization of the Bianca grape-vine roots by phylloxera root form ("Primorskoye" Private Corporation, 2015)

Table 1. Impact of elicitors on the phylloxera leaf form generation

Variant	17.06.2016		27.07.2016		11.08.2016		29.08.2016	
	P*	R**	P	R	P	R	P	R
Control	14.6	7.1	66.1	31.9	65.5	37.9	29.1	12.6
Elicitors	5.1	1.6	18.6	6.4	36.9	12.5	30.9	20.2
Farm processing	0	0	0	0	7.0	1.7	0	0

*P - generation of the phylloxera leaf form, %

** R - intensity of the leaves colonization by the phylloxera leaf form, %

Table 2. Abiogenic elicitors impact on formation of the Bianca grape-vine productivity, 2014-2015

Variant	Area of the shoot leaves, sq cm	Number of bunches, pcs./shoot	Leaves area, sq cm /bunch
Control	1740.1 - 2094.1	1.8 - 1.9	966.7-1102.1
Elicitors	2323.7 - 2848.2	1.8-2.2	1085.2-1582.2
Standard treatment	1406.4 -1970.8	1.7-1.9	1037.3-1097.9

Resulting from extensive damage of roots in 2015 in control and standard treatment variants, the emergence of nymphs from soil was more probable, than in elicitors variant. In 2016 only the phylloxera leaf form from among the hazardous organisms generated actively on Bianca grape-vine (see **Table 1**).

From the second half of June to the second decade of August the control showed the active colonization of the young leaves by the phylloxera leaf form, as this took place, the viticide count grew more than 4.5 times.

The three-time elicitor treatment of the grape-vine plants helped to reduce the count of phylloxera leaf form from 65 to 80 percent. In the standard treatment variant the application of pesticides in June resulted in burns to form on the leaves (from Leaf 14 through 18), that had an adverse impact on the vines colonization by quarantine pest.

50 days after the last application of elicitors their biological efficiency of the phylloxera leaf form containment lowered, which was due to the young leaves growth.

The abiogenic elicitors were good for productional process and, as a consequence – for the yield of the grape-vine berries (see **Table 2**).

The elicitors helped the grape-vine plants to form the leaf surface of larger area, enabling to attain the larger yield as compared with a control. Thus, the elicitors enhance the grape-vine yield and in so doing save its quality due to the larger area of leaf surface and lesser phylloxera affection of roots.

The microscopy of the grape-vine leaves permitted to establish, that the elicitors raise the palisadity index of leaves, making for the better photosynthetic activity and resistance of the summer period abiotic stressors (see **Table 3**).

Table 3. Leaf plate biometric parameters of the Bianca grape-vine plants in the different variants of experiment (July-August of 2014-2015)

Variant of experiment	Leaf plate thickness	Palisade layer thickness	Spongiosa thickness	Upper epidermis thickness	Palisadity index
Control	129.3-144.3	59.1-65.2	60.1-67.7	10.1-11.4	0.96-0.98
Elicitors	132.7-142.7	66.2-67.2	54.2-54.5	10.1-10.9	1.02-1.20
Standard treatment	133.9-137.0	60.2-61.7	68.3-64.4	9.9-10.9	0.94-0.95

Table 4. Elicitors impact on the Bianca grape-vine yield and quality, 2014-2015

Variant	Number of bunches per vine, pcs.	Mass of bunch, g	Productivity kg	Yield, hwt/ha	Quality of grapes	
					Sugar content, %	Juice acidity, g/l
Control	52.4-75.0	50.5-80.7	3.8-4.23	49.4-55.0	21.1-22.8	6.0-6.9
Elicitors	51.6-75.0	55.5-85.4	4.16-4.41	50.7-57.3	20.2-23.0	5.8-6.3
Standard treatment	56.6-80.0	51.6-84.8	4.13-4.80	53.7-62.4	17.3-22.0	6.8-7.3
$HCP_{0,5}$	3.6	3.5	0.4	3.2	0.7	0.7

Table 5. The parameters of protein, proline and glycine content of the Bianca grape-vine plants' leaves

Variant of experiment	Proline content, mg/kg	Glycine content, mg/kg	Protein content, mg/r	Sum of free amino acids protein
control	68.9-82.7	5.6-2.9	24.02-20.15	10-10.1
elicitors	99.9-91.2	17.4-1.3	18.56-16.71	32-13
standard treatment	52.0-87.8	12.1-8.2	20.58-15.54	13-14

Table 6. The parameters of the Ca^{2+}, Mg^{2+} and abscisic acid content of the Bianca grape-vine leaves

Variant of experiment	Ca cations content, mg/kg	Mg cations content, mg/kg	Abscisic acid content, mg/kg
control	0.28-0.97	0.35-0.63	1.4-1.6
elicitors	0.36-0.92	0.45-0.63	2.7-3.6
standard treatment	0.33-0.94	0.41-0.72	0.8-4.2

The development of diseases, such as mildew, oidium, blackspot on the leaves, inflorescences and bunches in all variants of experiment was singular and affected neither yield, nor its quality (see **Table 4**).

The three-time application of elicitors allowed to enhance the grape-vine yield by 1.3 - 2.3 hwt/ha, and that of standard treatment – by 4.3 - 7.4 hwt/ha, that is caused by the gain in bunch weight by 4.7 – 5.0 g and 1.1 - 4.1 g, respectively, and in the number of bunches per vine in the standard treatment variant by 4.2 – 5.0 pcs., as compared with the control variant. The application of elicitors has no significant impact on the grape-vine quality, and in standard treatment variant the acidity of juice grows by 0.4- 0.8 g/l.

The metabolome estimate of the grape-vine plants immunity against the phylloxera root form allowed to establish, that immunoresistance is caused by the roots' higher content of glycine amino acid, being a part of GRP-proteins, and proline being a part of the PRP-proteins – the special structural proteins, reinforcing the cell walls on exposure to pathogene. [16, 18].

It is also worth pointing out, that proline exhibits the osmoprotector properties and improves the desiccation resistance of cells, and, as a consequence, the resistance of plants to abiotic stressors.

See **Table 5** for parametric characteristic of these measures according to results of 2014-2016.

The elicitors enhance the proline content of the grape-vine leaves. The content of glycine also grows in elicitors-application variants, prompting the suggestions that the cell walls became more durable. The reduction of protein content in elicitors-application variants implies the activation of its hydrolysis, as a result of which the ratio between the content of the free amino acids sum and protein content of the leaves on exposure to elicitors grows. The larger content of magnesium cations, stabilizing mRNA, facilitates the activation of protein synthesis in the grape-vine leaves in elicitors-application variants (see **Table 6**).

On exposure to the stress-factors (BAS, pathogenes, etc.) in cytosol of cells, the content of Ca^{2+} and abscisic acid, activating the cascades of protective metabolic reactions, grows [24, 25].

Thus, in the variants with elicitor-treated grape-vine not only protein synthesis but also its hydrolysis is activated, characterizing the display of defense reactions.

One of the productional process indicators is intensity of growth. The elicitors activate the growth of the grape-vine shoots, as compared with control and standard treatment, that involves the higher content of indoleacetic acid (IAA), stimulating growth, and the lower content of abscisic acid (ABA) – an inhibitor of growth (see **Table 7**).

Table 7. The parameters of endogenous phytohormones content of the grape-vine shoots

Variant of experiment	Length of shoot, cm	IAA, mg/kg	ABA, mg/kg	IAA/ABA
Control	41.3-83.1	8.7-17.7	0.8-1.6	10.9-11.1
Elicitors	58.2-121.7	9.8-20.5	1.1-2.7	7.6-8.9
Standard treatment	36.6-75.8	7.1-14.7	1.5-4.2	3.5-4.7

Table 8. The parameters of K^+ and carbohydrates content in the Bianca grape-vine leaves

Variant of experiment	Saccharose , mg/g	Glucose, mg/g	Fructose, mg/ha	Starch, mg/g	K, mg/g
Control	0.07-0.10	0.33-1.70	0.59-1.67	1.01-5.55	0.28-2.99
Elicitors	0.76-18.95	1.93-5.21	1.8-19.42	2.08-5.02	0.3-3.34
Standard treatment	0.39-1.50	0.43-3.12	0.56-3.03	3.13-4.64	0.15-2.61

Table 9. The parameters of the pigments content and effectiveness of the primary photosynthesis processes in the Bianca grape-vine leaves

Variant of experiment	Chlorophyll a, mg/g	Chlorophyll b, mg/g	Carotin, mg/g	Carotin chlorophyll (a+b)	Effectiveness of the primary photosynthesis processes
control	4.1-4.89	1.36-1.91	2.37-3.04	0.43-0.45	1.21-1.61
Elicitors	5.13-6.22	1.48-2.56	2.80-4.54	0.40-0.46	0.97-1.54
Standard treatment	4.43-4.94	1.39-1.83	2.56-2.71	0.41-0.44	1.28-1.79

Table 10. The parameters of ascorbic and phenolcarbonic acids content in the Bianca grape-vine leaves

Variant of experiment	Content of ascorbic acid, mg/kg	Content of chlorogenic acid, mg/kg	Content of caffeic acid, mg/kg
Control	12.4-162.1	19-320	0.9-43
Elicitors	17.4-149.9	91-280	3.1-.68
Standard treatment	8.5-155.4	39-310	2.8-72

Photosynthesis is a basic process, in which the dry substance of plants is formed and a first free sugar, forming in the process of photosynthesis is saccharose, from which nonphosphorylated monosugars are formed - glucose and fructose. The basic transport form of sugars in the grape-vine plants is glucose. In unfavourable conditions starch accumulates in the leaves.

Standard treatment and elicitors enhance the content of starch in the grape-vine leaves, in which case its hydrolysis, generating such sugars, as saccharose, glucose and fructose, starts to work in all variants of experiment (see **Table 8**).

At a time when the content of potassium cations grows in elicitors variant, as compared with a control and standard treatment, it is fair to assume the larger part of synthetical processes in elicitors variant and hydrolitic processes in all the rest of experiment variants. The rise of saccharose content in all variants, as compared with a control, characterizes a more active photosynthesis process in the plants, whereas the higher monosugars content – their greater attraction to the generative organs. The more active photosynthetic activity is witnessed by the rise of the pigments content in the grape-vine leaves (see **Table 9**).

In elicitors-application variants the content both of chlorophyll and carotenoids grows, in which case a standard treatment negates the effectiveness of the primary photosynthesis processes, whereas the elicitors enhance.

The gain in the content of ascorbic and phenolcarbonic acids (chlorogenic and caffeic) in elicitors-application variant enhances the cellular membranes' resistance to oxidative stress (see **Table 10**).

CONCLUSION

The elicitors activate the immune processes and photosynthetic activity in the grape-vine plants, which in conjunction with the better condition of the root system and activation of the roots growth creates the conditions to increase productivity, yield of grape-vine and its quality. The enhanced resistance of the grape-vine plants to affection both by the leaf and root form of phylloxera is caused by activation of metabolic processes, increase in the content of phenolcarbonic (chlorogenic and caffeic) acids in leaves, creating the unfavourable conditions for feeding of pest. The application of elicitors in the grape-vine cultivation technology is highly competitive by its biological efficiency with a standard treatment variant, involving application of the multiple crop protection chemicals. The application of elicitors permits to enhance the productivity of plants without sacrifice of berries quality and yield ecologically clean production.

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