

Stress-metabolites phytoalexins -stilbenoids of grape skin Rkatsiteli variety (*Vitisvinifera* L.) in condition gray mildew

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Abstract

It has been studied Change of phytoalexins - stilbenes of white wine variety Rkatsiteli grape skin in the condition of the disease gray mildew (*botrytis cinerea*). The samples of health and infected grapes –with 60% gray mildew, were taken in 2018 year during the technical maturity, from the same vineyard planted in eastern Georgia. The vineyard soil belongs to meadow cinnamonic – Calcaricambisols and calcic kastanozem type. Vineyard is 16 years old. Based on the HPLC/MS analysis are established the stilbenoids profiles of healthy and infected skins and has been revealed Stress-metabolite stylbenoids - accumulated as a result of the action of botrytis cinearea. The dominant stress-metabolites Stilbenoides are resveratrol and its derivatives: trans-resveratrol, trans-piceid, cis-piceid, trans-piceatannol, trans-ε-viniferin. The variability of these stilbenoids concentrations in the condition of the disease -gray mildew, is different: trans-resveratrol 39.27mg kg⁻¹→57.33mg kg⁻¹; trans-piceid 13.72mgkg⁻¹→29.43mgkg⁻¹ ; trans-piceatannol 5.37mg kg⁻¹→19.45mg kg⁻¹; trans-ε-viniferin 7.22 mg kg⁻¹→5.13mg kg⁻¹. The stress-metabolite stilbenoids in the conditions of the disease with gray mildew, is a scientific novelty for the grape of Rkatsiteli variety. The results of the research are important for determination the correlation of the vine immunity with the phytoalexins-stilbenoids.

Keywords: grape, Stilbenoids, gray mildew, rkatsiteli.

Bezhushvili M, Tskhvedadze L, Surguladze M, Shoshiashvili G, Kharadze S, Gagunashvili L, Elanidze L, Vashakidze P (2019) Stress-metabolites phytoalexins -stilbenoids of grape skin Rkatsiteli variety (*Vitisvinifera* L.) in condition gray mildew. Eurasia J Biosci 13: 1-6.

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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. Stilbenoids act against different vine diseases caused by biotic factors. The following stilbenoids were identified in the extract of vine (*Vitisvinifera*) 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 *Plasmoparaviticola* by 50%, while the most active inhibitor of it turned out to be r2-viniferin

(Baydin et al. 2017, Gabaston, et al. 2017). Under the influence of *Botritiscinerea* 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 *Plasmoparaviticola* (Gindro et al. 2017). At three stages of the grape (*Vitisvinifera*) grain development, the grains were infected on purpose with *Botritiscinerea* 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 *VitisVinifera* cv. Barbera in the ripening period were infected with conidial suspension of *Aspergillusjannicus*, *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

Received: July 2018

Accepted: February 2019

Printed: April 2019

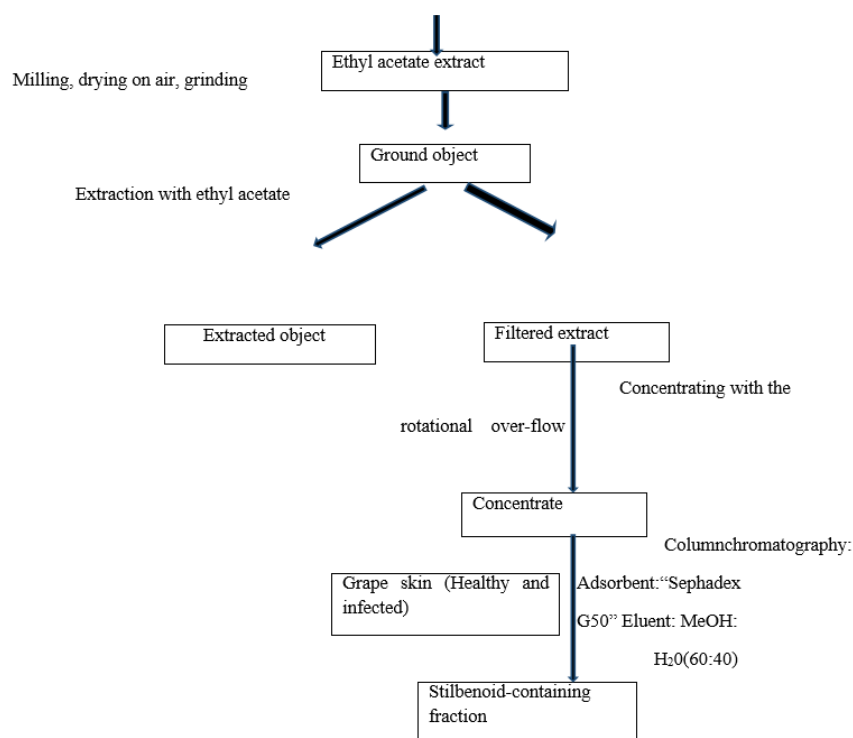


Fig. 1. Chart of isolating a stilbenoid-containing fraction from grape skin

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. carbonarius* was synthesized in the grain infected with *A. carbonarius* isolate and the anti-fungal activity occurred with the following concentrations: 300 mkgr^{-1} and 20 mkgr^{-1} , what was sufficient for the total inhibition of fungus *A. carbonarius* (Bavaresco et al. 2003). Besides above-mentioned biological activity stilbenoids have many other functional purposes (Bavaresco et al. 2003, Bavaresco and Fregoni 2001, Gabastoni et al. 2018, Jeandet et al. 2002, Langcake 1981, Langcake and Pryce 1976, Langcake et al. 1979, Mattivi et al. 2011, Sáeza et al. 2018). The vine and grape impact 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 et al. 2013, Surguladze and Bezhuashvili 2016, 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 (Alavi et al. 2013, Houlline et al. 2015).

Reason of study was to identify of stress-metabolites stilbenoids of grape skin Rkatsiteli variety (*Vitis Vinifera*) in condition gray mildew.

MATERIALS AND METHODS

Objects of research were health and infected white grape skin of Rkatsiteli variety. The samples of health and infected grapes were taken in 2018 year during the technical maturity, from the same vineyard planted in eastern Georgia. The vineyard soil belongs to meadow cinnamonic –Calcaricambisols and calcic kastanozemstype. Vineyard is 16 years old.

We isolated stilbenoid-containing fractions from the healthy and infected grape skin as a result of treatment according to the chart (Fig. 1)

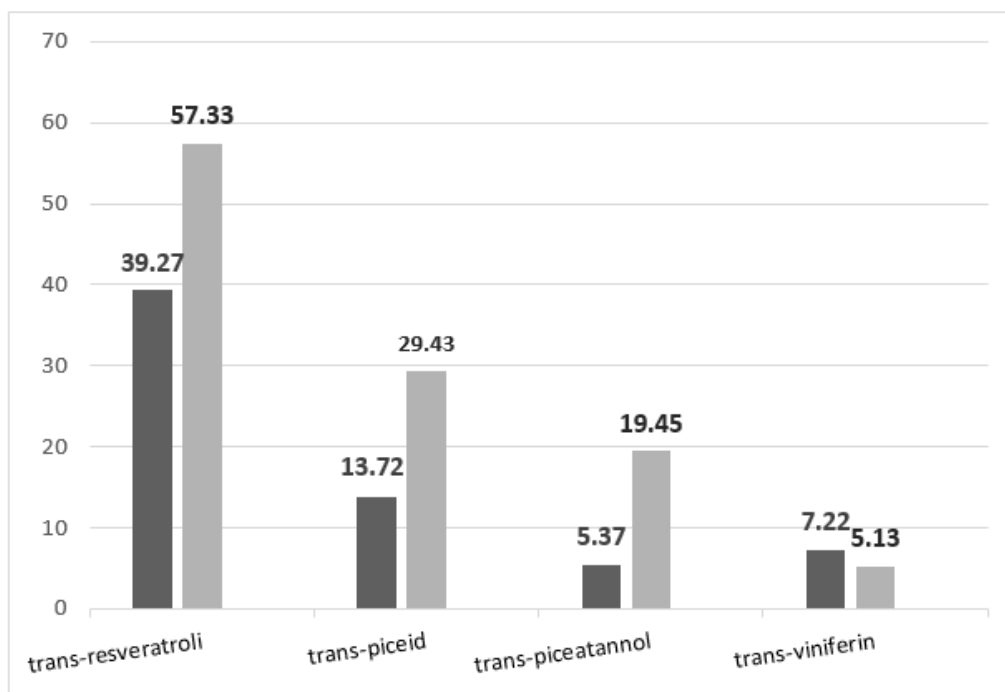


Fig. 2. change of stilbenoids of grape skin (mg kg⁻¹) Rkatsiteli in condition gray mildew.

■ - Health skin ■ - infected skin

Stilbenoids were determined by the method of high-performance liquid chromatography (HPLC) (Guebailia et al. 2006). For this purpose, we used the Varian 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 (0.45μ) 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

First of all, we need to mentioned our previous research results: In georgian red grape variety stilbenoids concentration is much higher compare to Georgian Wight wine grape variety. This total characteristic of Rkatsiteli wine grape variety describes low containing stilbenoids in the skin. It was identified in the health grape skin fraction resveratrol and its derivatives: glucosides, dimeric and trimeric stilbenoids. In concert: trans-resveratrol, (M+H)⁺-229.1; trans-piceid (M+H)⁺-391.1; trans-ε-viniferin (M+H)⁺-455.1; trans-piceatannol (M+H)⁺-245.2; trimeric stilbenoids (M+H)⁺-679.2; miyabenol C (M+H)⁺-681.2). Among identified stilbenoids dominant was trans-resveratrol. From above mentioned stilbenoids quantitation change suffered and

revealed following stress- metabolites in condition gray mildew of grape skin: trans-resveratrol, trans-ε-viniferin, trans-piceid, trans-piceatannol. To the action of Botrytis cinerea concentration of trans-resveratrol grow 39.27mg kg⁻¹→57.33mg kg⁻¹; trans-piceid 13.72 mg kg⁻¹→29.43mg kg⁻¹; trans-piceatannol 5.37mg kg⁻¹→19.45mg kg⁻¹; difference is trans-ε-viniferin, which concentration becomes lower 7.22 mgkg⁻¹→5.13mg kg⁻¹.

CONCLUSION

Established first researched results is scientific novelty for grape Rkatsiteli variety. Based on the basis of this and future research results will be found out the correlation of vine Rkatsiteli variety with phytoalexins – stilbenoids.

ACKNOWLEDGEMENT

This work was supported by Shota Rustaveli National Science Foundation of Georgia (SRNSFG) (grant number FR 17_486).

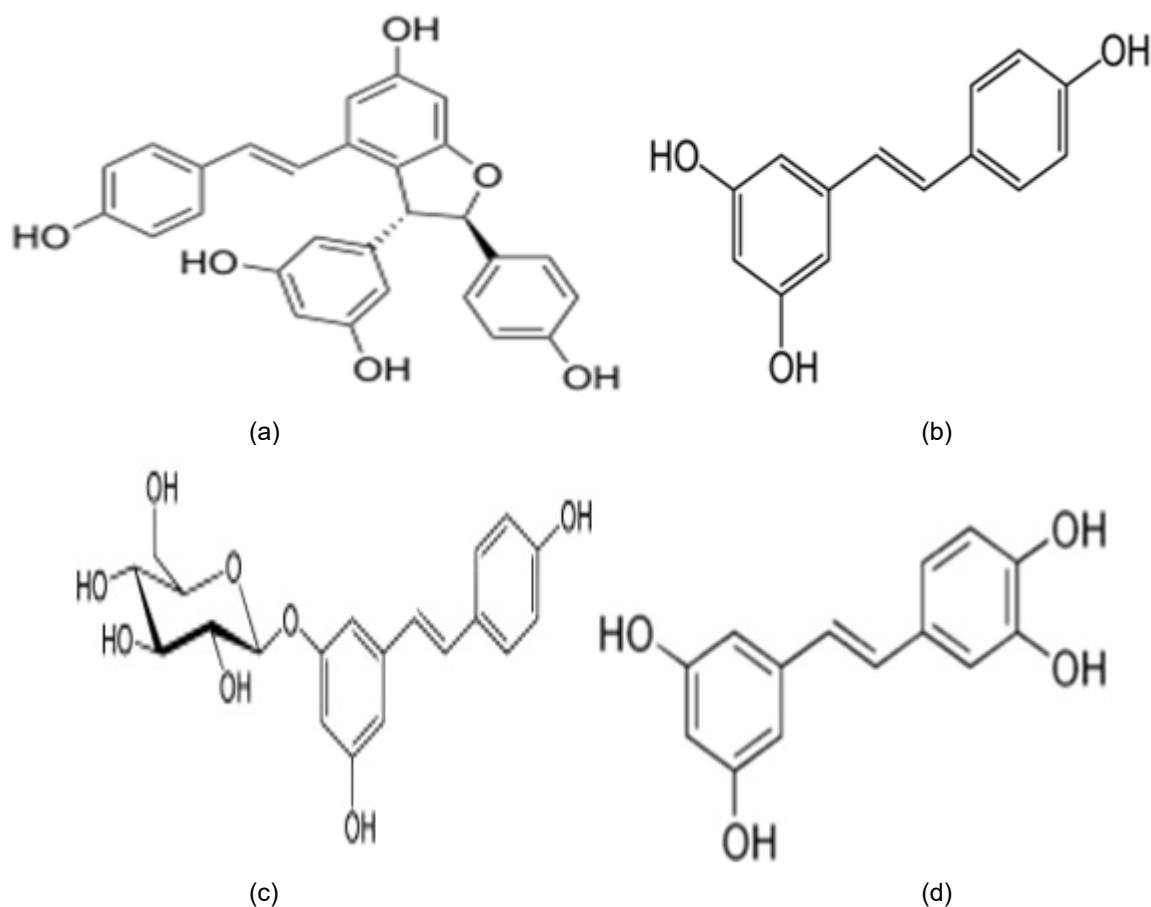


Fig. 3. a) Trans- ϵ -viniferin b) Trans-resveratrol c) trans-piceid d) trans-piceatannol

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