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Change of Phytoalexin Stilbenoids of Grape Skin Rkatsiteli (*Vitis vinifera L.*) in Condition of Powdery Mildew

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ABSTRACT

It has been studied a change of Georgian genome main white vine variety Rkatsiteli grape skin phytoalexins-stilbenoids in the condition of the disease Powdery mildew (*Uncinula nec.*). The samples of health and infected grapes –with 50% Powdery mildew, were taken in Beginning of September in 2018 year until the technical maturity, from the same vineyard planted in eastern Georgia. The vineyard soil belongs to meadow cinnamonic –Calcaric cambisols and calcic kastanozems type. Vineyard is 32 years old. Based on the HPLC/MS analysis are established the stilbenoids profiles of healthy and infected skins and has been revealed phytoalexin stylbenoids - accumulated as a result of the action of downy mildew. The concentration of stilbenoids increased during the diseases. The dominant stress-metabolites Stilbenoids are trans-resveratrol and trans- ϵ -viniferin. At the same time it was revealed as stress-metabolites: trans-piceid, cis-piceid, trans-piceatannol and oligomeric stilbenoids. The variability of these stilbenoids concentrations in the condition of the disease -Powdery mildew, is different: trans-resveratrol 27.7mg/kg→58.92 mg/kg(53,0%); trans- ϵ -viniferin 11.22 mg/kg→32.55mg/kg(65,5%); trans-piceid 5.36 mg/kg→7.27mg/kg(26,3%); trans-piceatannol 1.45mg/kg→2.04 mg/kg(28,9%); cis-piceid 17.75 mg/kg→17.79 mg/kg(0,2%); trans-astringin 14.45mg/kg →16.93 mg/kg(12,9%); cis-astringin 15.02 mg/kg→16.78 mg/kg(10,5%). The stress-metabolite stilbenoids in the conditions of the disease with Powdery 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.

KEY WORDS: Grape; Stilbenoids; Powdery Mildew; Rkatsiteli.

INTRODUCTION

The gene pool of Georgian vine incorporates up to 525 white- and red-grape varieties. According to the major requirements of vine-growing and wine-making, these

fields can be developed only by increasing the harvest of high quality and ecologically pure grapes what on its turn needs protecting the vine against bacterial and fungicidal diseases. The chemical content of vine and grape includes compounds of different classes. Among this compounds are important phenolic compounds, which characterized with difference biological activities. Phenolic compounds of grapevine are presented with flavonoids (procyanidins, phlavanols, antocians, phlavanols and et all) and nonphlavanoids (stilbenoids, phenolacids and phenolaldehyds). From nonphlavanoids compounds especially important are stilbenoids with high biological activity.

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; Adrian et al. 2000; Babieva et al.

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2018; Bavaresco, 2003; Bavaresco et al. 2003; Bavaresco and Fregoni 2001; Bavaresco et al. 2002; Bavaresco et al. 1997; Bavaresco et al. 2003). 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)- ω -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 (Bavaresco et al. 2007). 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* (Bezhuashvili, 1994). 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 (Bezhuashvili et al. 2013). 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 and 20 mkg/gr, what was sufficient for the total inhibition of fungus *A.carbonariuces* (Bezhuashvili and Surguladze 2016). Besides above mentioned biological activity stilbenoids have many other functional purposes (Gabaston et al. 2007; Gabastoni et al. 2018; Gindro et al. 2017; Guebailia et al. 2006; Houlline et al. 2015; Jalili et al. 2015; Jeandet et al. 2002; Langcake, 1981; Langcake et al. 1979). The vine and grape impacts some factor (Langcake and Pryce 1976; Larronde et al. 2005; Mattivi et al. 2011; Niesen Daniel et al. 2013). 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 (Özer et al. 2018; Pawlus et al. 2012; Reniero et al. 1996; Riviere et al. 2012; Sáeza et al. 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



Fig. 1: Rkatsiteli grape infected with Powdery Mildew.

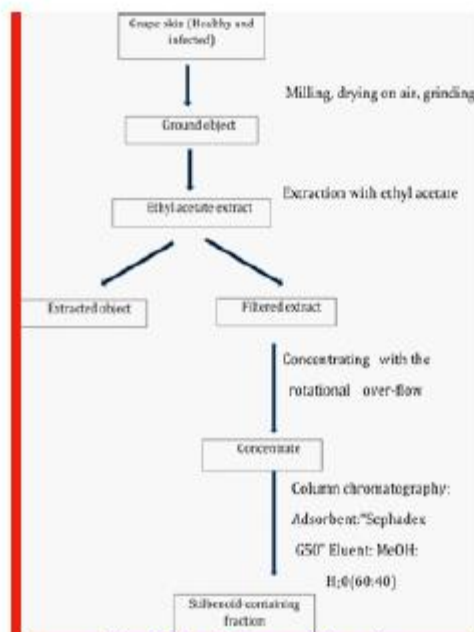


Fig. 1: Rkatsiteli grape infected with Powdery Mildew.

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 vinter-harvested grape canes and subsequently the potential

biological properties of the valuable extract (Surguladze and Bezhuashvi 2017; Surguladze and Bezhuashvili 2018; Vergara et al. 2012; Waffo Teguo et al. 1998).

Reason of Study

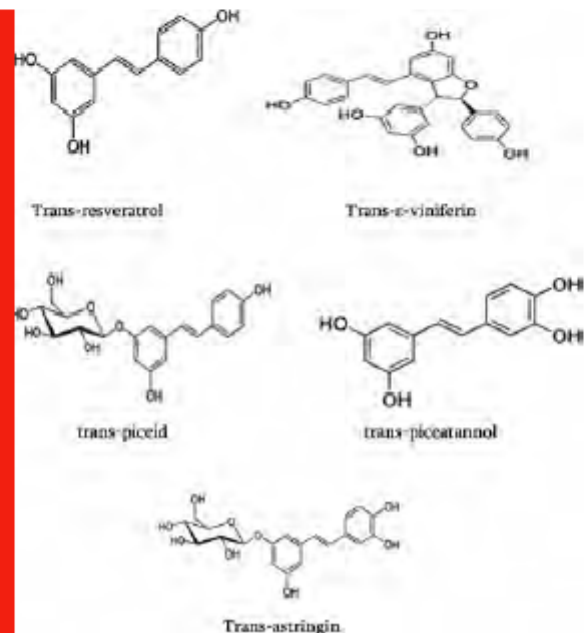
was to identify of stress- metabolites stilbenoids of grape skin Rkatsiteli variety (Vitis Vinifera L.) in condition Powdery mildew.

MATERIAL 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 Beginning of September in 2018 year until the technical maturity, from the same vineyard planted

Table 1: Content (mk/kg) of stress- metabolite stilbenoids in health and infected grape skin Rkatsiteli.

N.Name of stilbenoids	Health skin	Infected skin	Increase,%
1. trans-resveratrol	27.70	58.92	53,0
2. trans- ϵ -viniferin	11.22	32.55	65,5
3. trans-piceid	5.36	7.27	26,3
4. cis-piceid	17.75	17.79	0.2
5. trans-astringin	14.75	16.93	12,9
6. cis-astringin	15.02	16.78	10,5
7. trans-piceatannol	1.45	2.04	28,9



in eastern Georgia. The vineyard soil belongs to meadow cinnamonic –Calcic cambisols and calcic kastanozems type. Vineyard is 32 years old. (fig.1)

We isolated stilbenoid-containing fractions from the healthy and infected grape skin as a result of treatment according to the chart (fig .2)

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

On the basis of HPLC/MS analysis revealed stilbenoides profile of grape skin Rkatsiteli variety. This presented with trans- and cis-isomeric forms of resveratrol derivatives. In particular is identified trans-resveratrol, glucosides cis-, trans-piceids, cis-, trans-astringins; the metabolite of resveratrol trans-piceatannol(trans-astringinin); dimer of trans-resveratrol trans- ϵ -viniferin, trimeric and tetrameric stilbenoids. The grape disease –powdery mildew (*Uncinula necator*) is caused a change of concentration of stilbenoids. As a result, stress-metabolite stilbenoids have been detected, among them are dominant trans-resveratrol and trans- ϵ -viniferin. It is noteworthy their concentration increase. According to table data, concentrations of stress-metabolite stilbenoids are changing with different intensities. The largest quantity 31.22mg/kg increase a trans-resveratrol; concentration of cis-piceid has changed slightly – 0,04mg/kg; Concentration of trans-piceatannol increased by 0.59mg/kg. From oligomer stilbenoids of health and infected grape skin, identified trimeric stilbenoid with [M+H]⁺-681,2; tetrameric stilbenoids: a) [M+H]⁺-907,1(1,81% in total), b) [M+H]⁺-907,2(0,62% in total), c) [M+H]⁺-907,3(1,22% in total). Change of concentration of oligomer stilbenoids of grape skin in condition downy mildew is relatively minor.

CONCLUSION

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

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