

Change of phytoalexins -stilbenoids of vine leave Tsitska variety (*Vitis vinifera* L.) in condition Downy mildew

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Abstract

It is investigated change of phytoalexin-stilbenoids in the leaves of white grapevine Tsitska infected by Downy mildew. The Tsitska is cultivated in the west part of Georgia. Comparing the healthy and diseased grapevine leaves physiological concentrations of stilbenoids, in the infected grapevine leaves it is established following stress-metabolite stilbenoids: trans-resveratrol and ϵ -viniferin. The stress-metabolite stilbenoids in the conditions of the disease with Downy mildew is a scientific novelty for the vine leave Tsitska variety. The results of the research are important for determination the correlation of the vine immunity with the phytoalexins-stilbenoids.

Keywords: phytoalexin-stilbenoids, Tsitska, Downy mildew

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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 dimers, trimers, tetramers and glycosides (Aaviksaar et al. 2003, Bavaresco et al. 2002, Guebailia et al. 2006, Larronde et al. 2005, Niesen Daniel 2013, Pawlus et al. 2012, Reniero et al. 1996, Rivière et al. 2012, Waffo Teguo et al. 1998) Stilbenoids have diversified high biological activity and these compounds are very important for plants, as phytoalexins. Phytoalexin characteristics of grape vine is established by us in "in vitro" experiments by their inhibitory impact on the microorganisms, which are due to grape vine bacterial and fungus diseases. These diseases are: Grey mildew (*Botrytis cinerea*), Downy mildew (*Plasmopara viticola*), Powdery mildew (*Uncinula necator*), crown gall (*Agrobacterium tumefaciens*) (Bezhuashvili et al. 2019a, 2019b). 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 (Gabaston et al. 2017). Under the influence of *Botrytis 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* (Gindro et al. 2017). At three stages of the grape (*Vitis vinifera*) grain development, the grains were infected on purpose with *Botrytis 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 (Bavaresco et al. 1997). The grains of *Vitis Vinifera* L. cv. Barbera in the ripening period were infected with conidial suspension of *Aspergillus 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⁻¹ and 20 mkg gr⁻¹, what was sufficient for the total inhibition of fungus *A.carbonariuces* (Bavaresco et al. 2003). Besides above mentioned biological activity

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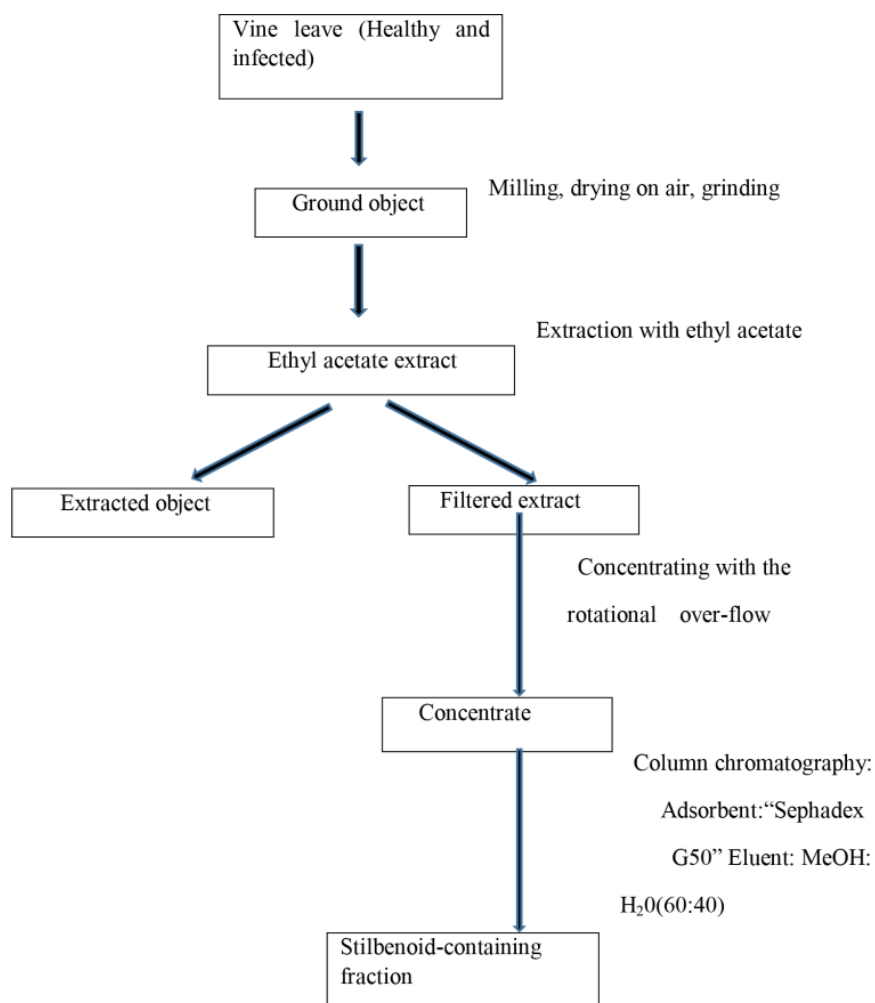


Fig. 1. Chart of isolating a stilbenoid-containing fraction from vine leaf

stilbenoids have many other functional purposes (Bavaresco and Fregoni 2001, Bavaresco et al. 2003, Gabastoni et al. 2018, Jeandet et al. 2002, Langcake and Pryce 1976, Langcake et al. 1979, Langcake 1981, Mattivi et al. 2011, Sáeza et al. 2018). The vine and grape impacts some factor (Adrian et al. 2000, Bavaresco 2003, Bavaresco et al. 2007, Vergara et al. 2012). 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 (Bezhuashvili 1994, Bezhuashvili and Surguladze 2016, Bezhuashvili et al. 2013, Surguladze and Bezhuashvili 2017, 2018). 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. It is established change of stilbenoids healthy and infected (95% and 50%) vine leaves and canes from 35 years old vineyard of cabernet franc (france). It is identified *E-piceatannol*, *E-resveratrol*, *E-ε-viniferin*, *ampelopsin A*, *E-miyabenol C*, *E-vitisin B*, *hopeaphenol*, *isohopeaphenol*. In infected grape skin was identified high quotation of *E-ε-viniferin* to compare with *E-resveratrol*. To point of view of authors: "These findings suppose that the health status in vineyards could modify the composition of stilbenoids in winter-harvested grape canes and subsequently the potential biological properties of the valuable extract (Houlline et al. 2015).

Research Aim

Research aim was to establish vine sort of *tsitska* leaves correlation with phytoalexin stilbenoid change infected by downy mildew.

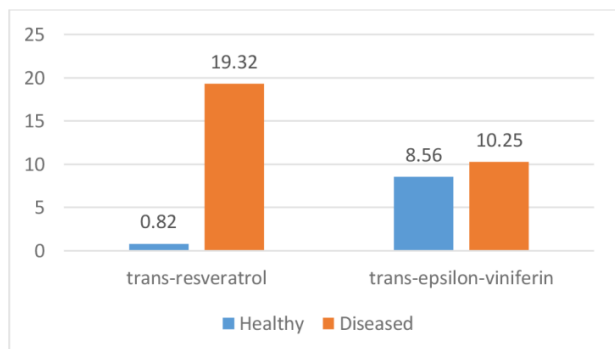


Fig. 2. Change of concentration (mg/kg) of trans-resveratrol and trans-epsilon-viniferin in condition Downy mildew in the leaves of Tsitska variety

MATERIALS AND METHODS

Research objects were healthy and infected by downy mildew leaves of Georgian white grape tsitska in west part of Georgia (Zestafoni region). Vineyards are located raw humus calcareous-rendzic-leptosols type of soil 30 years of vine. We isolated stilbenoid-containing fractions from the healthy and infected vine leave as a result of treatment according to the chart (**Fig. 1**).

Stilbenoids were determined by the method of high-performance liquid chromatography (HPLC) (Guebailia et al. 2006). For this purpose, we used the Varion chromatograph SupelcosilPM LC18 Column, 250x4,6mm, 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. The samples were analyzed three times and it is presented average results. Analyzed samples: isolated stilbenoid-containing fractions were filtered using a membrane filter (0,45 μ) before the chromatographic procedure. The chromat-mass-spectral investigations were carried out under the above-mentioned conditions; mass-spectra were detected by obtaining of positive ions.

RESULTS AND DISCUSSION

During the experiment it was determined quantitative change of phytoalexin-stilbenoids in condition of infected by Downy mildew vine leaves. Depending on the received results established main stress-metabolite stilbenoids: trans-resveratrol and trans- ϵ -viniferin. As diagram presents the physiological concentration of both compounds are increasing in condition downy mildew infect,in concret: trans-resveratrol 0.82 mg/kg-19.32 mg/kg and trans- ϵ -viniferin 8.56mg/kg-10.25mg/kg.

CONCLUSION

Established first researched results is scientific novelty for vine leave Tsitska variety. Belong on the basic of this and future research results will be found out the correlation of vine Tsitka variety with phytoalexins - stilbenoids.

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