COMMUNICATION

INFLUENCE OF STYLBENOIDS ON AGROBACTERIUM TUMEFACIENS

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ABSTRACT

A strain of Agrobacterium tumefaciens, isolated from the trunk of infected V. vinifera L. cv. Rkatsiteli was studied microscopically and its pathogenesis was established. The goal of the research was to study the role of stilbenoids on the bacterial growth. The bacterium strain was inoculated in two ways: a) the surface of the growth medium was covered by a watery suspension of stilbenoids; b) Stilbenoids was added to the growth medium before sterilization. In both experimental protocols the concentrations of the stilbenoids were: 1 mg/100 ml, 2 mg/100 ml, 3 mg/100 ml, 4 mg/100 ml, 5mg/100ml, 10 mg /100 ml, 15mg/100ml, 20 mg /100 ml and 30 mg /100 ml. The control version was the same medium without stilbenoids. The incubation period was 14-15 days at 270C and all the treatments completely (100%) inhibited Agrobacterium tumefaciens growth over the control. A second experiment was therefore set up in order to study the bacterial growth inhibition under stilbenoid concentrations lower than 1 mg/100 ml (ranging from 0.1 mg/100 ml to 0.9 mg/100 ml): the bacterial growth inhibition increased from 0.0 % to 88.0% by increasing the stilbenoid concentrations.

KEY WORDS
Vine, crown gall, stilbenoids

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INTRODUCTION

Vine and grape stilbenoids are one of the groups of a wide class of phenol compounds, which incorporates cis- and trans-isomers of resveratrol and their derivatives, as dimers, trimers, tetramers and glycosides [1-9]. Stilbenoids have diversified high biological activity and these compounds are very important for plants as phytoalexins. Stilbenoids act against different vine diseases caused by biotic factors. The following stilbenoids were identified in the extract of vine (*Vitis vinifera* L.) trunk, roots and canes: Ampelopsin A, (E)-piceatannol, Pallidol, E-resveratrol, hopeaphenol, isohopeaphenol, (E)-ε-viniferin, (E)-miyabenol C, (E) –w-viniferin, r- and r2-viniferin. It was established that the extract inhibits the growth of sporulation of fungus *Plasmopara viticola* by 50%, while the most active inhibitor of it turned out to be r2-viniferin [10]. Biotransformation of resveratrol, pterostilbene and a mixture of both occurred by the protein secretome of Botrytis cinerea; 21 analogous were obtained [11]. The reaction with pterostilbene afforded 5 new compounds while the reaction with a mixture of pterostilbene and resveratrol afforded 7 unusual stilbene dimers. The anti-fungal effect of these stilbenoids was evaluated against *Plasmopara viticola* [11]. At three stages of fruit development of *Vitis vinifera* L. cv. Huxelrebe and the hybrid Castor, the berries were in vitro infected by *Botrytis cinerea*, resulting in the synthesis of pterostilbene, (E)-ε-viniferin and trans-resveratrol, being (E)-ε-viniferin the most produced stilbenoid [12]. Berries of *Vitis vinifera* L. cv. Barbera were in vitro infected with conidial suspension of *Aspergillus japonicus*, *A. ochraceus, A. fumigatus* and *A. carbonarius* and the levels of ochratoxin A and stilbenoids were detected. All fungi except for *A. fumigatus* significantly increased the concentration of trans-resveratrol and at the same time trans-piceid was unaffected; only *A. ochraceus* was able to significantly increase the piceatannol concentration. Stilbenoids showed antifungal activity against *A. carbonarius* since trans-resveratrol (300 µg/g) and piceatannol (20 µg/g) totally inhibited the fungal growth [13]. Besides the above-mentioned biological activity stilbenoids have many other functional roles and they are affected by many viticultural factors [14-26]. The vine varieties of Georgia are rich in biologically active stilbenoids; trans-resveratrol, trans- ε-viniferin, 2 tetrameric stilbenes, including hopeaphenol, were isolated and identified from shoots of Rkatsiteli variety. These stilbenoids were identified in the Georgian red-grape wine varieties and their wines [26-29]. The study of stilbenoids in Georgian wine varieties in terms of qualitative and quantitative analyses, and their impact on bacterial and fungal disease is an urgent need. As a consequence, the goal of the paper is to test the role of stilbenoids on the activity of the crown gall agent Agrobacterium tumefaciens.

METHODS

Agrobacterium tumefaciens was isolated from infected trunks of 16-year-old vines of *V. vinifera* L., cv Rkatsiteli grafted on Kober 5BB, grown at a density of 2,850 vines/ha; the vineyard was located in Kakheti (Gurjaani) region of Eastern Georgia, at 600 m asl elevation, on alluvial soil. The pathogenic strain of *Agrobacterium tumefaciens* was first isolated in February 2018 and then modified in July 2018 and January 2019. Last modified pathogenic strain of Agrobacterium tumefaciens was applied to the experiment described in this article. The isolation was done by taking a little piece of the crown gall which was cleaned from the other microorganisms by ethyl alcohol. A suspension was then prepared and sowed in the culture media in petri dishes and the multiplication of bacteria colonies occurred, under standard conditions (24 hours at 27 °C) in petri dishes.

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The pathogenesis of bacterial strain was determined by the development on a carrot plant disc. The morphological characteristics of the strain were determined by gramm method on the base of microscopic analysis (Electronic microscope XSP-14).

The stilbenoids were extracted by acetone from air-dried vine pruning material, after grinding. The extract was concentrated in vacuo at 40 °C and then the stilbenoid containing fraction was isolated by column chromatography: a Sephadex G-25 gel filtration media was used, eluting with MeOH:H2O (60:40) and a concentration in vacuo occurred. The composition of the stilbenoid extract was detected by the method of high-performance liquid chromatography (HPLC/MS). A Varian chromatograph was utilized, equipped with a Supelcosil PM LC18 Column, 250 x 4,6 mm; eluents: A. 0,025% trifluoroacetic acid; B. Acetonitrile: A80/20. Gradient mode: 0-35 min, 20-50% B, 48-53min, 200% B. Flow rate of the eluent- 1 ml/min; wavelength-306 and 285nm. Isolated stilbenoid-containing fractions were filtered using a membrane filter (0,45μ) before the chromatographic procedure. The chromato-mass-spectral investigations were carried out under the above-mentioned conditions; mass-spectra were detected by obtaining positive ions.

The effect of stilbenoids on the activity of the bacterial strain was tested by two experimental approaches: A). A water suspension of the stilbenoids was used at different concentrations, as follows: 1.0-5.0 mg /100 ml, 10 mg /100 ml, 20 mg / 100 ml and 30 mg /100 ml; POTATO DEXTROZE AGAR was used to multiply the bacterial strain: 20 ml of steril PDA medium were placed in Petri dishes. Their surface was covered by the water suspension of the stilbenoids and then the bacterial strain was incubated. The incubation period was 14-15 days at 270C. Water without stilbenoids was used in some petri dishes, as a control. B). The stilbenoids were directly added into the medium (PDA) at different concentrations, as follows: 1.0-5.0 mg /100 ml, 10 mg /100 ml, 20 mg /100 ml and 30 mg /100 ml. PDA without stilbenoids was used as control. The incubation conditions were the same as in approach A.

After the analysis of the results a second experiment was set up, in order to test the role of lower stilbenoids concentrations (0.1 to 0.9 mg/100 ml) on the bacterial growth inhibition.

RESULTS

Based on morphological study, the Agrobacterium tumefaciens strain resulted gram-negative with a Stiff form. It multiplied both as units and groups and had a flagellum giving it the ability to move [Fig. 1].

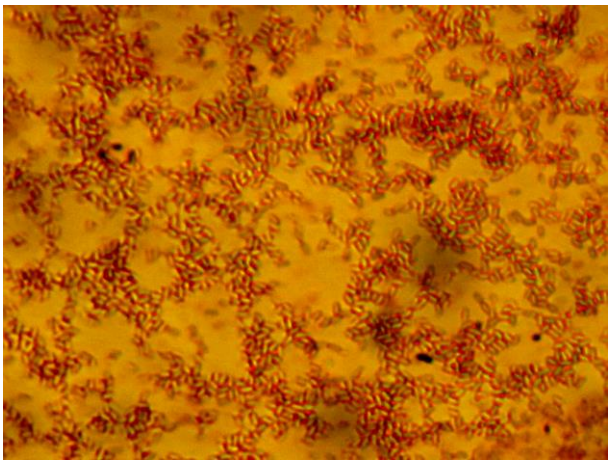

Fig. 1: Microscopic photograph of the *Agrobacterium tumefaciens* strain

The composition of the vine stilbenoids was represented by trans-resveratrol and its derivatives, as follows: trans-resveratrol 80.1%; trans- ε –viniferin 7.1%; dimers, trimers and tetramers 12.8%. The growth of the Agrobacterium tumefaciens strain was completely (100%) inhibited by the stilbenoids at concentration higher than 1 mg /100 ml. According to the Table the bacterial growth inhibition increased by increasing the stilbenoid concentrations from 0 to 0.9 mg/100 ml. Inhibition occurred at 0.2 mg/100ml, being 8%, and increased up to 1.0 mg/100 ml, being 100%.

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Table 1: *Agrobacterium tumefaciens* growth inhibition and multiplication rate depending on the stilbenoid concentration

N	Stilbenoids concentration mg/100ml	Bacterial growth inhibition %	Bacterial multiplication %
1	0.0	0	100
2	0.1	0	100
3	0.2	8	92
4	0.3	20	80
5	0.4	30	70
6	0.5	40	60
7	0.6	52	48
8	0.7	60	40
9	0.8	75	25
10	0.9	88	12
11	1.0	100	0

CONCLUSION

Stilbenoids from the vine trunk were effective in reducing the development of a pathogenic strain of Agrobacterium tume faciens, at a concentration above 1 mg/100 ml. The research result is very important for Georgian vine grape varieties for further researches on the role of stilbenoid phytoalexins on vine disease resistance.

CONFLICT OF INTEREST
There is no conflict of interest.

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FINANCIAL DISCLOSURE
None.

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