Phytoalexin Stilbenoids of Saperavi and Rkatsiteli(*Vitis vinifera* L.) as Biomarkers of Resistance to Some Fungal Diseases

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ABSTRACT---- *Among the biological activities of grape stilbenoids, phytoalexin activity is the most important. The paper deals with a study on the change of phytoalexin stilbenoids response of Saperavi and Rkatsiteli under powdery mildew and gray mold infection in different viticulture areas of Georgia: Mukuzni and Napareuli (for Saperavi), and , Tsarapi and Tibaani (for Rkatsiteli). In July 2024, the grape varieties were infected with a water suspension of powdery mildew. In August 2024, the grape varieties were infected with the conidial water suspension of Botrytis cinerea, and the development of diseases on the grape barries in the period of July-September was observed. In the experimental vineyards trans-resveratrol and its derivatives - glycosides and polymeric stilbenoids in the healthy grape skins of Saperavi and Rkatsiteli were detected. In Saperavi berries skins stilbenoids concentration were higher compared to Rkatsiteli. In both infected varieties the following stilbenoids were identified: trans-resveratrol, trans-ε-viniferin, trans-piceid, trans-astringin and cis-piceid. Stilbenoids concentration changes were observed depending on the vine variety and soil-climatic factors. The results represented the stilbenoids as important biomarkers for the Saperavi and Rkatsiteli for resistance against Powdery mildew and Gray mold.*

Keywords---- Saperavi, Rkatsiteli, Stilbenoids, Phytoalexins, Powdery mildew, Gray mold

1. INTRODUCTION

Stilbenoids (belonging to a wide class of phenolic compounds) include resveratrol and its derivatives (glucosides, dimers,trimers, tetramers, etc.) *cis*- and *trans*-isomeric forms[1, 2, 3, 4, 5]. Stilbenoids are characterized by several high biological activities and among them phytoalexin activity is important for the plant, especially for the grapevine. Phytoalexins, under plant infection, are actively synthesized and act against disease-causing microorganisms (for example *Botrytis cinerea* and *Plasmopara viticola*). Beside the biotic factors, phytoalexins also respond to abiotic stresses such as UV rays and AlCl3 [6]. Adrian et al. [6] studied the variability of stilbenoids in the berry skin of red (Pinot noir, Gamay) and white (Chardonnay)wine grape varieties infected by *Botrytis cinerea* and treated with UV rays. All samples infected with *B. cinerea* showed a decreased amount of resveratrol and an increased concentration after UV irradiation. Pterostilbene was found in low concentrations in infected berries of Chardonnay, Gamay, Castor and Huxelrebe [7, 8]. According to Pezet and Pont [9] , pterostilbene plays an important role in the resistance of immature grapes against disease-causing microorganisms..

According to Bezhuashvili et al [10] stilbenoids have been identified in healthy and naturally diseased Georgian winegrape varieties - Rkatsiteli (white), Tsolikouri (white), Alexandrouli (red), Mujuretuli (red).*Trans*-resveratrol and *trans*-ε-viniferin were dominant for red varieties; moreover, *trans*-resveratrol was lower than *trans*-ε-viniferin in healthy grape skins, and the concentration of *trans*-resveratrol was significantly higher under gray mold infection than *trans*-e-viniferin; it decreased under disease conditions [10].In white wine grape varieties (Rkatsiteli and Tsolikouri), the main stress metabolite was *trans*-resveratrol, which increased significantly under gray mold infection [11].The inhibitory effect of *trans*-resveratrol on *Botrytis cinerea* activity and consequently the spread of gray mold on grapes, has been established under laboratory conditions (in petri dishes) [12]. Stilbenoids had an inhibitory effect of the fungus- *Botrytis cinerea* pure culture in petri dishes and there was a negative correlation between the fungal propagation and the stilbenoids concentration. According to Adrian et al. [13] a resveratrol concentration inhibited the development of *B.cinerea* mycelium, while concentrations of pterostilbene at caused 50%, 80%, and 100% inhibition of *B.cinerea* mycelium development. According to Pezet and Pont [9] a concentration of pterostilbene caused a 50% inhibition of *B. cinerea* mycelium development.

Evidences have been obtained on the capability of some highly pathogenic *B. cinerea* strains to circumvent the defence by detoxifying resveratrol through an oxidative process [14]. Other stilbenoids can be detoxified by enzymatic (laccase) activity of *B. cinerea*, resulting in the release of compounds like pterostilbene *trans*-dehydrodimer, pterostilbene*cis*-dehydrodimer, resveratrol *trans*- dehydrodimer [15]. All the physiopathological aspects of stilbenoids are addressed in the review written by Jeandet et al [16]. Stress metabolite phytoalexin stilbenoids have been detected in the form of trans-resveratrol and its derivatives, under bacterial [17] and fungal [18,19] attack in Saperavi and Rkatsiteli.

The aim of the study was the variation of stilbenoids in the skin of Saperavi and Rkatsiteli grapes under powdery mildew and gray mold infection. Detection of stress metabolite phytoalexin stilbenoids and determination of their influence on the spread of these diseases was tested in lab ("in vitro") and in the vineyard ("in vivo") .

2. MATERIALS AND METHODS

The experiment included vineyards of Saperavi (red) and Rkatsiteli (white) located in specific viticultural areas in Eastern Georgia. Saperavi berries were sampled, as follows: a)Mukuzani area , from a 17-year-old vineyard on Eutric Cambisols and calcic Kastanozems type of soil; b) Napareuli area, from a 40- year-old vineyard on Eutric Cambisols and calcic Kastanozems type of soil; Rkatsiteli berries were sampled, as follows: a) Tsarapi area from a 40 -year- old vineyard on meadow cinnamonic-calcaric cambisols and calcic kastanozems type of soil ; b) Tibaani area from a 17- year- old vineyard on cinnamonic calcareous-calcaric cambisols and calcic kastanozemstype of soil.

Stilbenoids containing fractions were isolated from healthy and diseased grape skins according to Fig.1. Trans-resveratrol and ε-viniferin were individually isolated from one-year-old vine shoots by ethylacetate extraction and column separation as shown in Fig.1.

2.1 Stilbenoids were determined by the method of highperformance liquid chromatography (HPLC) [20]. For this purpose, we used the chromatograph Varian; Column- Supelcosil PM LC18, 250x4,6mm; Eluents: A. 0,025% trifluoroacetic acid,B.Acetonitrile: A, 80/20. Gradient mode: 0-35 min, 20-50% B; 35-40 min. 50-100% B ; 41- 46min. 100% B ; 46-48min 100-20% B; 48-53min, 20% B. Wave length: 306 nm for trans-stilbenoids, 285 nm for cis-stilbenoids. Flow rate of the eluent- 0,8ml/min; fractions were filtered using a membrane filter (0.45μ)before the chromatographic procedure.

2.3 Lab experiments. Healthy berries of red wine grape varieties (*V. vinifera* L.) Saperavi and Rkatsiteli were sampled at technological maturity (September, 2024) in the following environments of Georgia: a) Saperavi berries from vineyards located in Mukuzani and Nafareuli areas ; b)Rkatsiteli berries from vineyards located in Tsarapi and Tibaani areas. . The experimental design included the following treatments: 1) berry pre-treatment with water suspension of individual stilbenoids and then fungal infection by *Uncinula necator* and *Botrytis cinerea*; 2) control berries with fungal infection but without pre-treatments.The pre-treatments were done by soaking 12 berries per variety in the previously. described solutions (or just water in the case of the control), while fungal infection was done right away after the pre-tratments, by spraying *Uncinula necator* and *Botrytis cinerea* conidial suspension over the berries placed on damp filter paper inside petri dishes. The fungal inoculum was prepared by recovering conidia from infected berries grown in the field. The stilbenoid analysis was done 24 hours after the treatments.

2.4 Vineyard experiment As concerning the vineyard trial, healthy clusters of Saperavi and Rkatsiteli from the experimental (not sprayed by pesticides) vineyards, located in the above-mentioned areas were considered. An experiment for gray mold was conducted between August- September 2024, while for powdery mildew between July - August 2024. The experimental design was as follows: 1) Individual clusters pre-treatment with water suspension of *trans*-resveratrol and its derivatives and then fungal infection by water suspension of *Botrytis cinerea* and *Uncinula necator*. 2) Control vines with fungal infection but without any pre-treatment. Three clusters of different sizes were selected per each treatment. Biological efficiency (%) was studied.

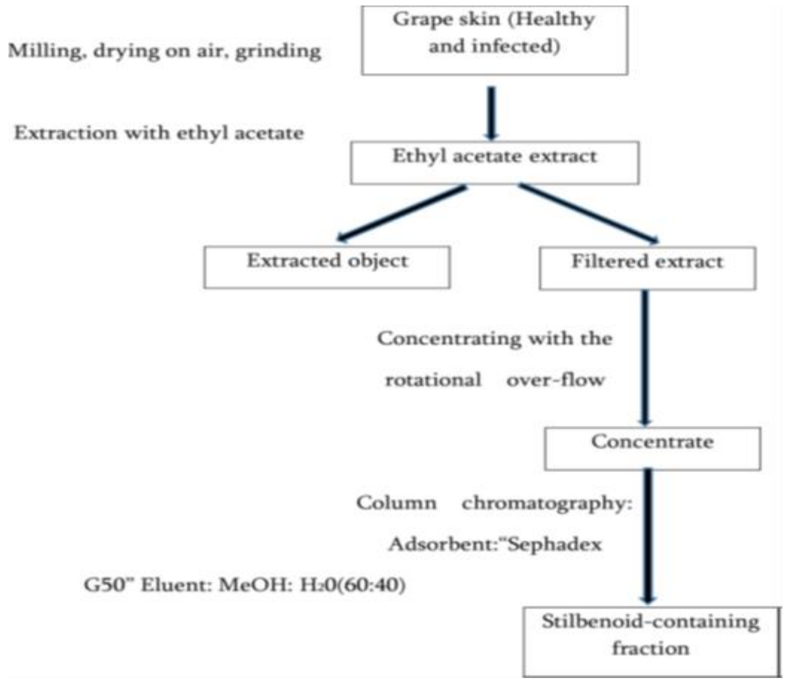


Figure 1: Chart of isolating a stilbenoid-containing fraction from vine trunk and grape skin

3. RESULTS AND DISCUSSION

In The daily air temperatures of July, August, September 2024, in the four areas are reported in Table 1.

Table 1: Daily air temperature (^{0}C) range in the experimental vineyards, depending on the growing area.

Months	Areas			
	Mukuzani	**Napareuli**	**Tsarapi**	**Tibaani**
July	17-33	17-37	16-36	17-36
August	21-37	23-39	23-37	23-38
September	17-30	16-30	17-28	16-30

Temperature is one of the important abiotic factors impacting the biosynthesis of stilbenoids in grapevine. The inhibitory effect of high temperature on the biosynthesis of stilbenoids has been established by a number of researchers [21,22,23,24].

The stilbenoid profiles, in the vineyard experiment, are dominated by glucosidic forms: trans-piceide, trans-astringin and cis-piceide. Phytoalexin stilbenoids: trans-resveratrol, trans-ε viniferin, trans-piceide, trans-astringin and cis-piceide were identified from the comparison of stilbenoids of healthy and gray mold-infected grape skins.

Trans-resveratrol and its derivatives, , were observed in different concentrations in healthy and under powdery mildew infected Saperavi and Rkatsiteli grape skins. For example, concentrations of the specified stilbenoids in healthy Saperavi grape skins were observed in the following order: *trans*- piceid >*trans*-astringin >*trans*-ε-viniferin > *trans*-resveratrol > *cis*-piceid > cis -resveratrol > *cis*-astringin (Table 2).

Table 2: Stilbenoids of grape skin of Saperavi and Rkatsiteli depending on the growing area and the treatments healthy grapes vs. powdery mildew infected grapes.

Stilbenoids,mg/kg	**Saperavi**				**Rkatsiteli**			
	Mukuzani		**Napareuli**		**Tsarapi**		**Tibaani**	
	Healthy	**Infected**	**Healthy**	**Infected**	**Healthy**	**Infected**	**Healthy**	**Infected**
Trans-resveratrol	6.7	11.2	5.3	10.8	4.7	4.3	4.3	3.1
Cis -resveratrol	2.0	1.8	2.5	2.0	1.1	0.7	1.4	1.0
Trans- piceid	11.0	7.3	7.8	10.9	6.3	8.0	8.7	9.5
Cis-piceid	2.2	2.6	1.5	2.5	0.9	0.5	0.9	0.5
Trans-astringin	10.5	6.0	9.4	13.2	2.4	2.6	2.0	2.4
Cis-astringin	0.5	0.7	3.0	1.3	0.7	0.5	0.5	0.8
Trans--ε-viniferin	7.8	4.0	6.2	3.4	4.2	4.5	4.3	4.7

The biological effect of individual and equal concentrations of stress metabolite stilbenoids in the lab experiment against *Uncinula necator* ranges from 55% to 98%. Trans-isomeric stilbenoids are characterized by high efficiency. Trans ε-viniferin has the maximum efficiency. (Figure 2).

The effectiveness of inhibition of powdery mildew with water suspension of a total stilbenoid preparation varies between lab and vineyard. Specifically, the spread of the disease in Saperavi grown in Mukuzani area is inhibited by 95% in the lab and 72% in the vineyard. Inhibition efficiency under lab conditions in Saperavi from Napareuli area was reduced from 94% to 65%. For Rkatsiteli in Tsarapi area the reduction was from 90% to 67% , while for Rkatsiteli in Tibaani area the reduction was from 94% to 67 % (Figure 3).

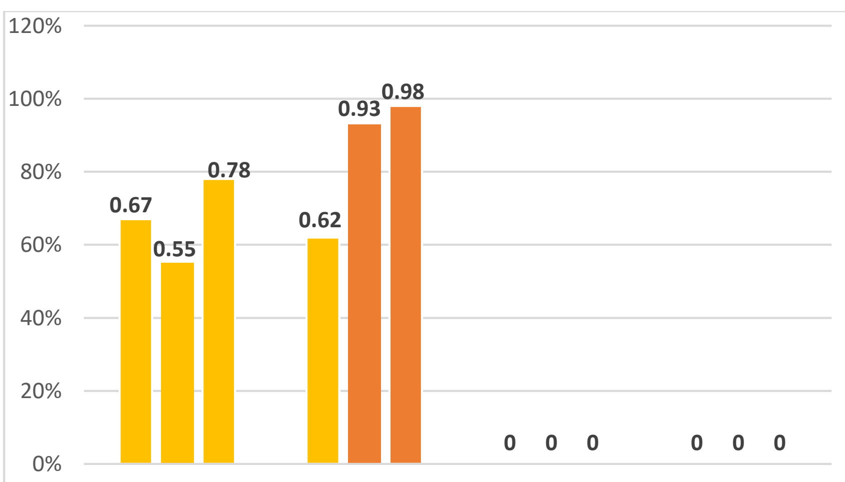
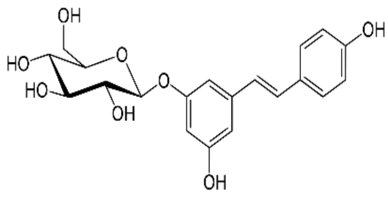


Figure 2: Biological efficiency of phytoalexin stilbenoids (at a concentration of 5 mg/100 ml) on the skin of Saperavi berries against the growth of powdery mildew under laboratory conditions . 1-*Trans*-piceid; 2-C*is*-piceid; 3.*Trans*-astringin;4. *Cis*-astringin; 5. *Trans*-resveratrol, 6. *Trans* ε-viniferin



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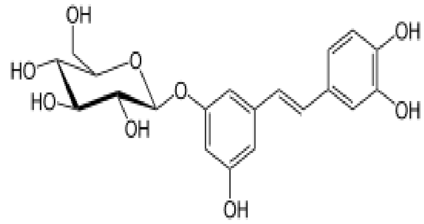

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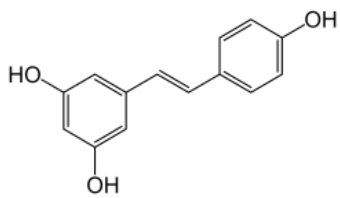

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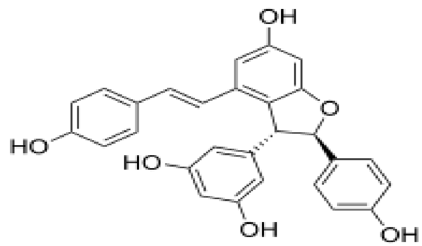

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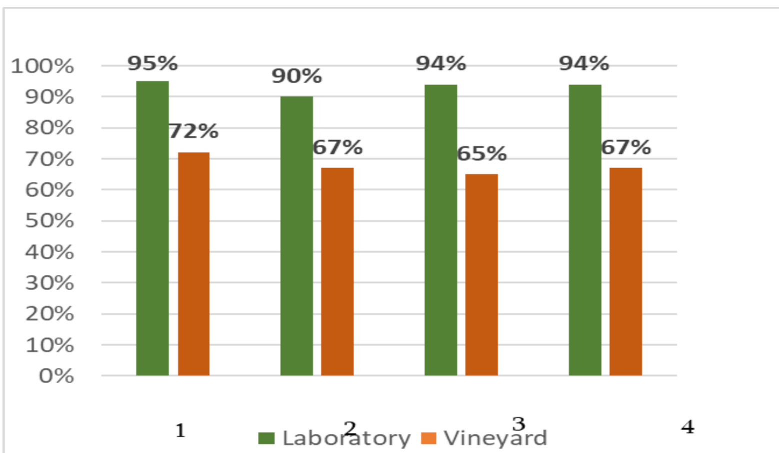

Figure 3: Biological efficiency of water suspension of total stilbenoid preparation on powdery mildew growth on the skin of Saperavi(1,3) and Rkatsiteli (2,4) grapes under laboratory and vineyard conditions.

The stress metabolite phytoalexin stilbenoids were detected in different concentrations in the skin of Saperavi and Rkatsiteli grapes under grey mold infection (Table 3). Trans-isomeric stilbenoid glucosides are dominant among them.

Table 3: Change of Stilbenoids of grape skin of Saperavi and Rkatsiteli under gray mold infection

Stilbenoids,mg/kg	**Saperavi**				**Rkatsiteli**			
	Mukuzani		**Napareuli**		**Tsarafi**		**Tibaani**	
	Healthy	**Infected**	**Healthy**	**Infected**	**Healthy**	**Infected**	**Healthy**	**Infected**
Trans-resveratrol	7.8	11.0	7.2	9.6	6.2	20.3	6.8	16.3
Trans- ε-viniferin	6.5	7.7	6.1	5.2	4.7	8.4	3.8	9.1
Trans-piceid	23.0	18.5	17.7	20.5	15.1	17.9	13.8	16.8
Trans- astringin	12.3	15.2	12.2	10.3	10.5	14.2	11.8	10.0
Cis-piceid	6.3	8.5	4.9	6.7	5.5	4.8	5.1	4.7

In lab conditions the biological efficiency of individual stilbenoids in inhibiting the spread of Botrytis cinerea (in Saperavi grape) varied between 75 and 91.7%, while for Rkatsiteli the variation was in the range of 66.7 - 83.3%. The biological effectiveness of the total preparation of stilbenoids, in contrast to individual stilbenoids, reaches a maximum -100% for both varieties of grapes (Table 4).

Table 4: Lab.trial. Impact of phytoalexins stilbenoids on the gray mold infection of Saperavi and Rkatsiteli grape berries

Pre-treatments	**Number of berries in each petri dish**		**Number on infected berries in each petri dish**		**Degree of infection (%)**		**Biological efficiency (%)**	
Saperavi	Mukuzani	Napareuli	Mukuzani	Napareuli	Mukuzani	Napareuli	Mukuzani	Napareuli
Control	12	12	12	12	100	100	0	0
R-5mg/100ml	12	12	2	2	16.7	16.7	83.3	83.3
V-5mg/100ml	12	12	1	1	8.3	8.3	91.7	91.7
trans-piceid 5mg/100ml	12	12	3	3	25	25	75	75
trans-astringin, 5mg/100ml	12	12	3	3	25	25	75	75
P, 5mg/100ml	12	12	0	0	0	0	100	100
Rkatsiteli	Tsarapi	Tibaani	Tsarapi	Tibaani	Tsarapi	Tiobaani	Tsarapi	Tibaani
Control	12	12	12	12	100	100	0	0
R-5mg/100ml	12	12	3	3	25	25	75	75
V-5mg/100ml	12	12	3	2	25	16.7	75	83.3
trans-piceid 5mg/100ml	12	12	4	3	33.3	25	66.7	75
trans-astringin, 5mg/100ml	12	12	3	4	25	33.3	75	66,7
P, 5mg/100ml	12	12	0	0	0	0	100	100

*R-*trans*-resveratrol, V- *trans*-ε-viniferin, P- suspension of total preparation of specified stilbenoids (Ratio to total equivalent quantity)

Table 5: Biological efficiency of stilbenoids on the growth of *Botrytis cinerea* in the laboratory and in the vineyard, depending on the variety and the area

Pre-treatments Biological Efficiency%				
	Saperavi		**Rkatsiteli**	
	Mukuzani	**Napareuli**	**Tsarapi**	**Tibaani**
	Lab. V-d	Lab. V-d	Lab. V-d	Lab. V-d
Control	0 48-50	0 47-48	0 43-45	0 42-44
R-5mg/100ml	83.3 61-63	83.3 59-62	75 47-52	75 48-50
V-5mg/100ml	91.7 64-65	91.7 63-64	75 53-55	83.3 53-56
Trans-piceid, 5mg/100ml	75 55-57	75 56-58	66.7 45-46	75 47-48
Trans-astringin, 5 mg/100ml	75 57-59	75 57-60	75 47-48	66.7 48-49
P, 5mg/100ml	100 68-70	100 65-67	100 57-58	100 57-58

*R- *trans*-resveratrol, V-*trans-ε*-viniferin, P- suspension of total preparation of specified stilbenoids (Ratio to total equivalent quantity), Lab.- In the laboratory, V-d. -in the vineyard

The biological effectiveness of individual stilbenoids and their total preparation in lab was significantly higher than the biological effectiveness of these stilbenoids in the vineyard. Among the stilbenoids, Trans-piceid and Trans-astringin were characterized by less biological effectiveness. Regarding to spread of *Botrytis cinerea*, non-treaded grape bunches with control-stilbenoids, the resistance of Saperavi prevailed to Rkatsiteli. This is caused by the higher resistance of Saperavi to *Botrytis cinerea* compared to Rkatsiteli (Table 5).

4. CONCLUSION

The stress metabolite stilbenoids were present in the form of trans-resveratrol and its derivatives in Saperavi and Rkatsiteli grape skins under fungal (powdery mildew and gray mold) infection. The biological effectiveness of phytoalexin stilbenoids in terms of inhibition of *Uncinula necator* and *Botrytis cinerea* in lab is higher to the effectiveness observed in the vineyard. The total preparation of stress metabolite phytoalexins stilbenoids showed high biological efficiency, both in Lab (100%) and vineyard conditions. Specifically, for Saperavi, this indicators were 68-70% in Mukuzani and 65-67% in Napareuli. The same variability was observed for Rkatsiteli in Tsarapi and Tibaani microzones, with the same efficiency of 57-58%. The biological efficiency of each stilbenoid revealed different. Significantly, less than the biological characteristic of their total preparation. The obtained results indicate the stilbenoids as a biomarker of Saperavi and Rkatsiteli resistance toward fungal diseases.

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