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ORIGINAL ARTICLE

Stress-metabolite Stilbenoids in Vine Trunk of Ojaleshi Grape Variety (*Vitis vinifera* L.) under Crown Gall Infection

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KEYWORDS

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ABSTRACT: The trunk stilbenoids of healthy and crown gall infected vines of Ojaleshi variety have been studied and identified as stress-metabolite compounds .Vine samples were taken from a 12-year-old vineyard cultivated on yellow soils type in Martvili region (Western Georgia). The stilbenoids were extracted by ethyl acetate and then separated in a chromatographic column and analyzed by the HPLC/MS method. The following stilbenoids have been detected: cis-piceid, trans-resveratrol, trans-ε-viniferin, cis-miyabenol C, cis-miyabenol. The concentrations were higher in infected vines as compared to the healthy ones. This is the first time that stilbenoids have been investigated in Ojaleshi grape variety.

INTRODUCTION

The most important physiological role of vine stilbenoids is to act as phytoalexins. According to previous studies developed by the Institute of Viticulture and Enology of the Agricultural University of Georgia, the stilbenoids are involved in the vine responses toward bacterial (*Agrobacterium tumefaciens*) and fungal diseases in Georgian grape varieties. Crown gall infected vines of the grape varieties – Rkatsiteli, Saperavi, Cabernet Sauvignon, Tsitska and Tsolikouri were identified in East and West regions of Georgia; healthy vines were considered, as well. Stilbenoid- containing fractions were isolated from the trunk of the infected and healthy vines and the single compounds were identified (namely trans-resveratrol and trans -ε- viniferin). The grape variety had a crucial role in the amount of detected stilbenoids. The obtained results were important for identifying the correlation of the immunity of the grape varieties with the phytoalexin stilbenoids [1]. Healthy and crown gall infected vines of *V. vinifera* L, cvs. Saperavi and Rkatsiteli were selected from vineyards in East region of Georgia, in 2018 and 2019. Samples of infected and healthy vines were taken in February – March 2018 in a 16-year-old vineyard and in January 2019 in a 24-year-old vineyard, both growing on an alluvial soil. Stilbenoids (*trans*-resveratrol and *trans*-ε-viniferin) were isolated from the infected and healthy vine trunks and analyzed by HPLC/MS, with three replicates. The concentration of *trans*-ε-viniferin in healthy trunks of the 24- year-old Saperavi and Rkatsiteli, was higher than the concentration of *trans*-resveratrol. On the other hand, in the healthy trunks of 16-year-old vines, the concentration of *trans*-resveratrol exceeded the concentration of *trans*- ε-viniferin. In the crown gall infected 24-year-old vines, the

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concentration of *trans*-resveratrol increased while the concentration of *trans*- ε- viniferin decreased [2].

A strain of *Agrobacterium tumefaciens*, isolated from the trunk of infected *V. vinifera* L. cv. Rkatsiteli was studied microscopically and its pathogenesis was established. The goal of the research was to study the role of stilbenoids on the bacterial growth. The bacterium strain was inoculated in two ways: a) the surface of the growth was covered by a watery suspension of stilbenoids; b) stilbenoids were added to the growth medium before sterilization. In both experimental protocols the concentrations of the stilbenoids were: 1mg/100ml, 2mg/100ml, 3mg/100ml, 4mg/100ml, 5mg/100ml, 10mg/100ml, 15mg/100ml, 20mg /100ml and 30mg/100ml. The control version was the same medium without stilbenoids. The incubation period was 14-15 days at 27°C and all the treatments completely (100%) inhibited *Agrobacterium tumefaciens* growth over the control. A second experiment was therefore set up in order to study the bacterial growth inhibition under stilbenoid concentrations lower than 1 mg/100 ml (ranging from 0.1mg/100 ml to 0.9mg/100ml): the bacterial growth inhibition increased from 0.0 % to 88.0% by increasing the stilbenoid concentrations [3].

It has also been studied the concentrations (in the berry skin) of stilbenoids of white wine variety Rkatsiteli under grey mould (*Botrytis cinerea*) attack. Samples of healthy and infected grapes –with 60% gray mould, were taken in 2018 during the technological maturity, from the same vineyard (16-year-old) planted in eastern Georgia. The vineyard soil belongs to meadow cinnamonic – Calcariccambisols and calcic kastanozems type. The stilbenoids profiles of healthly and infected skins were detected by HPLC/MS analysis. The dominant stress-metabolites were trans-resveratrol and its derivatives: trans-piceid, cis-piceid, trans-piceatannol, trans-ε-viniferin. The concentration variability of these stilbenoids under gray mould infection was different, as follows (healthy vs. infected):trans-resveratrol 39.27mg kg^{-1}→57.33mg kg^{-1}; trans-piceid13 72mg.kg^{-1}→29.43mg kg^{-1}; trans-piceatannol 5.37mg kg^{-1}→19.45mg kg^{-1}; trans-ε-viniferin 7.22 mg kg^{-1} →5.13mg kg^{-1}. These are the first evidences of the link stilbenoids –gray mould in the Rkatsiteli variety[4.].

Another trial on the interaction between grey mould infection and stilbene production of the white cv. Tsolikouri was carried out; the vineyard (30-year-old) was cultivated in the west part of Georgia (Zestafoni region) on raw humus calcareous-rendzic-leptosols soil. The production of trans-resveratrol, ε-viniferin, trans-piceid, cis-piceid, trans-piceatannol was elicited by grey mould. These are the first evidences of the link stilbenoids –grey mould in the Tsolikouri variety [5].

Moreover, a study on the effect of powdery mildew (*Uncinula necator*) infection on the synthesis of stibenoids in the grape white variety Rkatsiteli was developed. Samples of healthy and infected grapes (50% of powdery mildew attack) were taken at the beginning of September 2018 (technological maturity), from the same vineyard (32-year-old) planted in eastern part of Georgia; the vineyard soil belongs to meadow cinnamonic –Calcaric cambisols and calcic kastanozems type. The concentration of stilbenoids increased during the disease and the dominant stress-metabolites were trans- resveratrol and trans-ε-viniferin, while the minor compounds were trans-piceid, cis-piceid, trans-piceatannol and oligomeric stilbenoids. The concentrations of these stilbenoids changed from healthy to infected grapes, as follows: trans-resveratrol 27.7mg kg^{-1}→58.92 mg kg^{-1}; trans ε-viniferin 11.22 mg kg^{-1} →32.55mg kg^{-1}; trans-piceid 5.36 mg kg^{-1}→7.27mg kg^{-1} ; trans-piceatannol 1.45mg kg^{-1}→2.04 mg kg^{-1}; cis-piceid 17.75 mg kg^{-1}→17.79 mg kg^{-1}; trans-astringin 14.45mg kg^{-1} →16.93 mg kg^{-1}; cis-astringin 15.02 mg kg^{-1}→16.78 mg kg^{-1}. These are the first evidences of the link stilbenoids – powdery mildew in Rkatsiteli grape variety[6].

Another trial included the relationship between leaf stilbenoids of the white grape variety Tsitska, grown in the west part of Georgia, and downy mildew infection. The stilbenoids trans-resveratrol and ε- viniferin were higher in the infected vines than in the healthy ones. This is the first study done on Tsutska grape variety concerning this subject [7].

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 [8]. Under the influence of *Botrytis cinerea* on the mixture of pterostilbene and resveratrol 7 new stilbens were formed, while 5 new stilbenes were formed from pterostilbene under the same terms. The anti-fungal effect of these stilbenoids was fixed against *Plasmopara viticola* [9]. At three stages of the grape (*Vitis vinifera*) berry development, the berries were infected with *Botritis cinerea* spores, *in vitro*. In the infected berries, pterostilbene, (E)-ε-viniferin and trans-resveratrol were detected, being (E)-ε-viniferin the most produced [10].

Berries of *Vitis vinifera* L. cv. Barbera in the ripening period were infected with conidial suspension of *Aspergillus japonicus*, *A. ochraceus*, *A. fumigatus* and *A. carbonarius*. The process of formation of ochratoxin A and stilbenoids was assessed. It was found out that all fungi except for *A. fumigatus* significantly increased the concentration of trans-resveratrol and at the same time, trans-piceid stays unchanged. In the berries damaged by *A. ochraceus*, the concentration of piceatannol increased significantly. A large amount of ochatoxin A was synthesized in the berries infected by *A.carbonarius* isolate and the anti-fungal activity of stilbenoids was tested under the following concentrations: 300mg/g resveratrol and 20mg/g piceatannol, which were effective for an inhibition of the fungal (*A. carbonarius*) growth [11].

Besides to above mentioned trials we continue to study the response to bacterial and fungal disease of Georgian grape varieties, in terms of stilbenoid production. The goal of this research is to study the stilbenoids production of red grape variety Ojaleshi under bacterial cancer infection.

MATERIALS AND METHODS

The trial was carried out in a 12-year-old vineyard in the west part of Georgia (Martvili district) on yellow soil type. Healthy and crown gall infected vines of the Georgian red grape variety Ojaleshi were selected and from the healthy and infected trunks, stilbenoid fractions were isolated by the following scheme (Figure 1) and analyzed by HPLC/MS methods [12]. 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. Isolated stilbenoid-containing fractions were filtered using a membrane filter (0.45μ) before the chromatographic procedure. The chromato-mass-spectral investigations were carried out under the above-mentioned conditions; mass-spectra were detected as positive ions.

RESULTS AND DISCUSSION

trans- and cis-Isomers forms of stilbenoids (resveratrol and its dimers, trimers, tetramers and glucoside derivatives) were identified at the different wavelength (306nm and 285nm) by HPLC/MS in healthy and infected vine samples. The detected stilbenoids were: trans-resveratrol, trans-ε-vinifein, cis-piceid, cis-miyabenol and cis-miyabenol C, being trans-ε- viniferin the dominant form (Table 1, Figure 2). The concentrations of all stilbenoids were higher in the infected vines as compared to the healthy ones, as follows (healthy vs. infected): cis-piceid 0,65mg.kg^{-1} -0,92mg.kg^{-1}; trans-resveratrol 2,45mg kg^{-1} - 3,28mg kg^{-1}; trans-ε-viniferin 7,18mg kg^{-1} - 8,35mg kg^{-1}; cis-miyabenol 4,12mg kg^{-1} - 5,92mg kg^{-1}; cis-miyabenol C 1,15 – 1,67mg kg^{-1}. This is the first evidence of stilbenoids production in Ojaleshi grape variety, as affected by crown gall.

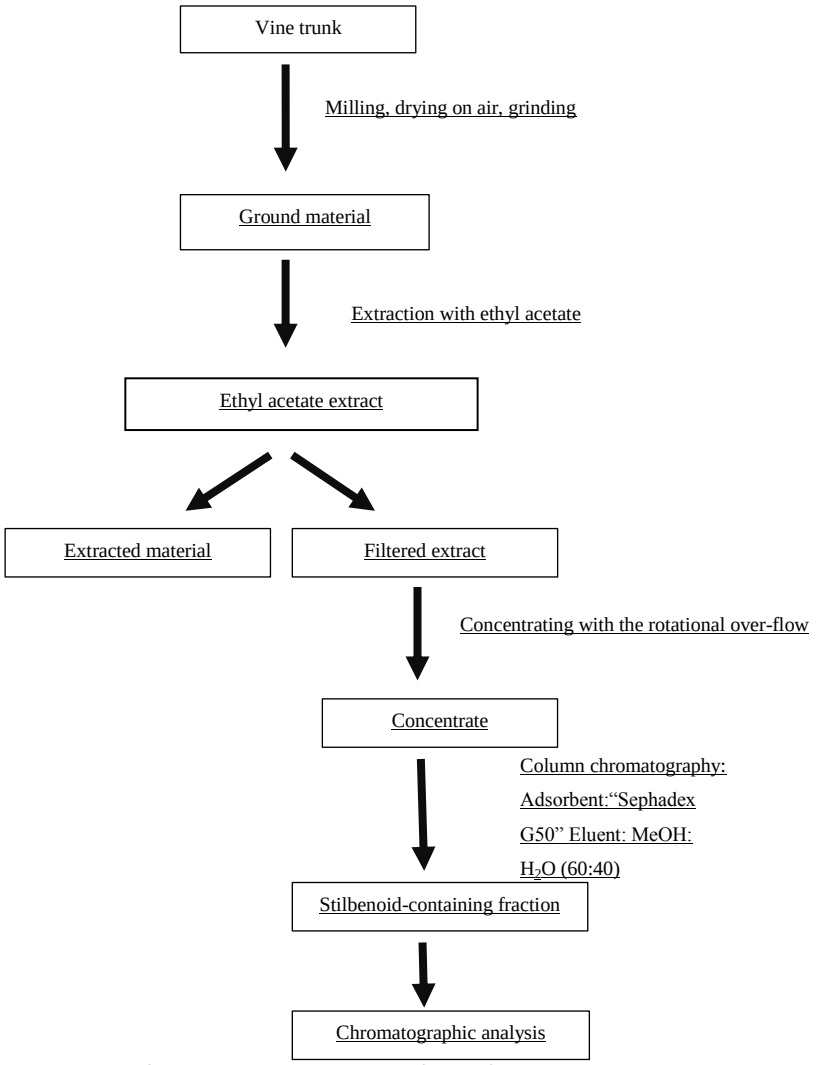

Figure 1. Chart of isolating a stilbenoid-containing fraction from vine trunk

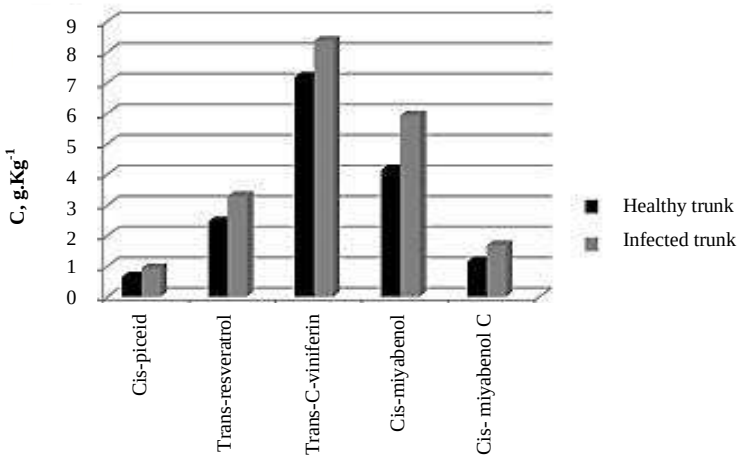

Figure 2. Concentration of vine trunk stilbenoids in healthy and crown gall infected vines

Table. 1. HPLC/MS characteristics of stilbenoids of healthly vine trunk of Ojaleshi variety.

NO.	Stilbenoid	RT, min	$[M+H]^+$
1	Cis-piceid	15.0	391.1
2	Trans-resveratrol	27.06	229.1
3	Dimmer	35.24	455.1
4	Trans-ε-viniferin	36.43	455.1
5	Cis-miyabenol	37.44	681.1
6	Miyabenol	38.56	681.0
7	Tetramer	39.31`	907.3
8	Cis-miyabenol C	41.33	681.1
9	Tetramer	41.63	907.2
10	Tetramer	42.56	907.2
11	Tetramer	42.88	907.2
12	Tetramer	43.41	907.3

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Conflict of interests

There is no conflict of interests.

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