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Occurrence of stilbenoids in grapevine under Crown gall infection

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A B S T R A C T

It is established occurrence of phytoalexin stilbenoids in white and red grape varieties infected with crown gall disease (Agrobacterium tumefaciens) in Georgia. The infected vine varieties – Rkatsiteli, Saperavi, Cabernet Sauvignon, Tsitska and Tsolikouri were identified in East and West regions of Georgia. Healthy vine varieties were taken from the same vineyards for studying. Stilbenoid-containing fractions were isolated from the infected and healthy vine trunks and was identified their stilbenoids profile. It is studied the variation of the physiological concentration of stress- metabolite stilbenoids – trans-resveratrol and trans -ε- viniferin in terms of crown gall disease. It is identified , that occurrence of the physiological concentration of stilbenoids -trans-resveratrol and trans -ε- viniferin in vine infected with crown gall disease depends on variety factor. The obtained results are important data to identify the correlation of the immunity of the grape varieties to the phytoalexin stilbenoids.

Keywords: Vine, Phytoalexin, Stilbenoids, Trunk, Crown gall, Grape varieties.

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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 monomer resveratrol and their derivatives, as dimmers, 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) trunk, roots and annual shoots: 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]. Under the influence of Botritis cinerea on the mixture of Pterostilben and Resveratrol 7 new stilbens were formed, while 5 new stilbens were formed from Pterostilben under the same terms. The anti-fungus effect of these stilbenoids was fixed against Plasmopara viticola [11]. At three stages of the grape (Vitis vinifera) grain development, the grains were infected on purpose with Botritis cinerea spores „in vitro“. In the infected grain, stilbenoids: Pterostilben, (E)-ε-viniferin and trans-resveratrol were fixed. Dominating among them was (E)-ε-viniferin. [12] The grains of Vitis Vinifera L. cv. Barbera in the ripening period were infected with conidial suspension of Aspergilus jannicus, A.ochraceus, A. fumigatus and A.carbonariuces. The process of formation of ochratoxin A and stilbenoids was supervised. It was found out that all experimental fungi except A. Fumigatus significantly increase the concentration of trans-resveratrol and at the same time, trans-Piceid stays unchanged. In the grape grain damaged by A.ochraceus, the concentration of piceatannol increased significantly. A large amount of A.carbonariuce was synthesized in the grain infected with A.carbonariuces isolate and

the anti-fungicidal activity occurred with the following concentrations: 300 mkg/gr and 20 mkg/gr, what was sufficient for the total inhibition of fungus A.carbonariuces [13]. Besides above mentioned biological activity stilbenoids have many other functional purposes[14-22]. The vine and grape impacts some factor[23-26]. The vine varieties of Georgia are rich in biologically active stilbenoids. trans-resveratrol, trans- ε-viniferin, 2 tetrameric stilbens, including hopeaphenol as one of them, were isolated and identified from an annual shoot of Rkatsiteli variety. These stilbenoids and new stilbenoids identified by us were identified in the Georgian red-grape vintage varieties and their wines [27-31]. The study of stilbenoids in Georgian vintage varieties as that of phytoalexins, qualitative and quantitative analyses of their physiological concentrations and stress-metabolites and their impact on the microorganisms causing bacterial and fungus diseases is an urgent issue of the research. Consequently, our goal was to identify the vine varieties infected with crown gall disease, identify and determine their stress-metabolite stilbenoids and compare them with healthy vine stilbenoid profile.

Objects and Methods

We used healthy trunks of white- and red-grape vine varieties and those infected with crown gall disease from the same vineyard as study objects. Simples were taken in period February – march in 2018 year. Rkatsiteli from Gurjaani region (Alluvial soil; vineyard 15-16 old); Saperavi from Kvareli region(Alluvial soil; vineyard 15-16 old); Cabernet Sauvignon from Akhmeta region(Cinamonic soil; vineyard 15-16 old); Tsitska and Tsolikouri from Zestaponi region(Yellow- brown Forest soil; vineyard 10-11 old) (Fig.1). For analyzing we used vertical parts of the trunks. We isolated stilbenoid-containing fractions from the healthy and infected vine trunks as a result of treatment according to the chart (Fig. 2).

Stilbenoids were determined by the method of high-performance liquid chromatography (HPHC) [9]. For this purpose, we used the Varion chromatograph SupelcosilPM LC18 Column, 250*4,6mm, 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. Analyzed samples: isolated stilbenoid-containing fractions were filtered using a membrane filter (0,45μ) before the chromatographic procedure. The chromate-mass-spectral investigations were carried out under the above-mentioned conitions; mass-spectra were detected by obtaining of nositive ions.

Results and Discussion

Ttrans-resveratrol and trans-ε-viniferin were identified as dominants in the stilbenoid profile trunks of healthy study vine varieties(fig.3). Therefore, in the present article we considered it purposeful to describe the quantitative variability of the said stilbenoids following the infection with crown gall disease. The physiological concentrations of trans-resveratrol and ε-viniferin in different varieties of healthy vine trunks vary a lot. The highest concentrations of trans-resveratrol of 7,8 g/kg were fixed in the vine trunk of Rkatsiteli variety, while the lowest concentration was fixed in the vine trunks of Tsitska and Tsolikouri of 0,83 g/kg and 0,81 g/kg, respectively. At the same time, it should be noted that the concentration of trans-ε-viniferin in the vine trunk of Tsitska and Tsolikouri much exceeds the concentration of trans-resveratrol. At the same time, the vine trunks of Rkatsiteli, Saperavi and Cabernet Sauvignon show much higher concentration of trans-resveratrol than the concentration of trans- εviniferin. Different results were obtained with the concentration changes of trans-resveratrol and trans-ε-viniferin, as stress-metabolites in the vine infected with crown gall disease. In particular, in Saperavi, the concentration of trans-resveratrol decreased from 2,6 g/kg to 0,85g/kg,while at the same time, the concentration of trans-ε-viniferin increased from 0,81g/kg to 2,1 g/kg; in Cabernet Sauvignon infected with crown gall disease, the concentration of trans-resveratrol decreased from 4,8 g/kg to 3,2gr/kg; at the same time, the concentration of trans-ε-viniferin also decreased from 2,2 g/kg to 1,5 gr/kg; infected Rkatsiteli variety with vine crown gall disease resulted in the reduction of the concentration of trans-resveratrol from 7,8 g/kg to 5,7g/kg and a quantitative increase in the amount of trans-ε-viniferin from 0,26 g/kg to 1,2 g/kg; in Tsitska infected with crown gall disease, the concentrations of both, trans-resveratrol and trans-εviniferin increased. In the trunk of Tsolikouri infected with crown gall disease, the concentration of the given stilbenoids showed a less quantitative reduction (Fig. 4,5).

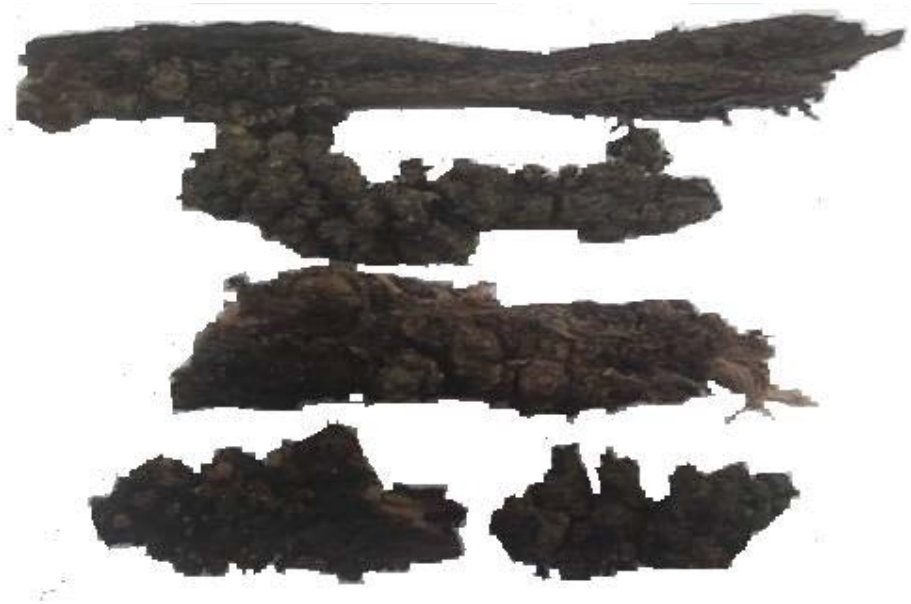


Fig. 1. *The trunk of Vine (Tsitska) infected by crown gall disease.*

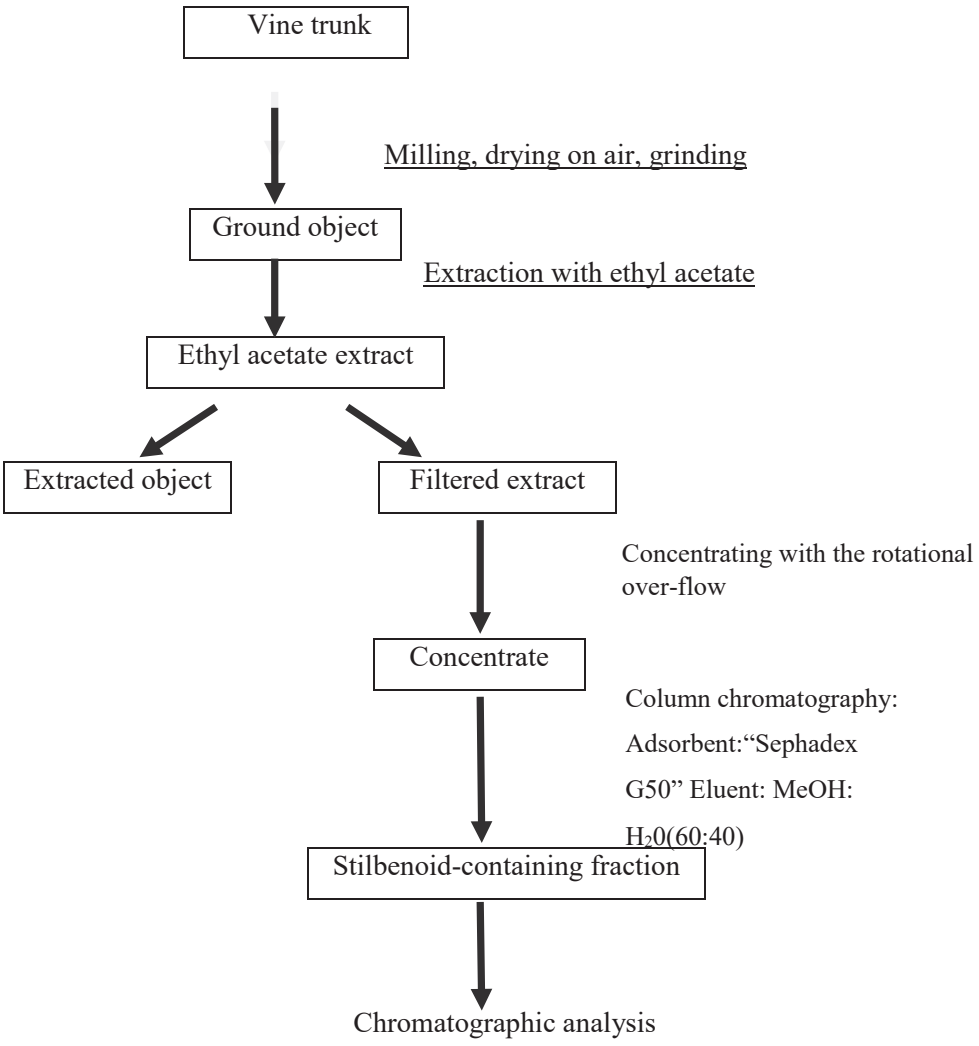


Fig. 2. *Chart of isolating a stilbenoid-containing fraction from vine trunk*

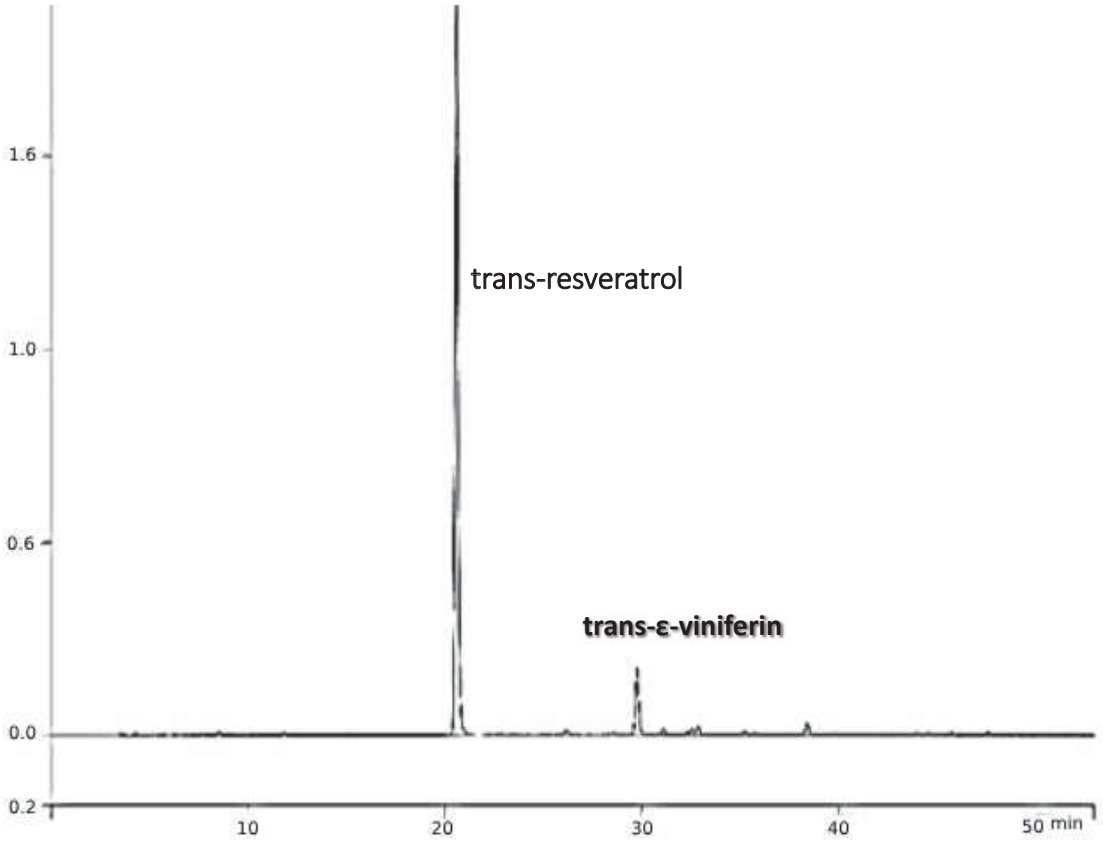


Fig. 3. *HPLC of stilbenoid-containing fraction of Vine trunk of Healthy Rkatsiteli*

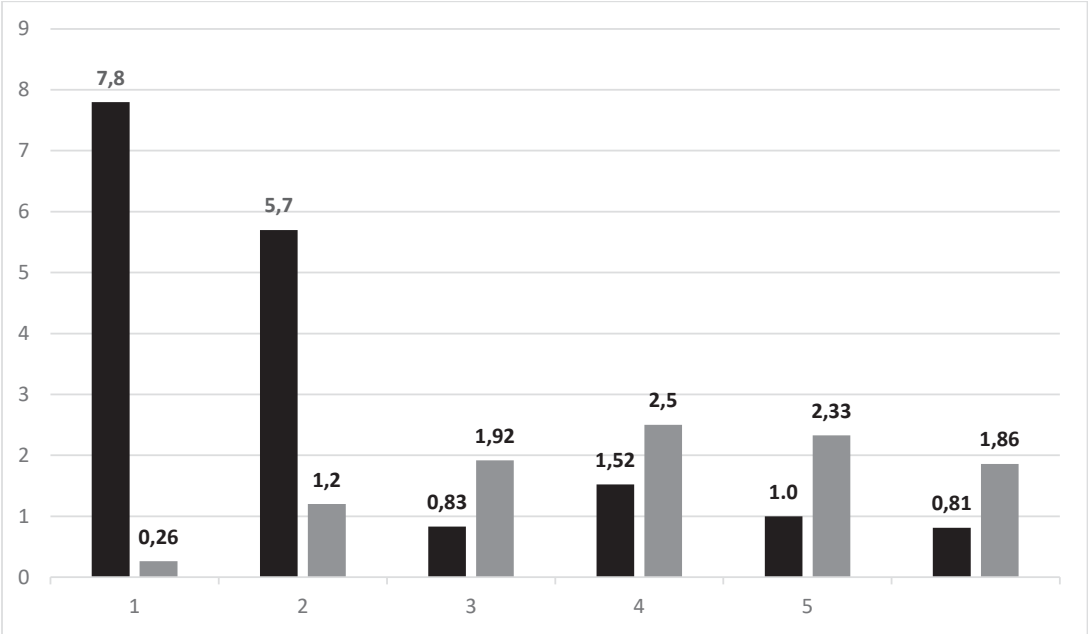


Fig. 4. *Quantitative variation of trans-resveratrol and ε-viniferin (g/kg) in white-grape vine varieties infected with crown gall disease. 1. Healthy Rkatsiteli, 2. Infected Rkatsiteli, 3. Healthy Tsitska, 4. Infected Tsitska, 5. Healthy Tsolikouri, 6. Infected Tsolikouri .*

-trans – Resveratrol, *- trans – ε-Viniferin*

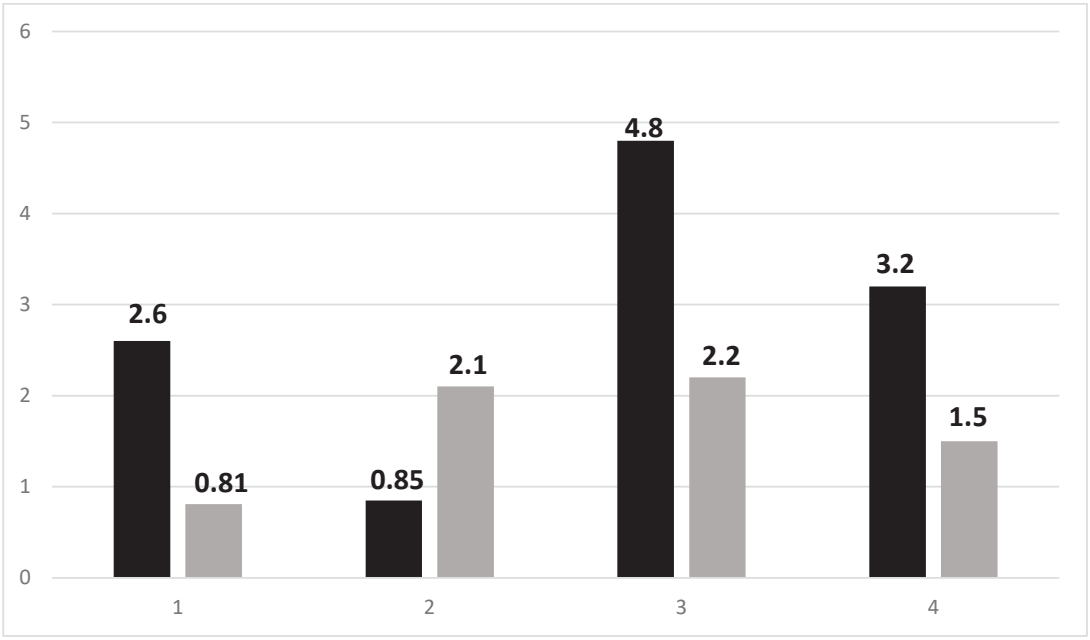
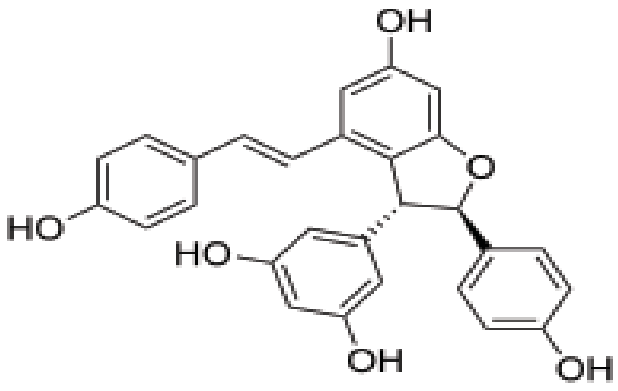
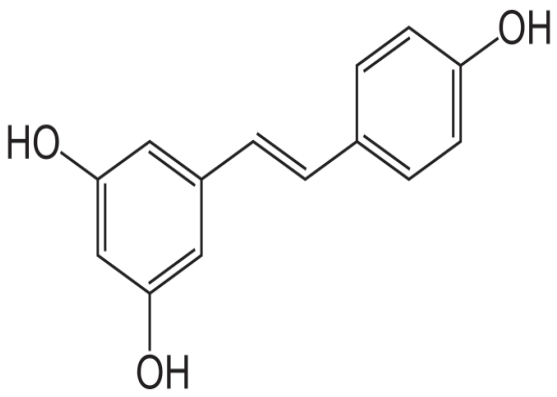


Fig. 5. *Quantitative variation of trans-resveratrol and ε-viniferin (g/kg) in red-grape vine. 1. Healthy Saperavi, 2. Infected Saperavi, 3. Healthy Cabernet Souvignon, 4. Infected Cabernet Souvignon.*

- trans – Resveratrol, *- trans – ε-Viniferin*



Trans-Ɛ-viniferin



Trans-resveratrol

Conclusion

Thus, as the accomplished study revealed the white- and red-grape vine varieties spread in Georgia: Rkatsiteli, Saperavi, Cabernet Sauvignon, Tsitska and Tsolikouri are characterized by the relevant phytoalexin-stilbenoid profile. The relevant vine varieties infected with crown gall disease differ with the qualitative and quantitative contents of stilbenoids. The variation of the physiological concentrations of stress-metabolite stilbenoids: trans-resveratrol and ε-viniferin in the vine infected with crown gall disease takes place under the influence of the generic factor. In particular, in Saperavi and Rkatsiteli vine varieties, the concentration of trans-resveratrol decreased and the concentration of ε-viniferin increased; in Tsitska infected with crown gall disease, the concentration of both phytoalexins increased; in Tsolikouri and Cabernet Sauvignon vine varieties, the amount of trans-resveratrol and ε-viniferin decreased. One of the reasons for such a quantitative reduction is the formation of other stress-metabolites of the derivatives of these compounds what helps the vine to fight against Agrobacterium tumefaciens. The obtained results are important data to establish the correlation of the vine varieties immunity with phytoalexins stilbenoids.

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