



## Change of Phytoalexins- Stilbenoids of grape skin Tsolikouri variety (*Vitis vinifera* L.) in condition Grey mildew

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### Abstract

It is investigated change of phytoalexins-stilbenoids in the Skin of white grapevine Tsolikouri infected by Grey mildew. The Tsolikouri is cultivated in the west part of Georgia (Zestafoni region). Vineyards are located raw humus calcareous-rendzic-leptosols type of soil 30 years of vine. Comparing the healthy and diseased grapevine Skins physiological concentrations of stilbenoids, in the infected grapevine Skin it is established following stress-metabolite stilbenoids: trans-resveratrol, ε-viniferin, trans-piceid, cis-piceid, trans-piceatannol. The stress-metabolite stilbenoids in the condition of the disease Grey mildew, is a scientific novelty for the grape skin Tsolikouri variety. The results of the research are important for determination the correlation of the vine immunity with the phytoalexins-stilbenoids.

**Keywords:** grape, stilbenoids, phytoalexins

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### INTRODUCTION

Vine stilbenoids characterized as an active phytoalexins. It is studied by us stilbenoids physiological concentration change in condition of bacterial (*Agrobacterium tumefaciens*) and fungicidal (*Botrytis cinerea*, *Plasmopara viticola* Bern., *Uncinula necator*) diseases of Georgian wine grape variety. After this it is established stress- metabolite stilbenoids (Bezhuashvili et al. 2019). Against this diseases vine stilbenoids are important inhibitors.

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 (Aaviksaar et al. 2003, Bavaresco et al. 2002, Guebailia et al., 2006, Larronde et al. 2005, Niesen Daniel et al. 2013, Pawlus et al. 2012, Reniero et al. 1996, Rivière et al. 2012, Waffo 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 (*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

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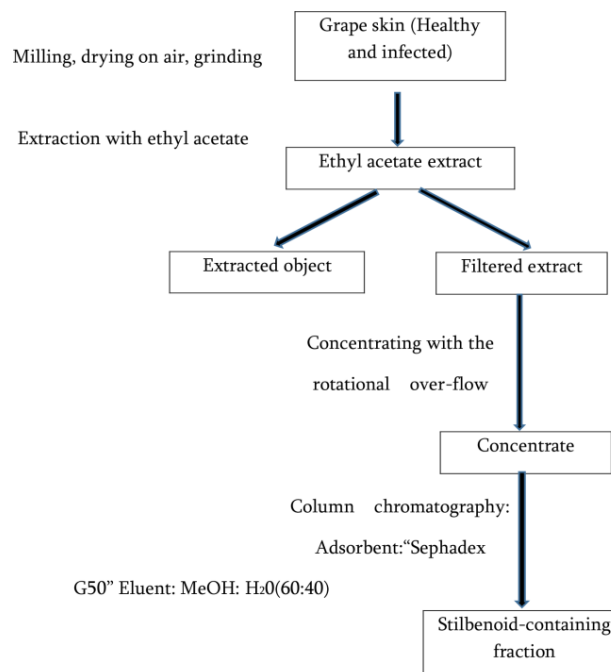
the same time, trans-Piceid stays unchanged. In the grape grain damaged by *A.ochraceus*, the concentration of piceatannol increased significantly. A large amount of *A.carbonariuce* was synthesized in the grain infected with *A.carbonariuces* isolate and the anti-fungicidal activity occurred with the following concentrations: 300 mkg/gr and 20 mkg/gr, what was sufficient for the total inhibition of fungus *A.carbonariuces* (Bavaresco et al. 2003). Besides above mentioned biological activity stilbenoids have many other functional purposes (Sáeza et al. 2018, Gabastoni et al. 2018, Langcake and Pryce 1976, Langcake et al. 1979, Langcake 1981, Bavaresco and Fregoni 2001, Mattivi et al. 2011, Jeandet et al. 2002, Bavaresco et al. 2003). 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-  $\epsilon$ -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, 2019a, 2019b, 2019c). 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- $\epsilon$ -viniferin, ampelopsin A, E-miyabenol C, E-vitisin B, hopeaphenol, isohopeaphenol. In infected grape skin was identified high quotation of E- $\epsilon$ -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 vinter-harvested grape canes and subsequently the potential biological properties of the valuable extract (Bezhuashvili et al. 2019d, Bezhuashvili and Surguladze 2016, Houillé et al. 2015, Surguladze and Bezhuashvili 2017, 2018).

### Reason of Study

Reason is to identify of stress- metabolites stilbenoids of grape skin Tsolikouri variety (*Vitis Vinifera* L.) in condition gray mildew.

### Objects and Methods

Objects of research were health and infected white grape skin of Tsolikouri variety. The samples of health and infected grapes were taken in 2019 year during the



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

technical maturity, from the same vineyard planted in west Georgia (Zestafoni region). The vineyard soil belongs to meadow calcareous-rendzic-leptosols type. Vineyard is 30 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)

Stilbenoids were determined by the method of high-performance liquid chromatography (HPLC) (Bavaresco et al. 1997). 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. 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 $\mu$ ) before the chromatographic procedure. The chromat-mass-spectral investigations were carried out under the above-mentioned conitions; mass-spectra were detected by obtaining of nositive ions.

## RESULTS AND DISCUSSION

First of all we need to mentioned our previous research results: In georgian red grape variety stilbenoids concentration is much more higher compare to Georgian Wight wine grape variety. This total characteristic of Tsolikouri wight grape variety describes low containing stilbenoids in the skin. It was identified in the health grape skin fraction resveratrol and its derivates: glucosides, dimeric and trimeric stilbenoids. In



**Fig. 2.** Change of physiological concentration of stilbenoids of the grape Tsolikouri in condition Grey Mildew

concret: trans-resveratrol, trans-piceid, cis-piceid, trans-ε-viniferin, trans-piceatannol et all. 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 14.55mg/kg→52.23mg/kg; trans-piceid 5.52→4.83mg/kg; cis-piceid 12,16mg/kg→12,03mg/kg, trans-piceatannol 4.27mg/kg→4.05 mg/kg; difference is trans-ε-viniferin, which concentration becomes increase 15,72 mg/kg→17.15mg/kg.

## CONCLUSION

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

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