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Biochemistry

Stilbenoids – Dimeric Derivatives of Resveratrol for the Resistance of Saperavi and Rkatsiteli (*Vitis vinifera* L.) against Gray Mold (*Botrytis cinerea*)

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(Presented by Academy Member Ramaz Gakhokidze)

Abstract. Grape stilbenoids are characterized by a variety of biological activities and among them phytoalexin activity is most significant. The role of stilbenoids against bacterial and fungal diseases affecting Georgian white and red grape varieties was identified. We studied the variability of phytalexin stilbenoids of Saperavi and Rkatsiteli in the Georgian viticulture microzones: in Mukuzani and Napareuli for Saperavi and in Tsarapi and Tibaani for Rkatsiteli. The stress metabolite stilbenoids in fungal and bacterial diseases like gray mold, powdery mildew and crown gall were identified. They are primarily found in significant concentration as *trans*-resveratrol, *trans*-piceid, *trans*-astringin, *cis*-piceid and *cis*-resveratrol. Other stilbenoids were also identified in small concentrations. In the current experiment the role of pallidol and *cis*-δ-viniferin against gray mold infection of Saperavi and Rkatsiteli berries was tested under *in vitro* conditions. Pallidol and *cis*-δ-viniferin were observed at higher concentrations in the skin of the Saperavi grape as compared to the skin of the Rkatsiteli grape. Pallidol and *cis*-δ-viniferin are characterized by high phytoalexin properties, being able to reduce the gray mold infection in both varieties. *© 2025 Bull. Natl. Acad. Sci. Georg.*

Keywords: Saperavi, Rkatsiteli, stilbenoids, phytoalexins, gray mold

Introduction

Stilbenoids are an important group of a wide class of phenolic compounds. They include resveratrol and its derivatives (glucosides, dimers, trimers, tetramers, etc.) *cis*- and *trans*-isomeric forms (Niesen et al., 2013; Flamini et al., 2013; Rivière et al., 2012). Stilbenoids are characterized by some high biological activities, mostly the phytoalexin activity for the plant, especially for the grapevine. The most important phytoalexin stilbenoids are: resveratrol (Langcake et al., 1976), pterostilbene (Langcake et al., 1979), piceid (Waterhouse et al., 1994), viniferins (Langcake, 1981; Flamini et al., 2016). Phytoalexins, in case of 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 $AlCl_3$ (Adrian et al., 1996; Błaszczyk et al., 2019). Adrian et al. (2000) studied the variability of stilbenoids in the berry skin of red (Pinot noir, Gamay) and white (Chardonnay) winegrape varieties infected by *Botrytis cinerea* and treated with UV rays. All samples infected with *B. cinerea* showed decreased amount of resveratrol and an increased concentration after UV irradiation. Pterostilbene was found in low concentrations in infected berries of Chardonnay and Gamay. Pterostilbene was also observed in low concentrations in grape skins by other authors (Bavaresco et al., 1997). According to Pezet et al., (1988), pterostilbene plays an important role in the resistance of immature grapes against disease-causing microorganisms. Other authors have studied the interaction between stilbenoids and *Botrytis cinerea* in grapevine.

According to Bezhuashvili et al., (2021), stilbenoids were identified in healthy and naturally infected Georgian varieties – Rkatsiteli (white), Tsolikouri (white), Aleksandrouli (red), Mujuretuli (red). *Trans*-resveratrol and *trans-ε*-viniferin were dominant for red varieties; *trans*-resveratrol was lower than *trans*-ε-viniferin in healthy grape skins, and the concentration of *trans*-resveratrol was significantly higher in case of gray mold infection than that of *trans*-e-viniferin; it decreased in case of a disease. In white wine grape varieties (Rkatsiteli and Tsolikouri), the main stress metabolite was *trans*-resveratrol, which increased significantly in case of gray mold attack (Bezhuashvili et al., 2019). The inhibitory effect of *trans*-resveratrol on *Botrytis cinerea* activity and consequently the effect of gray mold on grapes, were established in the laboratory conditions (in petri dishes) (Bezhuashvili et al., 2019). Stilbenoids had an inhibitory effect of *Botrytis cinerea* pure culture in food areas and there was a negative correlation between the fungal propagation and the concentration of stilbenoids. According to Adrian et al. (Adrian et al., 1997), a resveratrol concentration of 100 μg/ml completely inhibited the development of *B. cinerea* mycelium, while concentrations of pterostilbene at 20-40-60 μg/ml caused 50%, 80%, and 100% inhibition of *B. cinerea* mycelium development. According to Pezet et al. (1988), a concentration of pterostilbene of 18 μg/ml caused a 50% inhibition of *B. cinerea* mycelium development while a concentration of 52 μg/ml resulted in an inhibition of 52%.

Evidence was obtained on the capability of some highly pathogenic *B. cinerea* strains to circumvent the defence by detoxifying resveratrol, *trans*-dehydrodimer, pterostilbene *cis*-dehydrodimer and resveratrol *trans*-dehydrodimer through an oxidation process (Adrian et al., 1998; Breuil et al., 1999). All the physiopathological aspects of stilbenoids are addressed in the review by Jeandet et al (Jeandet et al., 2002).

As a result of our *in vitro* and *in vivo* studies in some viticultural microzones of Georgia, the stilbenoids were correlated to the resistance of Saperavi (in Mukuzani and Napareuli microzones) and Rkatsiteli (in Tsarapi and Tibaani microzones) to gray mold. The mentioned compounds were represented by the following main stilbenoids: *trans*-resveratrol, *cis*-resveratrol, trans-piceid, *cis*-piceid, *trans*-astringin, *cis*-astringin, *trans-ε*-viniferin (Bezhuashvili et al., 2025; Bezhuashvili et al., 2023). The experiments in the same field continued, by focusing on the activity of resveratrol dimers – pallidol and *cis*-δ-viniferin in Saperavi and Rkatsiteli grape skins infected with *Botrytis cinerea in vitro* conditions.

Materials and Methods

The experimental design included healthy grape skins of Saperavi (red) and Rkatsiteli (white) varieties grown in some areas of Eastern Georgia and the same material artificially infected with gray mold as follows: Saperavi in vineyards located Mukuzani (17-year-old vines) on Eutric Cambisols and calcic Kastanozems, and Napareuli (40-year-old vines) on Eutric Cambisols and calcic Kastanozems; Rkatsiteli – in vineyards located at Tsarapi (40-year-old vines) on meadow cinnamonic-calcaric cambisols and calcic kastanozems, and Tibaani (17-year-old vines) on cinnamonic calcareous-calcaric cambisols and calcic kastanozems. Berries were sampled during harvesting (2024).

Stilbenoids containing fractions were isolated from healthy and infected grape skins according to Fig. 1. Pallidol and *cis*-δ-viniferin were individually isolated from ethyl acetate extract by column separation as shown in Fig. 1.

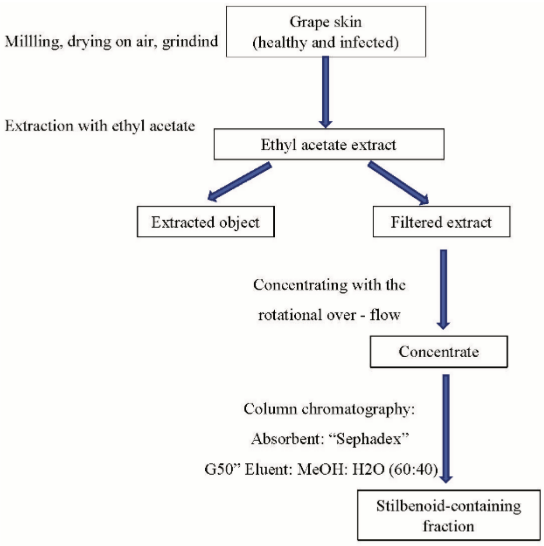


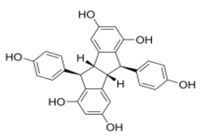
Fig. 1. Chart of isolating a stilbenoid-containing fraction from the grape skin.

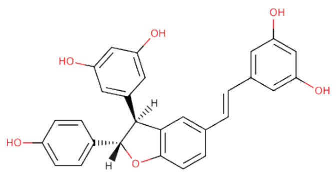
Stilbenoids were determined by the method of high-performance liquid chromatography (HPLC) (Guebailia et al., 2006). For this purpose, the following was used: Column- Supelcosil PM LC18, 250 x 4.6 mm; Eluents: A. 0.025% trifluoroacetic acid, B. Acetonitrile**:** A 80/20. Gradient mode: 0-35 min, 20-50% B, 48-53 min, 200% B. Flow rate of the eluent- 0.8 ml/min; fractions were filtered using a membrane filter (0.45 µ) before the chromatographic procedure.

Lab-*in vitro*-experiment. Healthy of winegrape varieties (*V. vinifera* L.) Saperavi and Rkatsiteli were sampled by technological maturity (September, 2024) at the locations described above. The experimental design included: 1) berry pretreatment with 3 mg/100 mL, 5 mg/100 mL and 10 mg/100 ml of pallidol and *cis*-δ-viniferin, followed by the infection with *Botrytis cinerea*; 2) control berries with fungal infection but without pretreatment. The pre-treatment was done by soaking 12 berries per variety in the solutions described above (or just water in case of the control group), while fungal infection was done by spraying *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.

Results and Discussion

The physiological concentration of pallidol in the skin of healthy Saperavi and Rkatsiteli grapes was higher than the concentration of *cis*-δ -viniferin. In the presence of gray mold, the concentrations of these phytoalexins – dimeric stilbenoids, increase in the skin of infected Saperavi and Rkatsiteli grapes (Table 1).





Pallidol, $C_{28}H_{22}O_6$ *Cis*-δ-viniferin, $C_{28}H_{22}O_6$

The phytoalexin activity of pallidol and cis-δ-viniferin was confirmed by their effect on the growth and development of *Botrytis cinerea in vitro* (in lab) conditions. Pallidol showed high biological efficacy in inhibiting the growth and development of *Botrytis cinerea* compared to *cis-δ* -viniferin. Meanwhile, the phytoalexin activity of both stilbenoids increased directly proportionally within their concentration variability range from 3 mg/100 ml to 10 mg/100 ml. A higher biological efficacy was exhibited by the total preparation of these stilbenoids, the suspension of which at a concentration of 10 mg/100 ml caused a 91.7% inhibition of *Botrytis cinerea* (Table 2).

Table 1. Change of pallidol and *cis*- δ-viniferin of grape skin of Saperavi and Rkatsiteli (*Vitis vinifera* L.) in case of gray mold infection

Stilbenoids, mg/kg	Saperavi				Rkatsiteli			
	Mukuzani		Napareuli		Tsarapi		Tibaani	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
Pallidol	2.7	3.5	2.3	3.1	1.9	2.6	2.4	3.2
Cis- δ-viniferin	1.8	3.0	1.5	2.8	0.9	2.3	1.5	2.3

Table 2. Impact of pallidol and *Cis*- δ-viniferin on the gray mold infection (*Botrytis cinerea*) of Saperavi and Rkatsiteli grape berries *in vitro* condition (lab experiment)

Pre-treatments	Number of berries in each petri dish		Number of 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
Pallidol, 3mg/100ml	12	12	5	5	41.7	41.7	58.3	58.3
Pallidol, 5mg/100ml	12	12	4	4	33.3	33.3	66.7	66.7
Pallidol, 10mg/l00ml	12	12	2	2	16.7	16.7	83.3	83.3
Cis- δ-viniferin, 3 mg/100ml	12	12	6	6	50.0	50.0	50.0	50.0
Cis- δ-viniferin, 5 mg/100ml	12	12	4	4	33.3	33.3	66.7	66.7
Cis- δ-viniferin, 10mg/100ml	12	12	3	3	25.0	25.0	75.0	75.0
P-1, 3mg/100ml	12	12	4	4	33.3	33.3	66.7	66.7
P-2, 5mg/100ml	12	12	2	2	16.7	16.7	83.3	83.3
P-3, 10mg/100ml	12	12	1	1	8.3	8.3	91.7	91.7
Rkatsiteli	Tsarapi	Tibaani	Tsarapi	Tibaani	Tsarapi	Tibaani	Tsarapi	Tibaani
Control	12	12	12	12	100	100	0.0	0.0
Pallidol, 3mg/100ml	12	12	6	5	50.0	41.7	50.0	58.3
Pallidol, 5mg/100ml	12	12	4	4	33.3	33.3	66.7	66.7
Pallidol, 10mg/l00ml	12	12	2	2	16.7	16.7	83.3	83.3
Cis- δ-viniferin, 3 mg/100ml	12	12	6	6	50.0	50.0	50.0	50.0
Cis- δ-viniferin, 5 mg/100ml	12	12	5	5	41.7	41.7	58.3	58.3
Cis- δ-viniferin, 10mg/100ml	12	12	3	2	25.0	16.7	75.0	83.3
P-1, 3mg/100ml	12	12	4	4	33.3	33.3	66.7	66.7
P-2, 5mg/100ml	12	12	2	2	16.7	16.7	83.3	83.3
P-3, 10mg/100ml	12	12	1	1	8.3	8.3	91.7	91.7

P-Total preparation (pallidol+*cis*-δ-viniferin).

Conclusion

In the skin of healthy and infected Saperavi and Rkatsiteli grapes harvested from selected micro-zones, pallidol and *cis*-δ-viniferin were identified as phytoalexin stilbenoids. Their phytoalexin properties against gray mold were determined in *in vitro* (lab) conditions. The mentioned dimers were indicated as stilbenoid bio-markers in Saperavi and Rkatsiteli for their resistance against the fungal disease – gray mold.

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(წარმოდგენილია აკადემიის წევრის რ. გახოკიძის მიერ)

ვაზის სტილბენოიდები მრავალფეროვანი ბიოლოგიური აქტივობებით ხასიათდება, რომელთა შორის მნიშვნელოვანია ფიტოალექსინური აქტივობა. ჩვენ მიერ დადგენილია საქართველოს თეთრ- და წითელყურძნიანი საღვინე ვაზის ჯიშების იმუნიტეტის კორელაცია ფიტოალექსინ სტილბენოიდებთან ბაქტერიული და სოკოვანი დაავადებების პირობებში. შევისწავლეთ საფერავის და რქაწითელის ფიტოალექსინი სტილბენოიდების ცვალებადობა საქართველოს მევენახეობის მიკროზონებში, კონკრეტულად, საფერავისთვის მუკუზანსა და ნაფარეულში; რქაწითელისთვის – წარაფსა და ტიბაანში. სოკოვანი – ნაცრის და ნაცრისფერი სიდამპლის და ბაქტერიული – ბაქტერიული კიბოთი დაავადებების პირობებში გამოვლენილია სტრეს-მეტაბოლიტი სტილბენოიდები. ისინი მნიშვნელოვანი კონცენტრაციით, ძირითადად, წარმოდგენილია ტრანს-რეზვერატროლის, ტრანს-პიცეიდის, ტრანს-ასტრინგინის, ცის-პიცეიდის, ცის-რეზვერატროლის სახით. სტილბენოიდურ პროფილში დაფიქსირდა ასევე სხვა სტილბენოიდები მცირე კონცენტრაციით. საფერავისა და რქაწითელის სტილბენოიდური პროფილის, როგორც რეზისტენტობის ბიომარკერის, კვლევით გამოვლინდა რეზვერატროლის დიმერული წარმოებულების – პალიდოლის და ცის-δ-ვინიფერინის ფიტოალექსინური აქტივობა *Botrytis cinerea*-ს მიმართ *in vitro* (ლაბორატორიულ) პირობებში. პალიდოლის და ცის-δ-ვინიფერინის ფიზიოლოგიური და სტრეს-მეტაბოლიტური კონცენტრაციები საფერავის კანში მეტი კონცენტრაციით დაფიქსირდა რქაწითელის კანთან შედარებით. დადგინდა, რომ პალიდოლი და ცის-δ-ვინიფერინი ხასიათდება ფიტოალექსინური თვისებებით და სტილბენოიდურ ბიომარკერებში, ძირითადად, მაღალი კონცენტრაციის სტრეს-მეტაბოლიტებთან ერთად დაფიქსირებული მათი დაბალი კონცენტრაციები გარკვეულწილად განაპირობებს საფერავის და რქაწითელის რეზისტენტობას ნაცრისფერი სიდამპლის მიმართ.

REFERENCES

Adrian, M. Jeandet, P., Douillet-Breuil, A.C., Tesson, L., Bessis, R. (2000). Stilbene content of mature Vitis vinifera berries in response to UV-C elicitation. *J. Agric. Food Chem*., *48*, 6103-6105.

Adrian, M., Jeandet, P., Bessis, R., Joubert, J. M. (1996). Induction of phytoalexin (resveratrol) synthesis in grapevine leaves treated with aluminum chloride (AlCl3*). J. Agric. Food Chem*., *44*,1979-1981.

Adrian, M., Jeandet, P., Veneau, J., Weston, L. A. and Bessis, R. (1997). Biological activity of resveratrol, a stilbenic compound from grapevines, against Botrytis cinerea, the causal agent for gray mold. *J. Chem. Ecol*., *23* (7), 1689-1702.

Adrian, M., Rajaei, H., Jeandet, P., Veneau, J. and Bessis, R. (1998). Resveratrol Oxidation in Botrytis cinerea Conidia. *Phytopathology*, *88* (5),472-476.

Bavaresco, L; Petegolli, D; Cantu, E; Fregoni, M; Chiusa, G; Trevisan, M. (1997). Elicitation and accumulation of stilbene phytoalexins in grapevine berries infected by Botrytis cinerea. *Vitis*, *36* (2),77-83.

Bezhuashvili, M., Bavaresco, L., Surguladze, M., Tskhvedadze, L., Shoshiashvili, G., Gagunashvili, L., Elanidze, L.,Vashakidze, P. (2021). Stress-metabolite stilbenoids of grape skin of varieties Aleksandrouli and Mujuretuli (*Vitis vinifera* L.) in condicion of gray mold. *AGRICULTURE & FOOD, 9th International Conference,* August 16-19, Burgas, Bulgaria.

Bezhuashvili, M., Tskhvedadze, L., Surguladze, M., Shoshiashvili, G., Kharadze, Sh., Gagunashvili, L., Elanidze, L.,Vashakidze, P. (2019). Stress-metabolites phytoalexins -stilbenoids of grape skin Rkatsiteli variety (*Vitis vinifera* L.) in condition gray mildew. *Eurasian Journal of Biosciences*, *13*, 1-6.

Bezhuashvili, M., Tskhvedadze, L., Surguladze, M., Shoshiashvili, G., Elanidze, L., Vashakidze, P. (2019). Impact of trans-resveratrol on the activity of conidium of the Botrytis cinerea. *International Conference on Plant Physiology and Biotechnology,* May 06-07, Prague, Czech Republic. Abstract book, p. 7-8.

Bezhuashvili, M., Bavaresco, L., Tskhvedadze, L., Surguladze, M., Shoshiashvili, G., Darchiashvili, N., Vashakidze, P. (2023). Phytoalexin stilbenoids of Saperavi and Rkatsiteli (*Vitis vinifera* L.) as biomarkers of resistance to gray mold (*Botrytis cinerea*). *Bull. Georg. Natl. Acad. Sci., 17* (2),136-141.

Bezhuashvili, M., Bavaresco, L., Surguladze, M., Tskhvedadze, L., Shoshiashvili, G., Darchiashvili, N., Vashakidze, P. (2025). Phytoalexin stilbenoids of Saperavi and Rkatsiteli (*Vitis vinifera* L.) as biomarkers of resistance to some fungal diseases. *Asian Journal of Agricultural and Food Sciences, 13*(1), 1-9.

Błaszczyk, A., Sady, S., Sielicka, M. (2019). The stilbene profile in edible berries. *Phytochemistry Reviews, 18* (1), 37-67.

Breuil, A.C., Jeandet, P., Adrian, M., Chopin, F., Pirio, N., Meunier, P. and Bessis R. (1999). Characterization of a pterostilbene dehydrodimer produced by laccase of Botrytis cinerea. *89* (4), 298-302.

Flamini, R., De Rosso, M., De Marchi, F., Vedova, A.D., Panighel, A., Gardiman, I. Maoz, M., Bavaresco, L. (2013). An innovative approach to grape metabolomics: Stilbene profiling by suspect screening analysis. *Metabolomics, 9* (6), 1243-1253.

Flamini, R., Zanzotto, A., de Rosso, M., Lucchetta, G., Vedova, A.D., Bavaresco, L. (2016). Stilbene oligomer phytoalexins in grape as a response to Aspergillus carbonarius infection. *Physiological and Molecular Plant Pathology,* 93, 112-118.

Guebailia, H. A., Chira, K., Richard, T., Mabrouk, T. and Furiga, A. (2006). Hopeaphenol: the first resveratrol tetrameric wines from North Africa. *J. Agric. Food Chem.*, *54*, 9559-9564.

Jeandet, P., Douillet-Breuil, A., Bessis, R., Debord, S., Sbaghi, M., Adrian, M., (2002). Phytoalexins from the Vitaceae: Biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. *J. Agric. Food Chem*., 50, 2731-2741.

Langcake, P., Cornford, C. A., Pryce, R. J. (1979). Identification of pterostilbene as a phytoalexin from Vitis vinifera leaves. *Phytochemistry*, 18,1025-1027.

Langcake, P., (1981). Disease resistance of Vitis spp and the production of the stress metabolites resveratrol, E-viniferin, R-viniferin and pterostilbene. *Physiol. Plant Pathol*., 18, 213-226.

Langcake, P., Pryce, R. J. (1976). The production of resveratrol by Vitis Vinifera and other members of the Vitaceae as a response to infection or injury. *Physiol. Plant Pathol.,* 9, 77-86.

Niesen D., Hessler, C. and Navindra, S. P. (2013). Beyond resveratrol: A review of natural stilbenoids identified from 2009-2013. *J.of Berry Research,* 3, 181-196.

Pezet, R., Pont, V. (1988). Mise en evidence de pterostilbene dans les grappes de Vitis vinifera. *Plant Physiol. Biochem*., *26*, 603-607.

Rivière, C., Pawlus, A.D., Mérillon, J.M. (2012). Natural stilbenoids: Distribution in the plant kingdom and chemotaxonomic interest in Vitaceae. *Natural Product Reports, 29* (11), 1317-1333.

Waterhouse, A. L; Lamuela Raventos, R. M. (1994). The occurrence of piceid, a stilbene glucoside in grape berries. *Phytochemistr*y, 37, 571-573.

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