Erwerbs-Obstbau (2020) 62(Suppl 1):S81–S85
https://doi.org/10.1007/s10341-020-00500-2

ORIGINAL ARTICLE/ ORIGINALBEITRAG

Clonal *trans*-Resveratrol Potential in the Ripened Grapes of *Vitis vinifera* L. cv 'Kalecik Karası'

Nurhan Keskin[1] · Birhan Kunter[2] · Hasan Çelik[3]

Received: 6 April 2020 / Accepted: 21 April 2020 / Published online: 26 May 2020

Abstract

In this study, *trans*-resveratrol (*t*-RSV; 3,5,4′-trihydroxy-*trans*-stilbene) contents of the ripened berries of 23 clones of 'Kalecik Karası' which is one of Turkey's leading red wine grape cultivars, grown in Ankara (Kalecik) were determined by HPLC. For this purpose; the berries were collected at 22–24 °Brix during 2016–2017 vintage seasons. Descriptive statistics for *t*-RSV content of clones were expressed as mean with their standard errors. One-way analysis of variance was performed for the comparison. Duncan multiple comparison test was used to determine the significant variances among clones. In addition, Analysis of Mean (ANOM) was also applied to determine the differences of the clones from the grand mean. As a result, *t*-RSV content of the clones ranged from 0.31 mg kg^{-1} (Clone 22) to 1.67 mg kg^{-1} (Clone 7) followed by Clone 6 with 1.12 mg kg^{-1} and differences among the clones were statistically significant ($p < 0.05$).

Keywords Grape · clone · HPLC · phytoalexin · stilbene

Trans-Resveratrol Potential in ausgereiften Trauben von Klonen der Rebsorte (*Vitis vinifera* L.) 'Kalecik Karası'

Schlüsselwörter Traube · Klon · HPLC · Phytoalexin · Stilbene

Introduction

Trans-resveratrol (*t*-RSV; 3,5,4′-trihydroxy-*trans*-stilbene) is a phytoalexin of stilbenes group. It is found at considerable amounts in grape berries and products. *t*-RSV has been detected in different parts of the grapevines such as seed and pulp (Pezet and Cuneat 1996), cluster stems (Bavaresco et al. 1997a), cane (Karacabey and Mazza 2008), and especially berry skin (Creasy and Coffee 1988; Jeandet et al. 1991; Roggero and Garcia-Parrilla 1995; Romero-Pérez et al. 2001). The genotypic potential is one of the most important factors affecting *t*-RSV production in grape berries (Okuda and Yokotsuka 1996). Preliminary data clearly revealed that some grape cultivars describe to have low *t*-RSV production capacity ('Kalecik Karası', 'Aglianico' 'Schiava Grossa' and 'Nebbiolo') while some ('Erciş' 'Öküzgözü', 'Cabernet Sauvignon', 'Pinot noir', 'Lemberger', 'Marsanne') have higher contents (Keskin and Kunter 2007, 2008, 2009, 2010; Castellarin et al. 2012). Environmental factors and cultural practices notably affects *t*-RSV production potential not only among the grape cultivars but also in a cultivar depending on the vintage year (Negri et al. 2008; Gökçen et al. 2017; Keskin 2017; Keskin and Kunter 2017). So, *t*-RSV accumulation in the berries of a cultivar can increase as a reply of biotic and abiotic stress conditions (Revilla and Ryan 2000; Adrian et al. 2000).

The purpose of clonal selection is to select superior individuals of a grape variety in its original populations

✉ Nurhan Keskin
keskin@yyu.edu.tr

Birhan Kunter
marasali@agri.ankara.edu.tr

Hasan Çelik
hcelik@eul.edu.tr

[1] Faculty of Agriculture, Department of Horticulture, Van Yüzüncü Yıl University, 65080 Van, Turkey

[2] Faculty of Agriculture, Department of Horticulture, Ankara University, 06110 Ankara, Turkey

[3] Faculty of Agricultural Sciences & Technologies, Department of Horticultural Production & Marketing, European University of Lefke, Gemikonağı, Lefke, Cyprus

considering the yield and quality characteristics. Therefore, variations among the clones of a grape cultivar may contribute to a better understanding of their genetic potential for *t*-RSV accumulation (Bavaresco and Fregoni 2001). 'Kalecik Karası' is one of the leading red wine grape variety of Turkey, grown along the valley of Kızılırmak river near Kalecik located at 70 km east of Ankara. This cultivar with its violet-ruby colored and aromatic berries gives soft, lively, easy-to-drink and medium-sized bodied wines with elegant and delicate structure (Çelik et al. 2019). *T*-RSV content in ripened grapes and wines of 'Kalecik Karası' has been studied in some previous studies (Anlı and Vural 2009; Çelik et al. 2019). Although agronomic traits of 'Kalecik Karası' clones are well known, our knowledge on their *t*-RSV capacity is still limited. A unique and detailed clone selection study was conducted by Çelik et al. (2019) on 23 clones of 'Kalecik Karası' including the *t*-RSV contents of their berry skins and seeds in its original ecology.

The objectives of the present study were to determine the *t*-RSV production performance of 23 'Kalecik Karası' clones by use of total berry extraction method and to compare the performances with statistical comparison technique (Analysis of mean ANOM).

Material and Method

Material

The study was carried on the 23 Clones of 'Kalecik Karası' (*Vitis vinifera* L. cv.) which were selected as the result of clone selection project (Fidan et al. 1975, 1988, 1991; Çelik et al. 2019) and were cultivated in the clonal selection vineyard which was established in 1999 at the Research Station for Viticulture in Kalecik, Ankara, Turkey.

Method

Collecting the Grape Berry Samples

Berries were collected randomly when total soluble solids contents of the berries reached at 22–24 °Brix, according to Amerine and Cruess (1960) during the vintage of 2016–2017, and then were stored in a deep freezer at –25 °C within plastic bags, up to extraction.

Determination of t-RSV Content of the Berries

Grape berries were thoroughly ground with a mortar and pestle, then extracted in darkness at room temperature with an orbital shaker vigorously for 20 min in 30 ml of 95% methanol. After the mixture was filtered through GF/A Whatman filter paper, the filtrate was dried in vacuum at 40 °C using a rotary evaporator. During the drying process, the water phase was extracted twice with ethyl acetate and 5 ml of 5% $NaHCO_3$. The organic phase was evaporated till to dryness and *t*-RSV was recovered twice with 1 ml of methanol (100%). The extract was stored in brown glass bottles at 4 °C until analysis (Bavaresco et al. 1997b).

T-resveratrol was analyzed with HPLC (High Pressure Liquid Chromatography) and standard for this analysis was supplied by Sigma (Germany).

HPLC device was used with Agilent 1100 (Agilent, USA), Phenomenex/Luna guard column (5 µm, 12.5 × 4.6 mm, ID), Phenomenex/Luna C18 column (5 µm, 250 × 4.6 mm, ID) and UV-VIS detector system in accordance with Jeandet et al. (1997). *T*-RSV values were obtained at 330 nm wavelength.

Statistical Analysis

Descriptive statistics for *t*-RSV content of the clones were expressed the mean and standard error. One-way analysis of variance (ANOVA) was performed to compare means of clones. Following the ANOVA, Duncan multiple comparison test was used to determine the comparative performances of the clones. Additionally, the ANOM test was also performed to determine the differences of the clones from the overall mean and to present visually the results. For all statistical comparisons, the significance level was considered as 5% and MINITAB statistical package program was used for all statistical computations.

Results and Discussion

The *t*-RSV contents of 23 clones of 'Kalecik Karası' red wine grape cultivar are shown in Table 1. The highest *t*-RSV content was measured in Clone 7 (1.67 mg kg^{-1}) and the lowest in Clone 22 (0.31 mg kg^{-1}).

Fig. 1 has also presented the similarities of the clones in terms of *t*-RSV. Upper and lower decision lines were 0.807 mg kg^{-1} and 0.616 mg kg^{-1}, respectively. Clones numbered as 1, 2, 3, 5, 6, 7, 8, 10, 16 and 21 were significantly different from the 0.712 mg kg^{-1} overall mean with the outside of the upper decision line. Similarly, clones numbered as 4, 9, 11, 12, 13, 14, 15, 17, 18, 20, 22 and 23 were found to be significantly different from the overall mean by out of the lower decision line. In addition, the differences among the clones that below and above of the decision lines were also statistically significant. Thus, it can be stated that there is a considerable difference among the clones in terms of *t*-RSV content.

Clone selection studies have focused more on yield and quality. *T*-RSV content of the grape berries that can be

Table 1 Descriptive statistics and comparative performances of the clones of 'Kalecik Karası' in terms of *t*-RSV contents of the berries

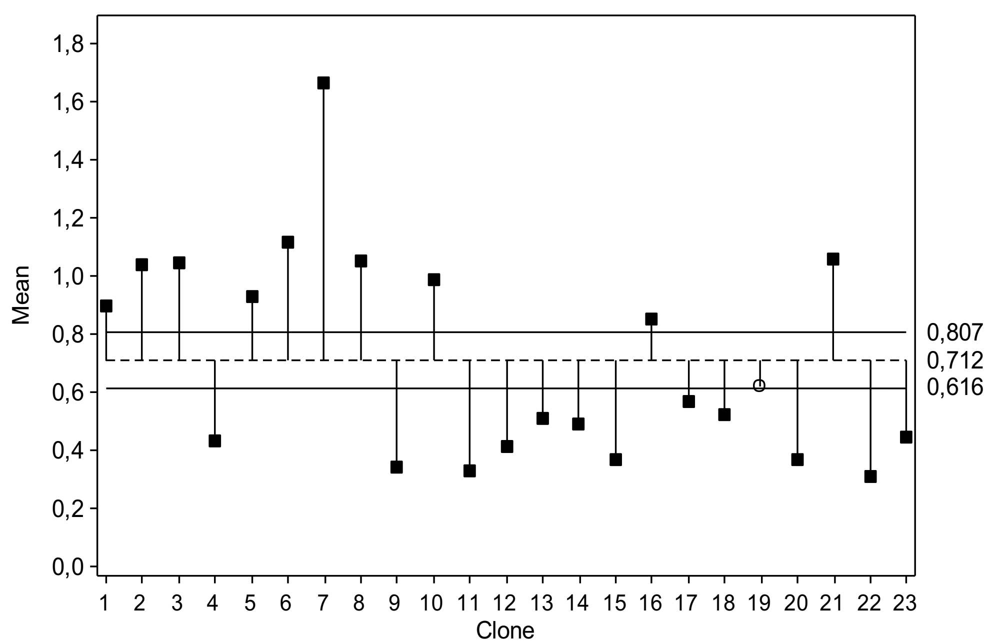
Clone number	*t*-RSV(mg kg^{-1})	Standard Error
1	0.89 e*	0.01
2	1.04 bc	0.01
3	1.05 bc	0.01
4	0.44 hijk	0.01
5	0.93 cd	0.01
6	1.12 b	0.03
7	1.67 a	0.02
8	1.05 bc	0.03
9	0.35 klm	0.02
10	0.99 cd	0.01
11	0.33 lm	0.01
12	0.41 ijkl	0.01
13	0.51 gh	0.01
14	0.49 ghj	0.01
15	0.37 jklm	0.01
16	0.85 e	0.05
17	0.57 fg	0.02
18	0.53 gh	0.03
19	0.62 f	0.02
20	0.37 jklm	0.05
21	1.06 bc	0.07
22	0.31 m	0.01
23	0.45 hij	0.05

*Different lower cases represent statistically significant differences among the clones' means ($p < 0.05$)

considered as a quality factor in grape cultivars has been affected by cultivars (Bavaresco et al. 2007; Gatto et al. 2008), clone (Gatti et al. 2014), meteorological conditions (Bavaresco et al. 2007), soil (Bavaresco et al. 2005) and cultural practices (Gebbia et al. 2003; Bavaresco et al. 2007, 2008; Gatti et al. 2011). There are almost no studies to compare the clones in terms of *t*-RSV content.

Iacopini et al. (2008) found quite low *t*-RSV content in the clones of 'Italian Sangiovese' cultivar (AP SG 1, F9, ISV 2, ISV, RC1). There was no difference in *t*-RSV content of 'Cabernet Sauvignon' clones (169, 191, 338, 341, 685, ISV105, ISV117, ISV2, R5, VCR8) although there were significant differences between the clones in terms of other stilbene compounds such as piceid, piceatannol and ε-viniferin (Gatti et al. 2014). Çelik et al. (2019), compared 23 clone of 'Kalecik Karası' grape cultivar for *t*-RSV content in the berry skin and seed separately. According to their data, the berry skin *t*-RSV content showed significant difference between years. Clone 1 had the highest value with 1.54 µg g skin FW^{-1} in 2008 while Clone 23 with 1.67 µg g skin FW^{-1} in 2009. Similarly, *t*-RSV content in the seed ranged from 1.10 µg g skin FW^{-1} (Clone 1) to 0.40 µg g skin FW^{-1} (Clone 17) in 2008.

Fig. 1 ANOM diagram for *t*-RSV content of 'Kalecik Karası' clones



Conclusion

Genotype, rootstock, climate, soil and cultural practices as well as biotic and abiotic stress factors affect directly or indirectly the production of *t*-RSV in grapes. The investigation of *t*-RSV content whether in lower and upper limits can be a criterion for improving clonal selection. Therefore, this study was carried out to contribute better understanding of the *t*-RSV production whether depends on the genetic performance of the clones of 'Kalecik Karası' that is one of the leading red wine cultivars of Turkey. *t*-RSV content in 23 clones of 'Kalecik Karası' varied between 0.31 mg kg^{-1} and 1.67 mg kg^{-1}. It was concluded that clonal variation is considerable in 23 clones and Clone 7 has the highest capacity with 1.67 mg kg^{-1} for *t*-RSV content. Also statistical similarity analysis showed that selected clones of 'Kalecik Karası' can be grouped for their *t*-RSV accumulative capacities.

Conflict of interest N. Keskin, B. Kunter and H. Çelik declare that they have no competing interests.

References

Adrian M, Jeandet P, Douillet-Breuil AC, 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

Amerine MA, Cruess MV (1960) The technology of wine making. Avi Publishing, Westport

Anli RE, Vural N (2009) Antioxidant phenolic substances of Turkish red wines from different wine regions. Molecules 14:289–297

Bavaresco L, Fregoni C (2001) Physiological role and molecular aspects of grapevine stilbenic compounds. In: Roubelakis-Angelakis KA (ed) Molecular biology and biotechnology of the grapevine. Kluwer, Netherlands, pp 153–182

Bavaresco L, Cantu E, Fregoni M, Trevisan M (1997a) Constitutive stilben contents of grapevine cluster stems as potential source of resveratrol in wine. Vitis 36:115–118

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

Bavaresco L, Civardi S, Pezzutto S, Vezzulli S, Ferrari F (2005) Grape production, technological parameters, and stilbenic compounds as affected by lime-induced chlorosis. Vitis 44(2):63–65

Bavaresco L, Gatti M, Pezzutto S, Fregoni M, Mattivi F (2008) Effect of leaf removal on grape yield, berry composition, and stilbene concentration. Am J Enol Vitic 59:292–298

Bavaresco L, Pezzutto S, Gatti M, Mattivi F (2007) Role of the variety and some environmental factors on grape stilbenes. Vitis 46:57–61

Castellarin SD, Bavaresco L, Falginella L, Gonçalves MIVZ, Di Gaspero G (2012) Phenolics in grape berry and key antioxidants. In: Gerós H, Chaves MM, Delrot S (eds) The biochemistry of the grape berry. Bentham, Dubai, pp 89–110

Creasy LL, Coffee M (1988) Phytoalexin production potential of grape berries. J Am Soc Hortic Sci 113:230–234

Çelik H, Kunter B, Selli S, Keskin N, Akbaş B, Değirmenci K (2019) Kalecik Karası üzüm çeşidinde klon seleksiyonu ve seçilen klonlara ait ana damızlık parselinin oluşturulması. In: Kunter B, Keskin N (eds) Tarım Bilimlerinde Güncel Araştırma ve Değerlendirmeler. Stamparija Ivpe, Cetinje, pp 59–95

Fidan Y, Eriş A, Şeniz V (1975) Kalecik Karası üzüm çeşidinde seleksiyon. TÜBİTAK-TOAG, Proje No: TOAG-157. Sonuç Raporu, Ankara, p 37

Fidan Y, Yavaş İ, Özışık S (1991) Kalecik Karası üzüm çeşidinde teksel seleksiyon. TÜBİTAK-TOAG, Proje No: 634. Sonuç Raporu, Ankara

Fidan Y, Çelik H, Eriş A, Çelik S, Şeniz V, Yavaş İ, Demir İ, Özışık S (1988) Kalecik Karası üzüm çeşidinde teksel seleksiyon, Türkiye. In: III. Bağcılık Simpozyumu Bildiri Özetleri Bursa, 31 May–3 June, p 28

Gatti M, Civardi S, Ferrari F, Fernandes N, van Zeller de Basto Gançalves MI, Bavaresco L (2014) Viticultural performances of different Cabernet Sauvignon clones. Acta Hortic 1046:659–664

Gatti M, Civardi S, Zamboni M, Ferrari F, Elothmani D, Bavaresco L (2011) Preliminary results on the effect of cluster thinning on stilbene concentration and antioxidant capacity of V. vinifera L. "Barbera" wine. Vitis 50:43–44

Gatto P, Vrhovsek U, Muth J, Segala C, Romualdi C, Fontana P, Pruefer D, Stefanini M, Moser C, Mattivi F, Velasco R (2008) Ripening and genotype control stilbene accumulation in healthy grapes. J Agric Food Chem 56:11773–11785

Gebbia N, Bavaresco L, Fregoni M, Civardi S, Crosta L, Ferrari F, Grippi F, Tolomeo M, Trevisan M (2003) The occurrence of the stilbene piceatannol in some wines from Sicily. Vignevini 30:87–94

Gökçen İS, Keskin N, Kunter B, Cantürk S, Karadoğan B (2017) Üzüm fitokimyasalları ve Türkiye'de yetiştirilen üzüm çeşitleri üzerindeki araştırmalar. Turk J For Sci 1:93–111

Iacopini P, Baldi M, Storchi P, Sebastiani L (2008) Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: content, in vitro antioxidant activity and interactions. J Food Compos Anal 21:589–598

Jeandet P, Bessis R, Gautheron B (1991) The production of resveratrol (3,5,4-trihydroxy-stilbene) by grape berries in different developmental stages. Am J Enol Vitic 42:41–46

Jeandet P, Breuil AC, Adrian M, Weston LA, Debord S, Meunier P, Maume G, Bessis R (1997) HPLC analysis of grapevine phytoalexins coupling photodiode aray detection and fluorimetry. Anal Chem 69(24):5172–5177

Karacabey E, Mazza G (2008) Optimization of solid-liquid extraction of resveratrol and other phenolic compounds from milled grape canes (Vitis vinifera). J Agric Food Chem 56(15):6318–6325

Keskin N (2017) Multidimensonal scaling (MDS) to visual representation of proximites for quality and phytochemical characterstics in Vitis vinifera L. cv. 'Ercis. Prog Nutr 19:305–311

Keskin N, Kunter B (2007) Induction of resveratrol via UV irradiation effect in Ercis callus culture. J Agric Sci 13(4):379–384

Keskin N, Kunter B (2008) Production of trans-resveratrol in 'Cabernet Sauvignon' (Vitis vinifera L.) callus culture in response to ultraviolet-C irradiation. Vitis 47(4):193–196

Keskin N, Kunter B (2009) The effects of callus age, UV irradiation and incubation time on trans-resveratrol production in grapevine callus culture. J Agric Sci 15(1):9–13

Keskin N, Kunter B (2010) Production of trans-resveratrol in callus tissue of Öküzgözü (Vitis vinifera L.) in response to ultraviolet-c irradiation. J Animal Plant Sci 20(3):197–200

Keskin N, Kunter B (2017) Stilbenes profile in various tissues of grapevine (Vitis vinifera L. cv. 'Ercis'). JEPE 18:1259–1267

Negri AS, Prinsi B, Rossoni M, Failla O, Scienza A, Cocucci M, Espen L (2008) Proteome changes in the skin of the grape cultivar Barbera among different stages of ripening. BMC Genom 9:378

Okuda T, Yokotsuka K (1996) Trans-resveratrol concentrations in berry skins and wines from grapes grown in Japan. Am J Enol Vitic 47:93–99

Pezet R, Cuneat P (1996) Resveratrol in wine: extraction from skin during fermentation and post-fermentation standing of must from Gamay grapes. Am J Enol Vitic 47:287–290

Revilla E, Ryan JM (2000) Analysis of several phenolic compounds with potential antioxidant properties in grape extracts and wines by high-performance liquid chromatography-photodiode array detection without sample preparation. J Chromatogr A 881:461–469

Roggero JP, Garcia-Parrilla C (1995) Effects of ultraviolet irradiation on resveratrol and changes in resveratrol and various of its derivatives in the skins of ripening grapes. Sci Aliments 15:411–422

Romero-Pérez AI, Lamuela-Raventós RM, Andrés-Lacueva C, de la TorreBoronat MC (2001) Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. J Agric Food Chem 49:210–215