

SKIN PHENOLS IN SEEDLESS GRAPES (*VITIS VINIFERA* L.) AS INFLUENCED BY GENOTYPE AND IRRIGATION

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ABSTRACT

Black/red grapes are rich in phenol compounds. Many clinical studies have underlined the health benefits of consuming phenol-rich foods in order to reduce the risk of developing degenerative diseases. The berry phenols accumulation has a genetic base, but, its final expression is influenced by soil, climate, grape maturity and cultural practices. A trial was run in Southern Italy to assess berry skin phenols in three seedless cultivars, at a same degree of grape maturity (sugar concentration): Black Emerald (early ripening), Autumn Royal (medium-late ripening) and Crimson Seedless (late ripening). Moreover, the seeded cv Black Pearl was included in the study as a reference. It was evident that the two cultivars with darker berry skin color (Black Emerald and Black Pearl) accumulated a higher amount of phenols per gram of fresh berry, while the cultivar with lighter (red) skin color (Crimson Seedless) performed at the opposite; the cultivar with dark blue skin color (Autumn Royal) performed intermediately. However, the difference in phenol amounts was not limited to anthocyanins, but concerned also others phenol compounds. Autumn Royal and Crimson Seedless were also tested to evaluate phenol differences in grapes produced by “normally-irrigated” and “deficit-irrigated” vines. By comparing the phenol accumulation as related to the irrigation level, a genotype-irrigation interaction was noticed: grapes from deficit-irrigated Autumn Royal vines showed a moderate decrease in berry weight but a high increase in phenol amount per kilogram of grape, while Crimson Seedless, in this trial, did not show a relevant difference of berry skin phenols related to the irrigation level.

RESUME

Les raisins à peau noire ou rouge sont riches en polyphénols, composés que plusieurs études cliniques ont montré les bienfaits sur la santé humaine par la réduction des risques de maladies dégénératives. La richesse en polyphénols est déterminée génétiquement, mais elle est également grandement influencée par le terroir, le stade de maturité et les pratiques culturales. Une étude a été menée en Italie du sud pour déterminer la richesse en polyphénols, à maturité équivalente (exprimée par le degré Brix) chez quatre variétés de raisin de table sans pépins : Black Emerald (maturité précoce), Autumn Royal (maturité moyenne) et Crimson Seedless (maturité tardive). La variété Black Pearl, avec pépins, a été indiquée comme référence. Cette étude a aussi permis de déterminer l'effet de l'irrigation déficitaire sur la richesse en polyphénols par comparaison à une irrigation classique chez les deux cépages

Autumn Royal et Crimson Seedless. Il apparait donc que cette teneur est « proportionnelle » à l'intensité de la couleur de la peau du raisin. Cette différence de teneur en phénols n'a pas concerné uniquement les anthocyanes mais également autres paramètres mesurés. La teneur en phénols des raisins semble être dépendante de l'interaction génotype/irrigation. En effet, pour le cépage Autumn Royal, l'irrigation déficitaire a donné des baies plus petites mais avec une teneur en phénols plus élevée, alors que chez le cépage Crimson Seedless, il n'y a pas eu d'effets visibles en phénols.

INTRODUCTION

Italy is one of the major producers and exporters of table grapes; seeded varieties are traditionally the most grown, but nowadays there is a wide interest for seedless ones due to the increase of world market demand for this type of grapes.

Black/red grapes are known to be rich in phenol compounds. Many clinical studies have underlined the health benefits of consuming phenol-rich foods and beverages, especially to reduce the risk of developing degenerative diseases. The grape phenols are characterized by functional properties that bring benefits to human health. When different parts of grape are analyzed, the greatest antioxidant capacity is generally found in seeds, followed by skin and then by flesh (Pastrana-Bonilla *et al.*, 2003; Xia *et al.*, 2010). It is known that the amount of berry phenols have a genetic base but, at same time, its final expression is influenced by soil, climate, maturity and cultural practices (Downey *et al.*, 2006).

Among cultural practices, irrigation is critical for table grapes, since tablegrape vineyards need consistent water supply in order to achieve satisfying results in terms of grape quality and quantity. Nevertheless, presently there is a renovate interest for deficit-irrigation, aiming to save water resource and limit the production costs (Du *et al.*, 2008; El-Ansary *et al.*, 2005).

Basing on these considerations, a trial was run in Southern Italy to assess the amount of berry skin phenols in three seedless cultivars: Black Emerald, that is early ripening, has dark berry skin and round to oval berry shape, Autumn Royal, that is medium-late ripening, has dark purple to dark blue skin color and a naturally large berry of elongated shape, Crimson Seedless that is late ripening, has a red-violet skin color, berry shape from cylindrical to oval in shape, and is the main variety in many markets (Ramming and Taraillo, 1995; Ramming and Taraillo, 1995, Ramming and Taraillo 1998; USDA ARS, 1996). Moreover, Black Pearl that is a late ripening seeded cultivar having dark berry skin color, elongated berry, and a good storage performance (Isbat and Zeba, 2011) was also included in the trial, as a reference.

Autumn Royal and Crimson Seedless that are, among the four genotypes, the most popular in Italy, were tested also to evaluate differences in the amount of berry skin phenols of grapes produced by “normally-irrigated” and “deficit-irrigated” vines.

In both the experiments, the indices of the berry skin total content of polyphenols, flavonoids, anthocyanins and proanthocyanidins were analyzed, together with other parameters of the berry quality, including the skin color.

MATERIALS AND METHODS

The experiment was carried out in 2010, at a vineyard located in the Apulia region to the North West of the province of Bari (Corato, Lat. N. 41° 8'; Long E 16° 24'; 13 km from the sea; 230 m above sea level). The environment has semi-arid conditions, with warm summer (maximum average temperature 31.6 °C in July) and low annual rainfall (550 mm).

The vineyard was set up in 2002 with *Vitis berlandieri* x *Vitis rupestris* 1103 P, spaced at 2.60 x 2.60 m, grafted with the *V. vinifera* seedless tablegrape cultivars Black Emerald,

Autumn Royal, Crimson Seedless, and with the seeded cultivar Black Pearl. The soil has silty-sandy texture, strong presence of gravel, slightly alkaline reaction (pH 7.5), organic matter (1.45 %) and macronutrient content suitable for viticulture.

The vines were trained to overhead tendone system; according to the cultivar characteristics (Ramming and Taraillo, 1998; Ramming, 1999; Dokoozlian *et al.*, 2000), Black Emerald, Autumn Royal and Black Pearl were pruned to four 10-bud canes, while Crimson Seedless was pruned to six 15-bud canes. Viticultural practices commonly used for tablegrape cultivars in that growing area were applied, except for physical or chemical treatment aiming at increasing berry size. The seasonal irrigation volume ranged from 1600-1800 m³ ha⁻¹ for early-ripening varieties to 2500-3000 m³ ha⁻¹ for late varieties. In particular, Autumn Royal and Crimson Seedless received 2000 and 2500 m³ ha⁻¹, respectively. Two separate blocks of these two cultivars were deficit-irrigated by lowering at 50% the amount of water supplied at each irrigation.

Grapes were harvested when they reached 18 °Brix total soluble solid concentration. Per each cultivar, 5 replicates of 10 clusters were weighted. From each replicate, 100 berries were randomly sampled. In the laboratory, they were weighted and a sub-sample of 50 berries was used to measure the following parameters: berry length and width, by means of an electronic calliper (length/width ratio was also calculated, as an indicator of berry shape), chromatic coordinates in the CIE L*a*b* space, by means of a portable colorimeter (CR 400 Minolta Co, Osaka, J). Thirty berries of each sub-sample were manually crushed. The juice was centrifuged and used to assess: total soluble solid concentration (TSS), by means of a digital refractometer (Digital wine VM-7 Atago Co. LTD, J), pH by means of a pH-meter (Titrex Universal Potentiometric Titrator, Steroglass S.r.l., San Martino in Campo (PG), I), and titratable acidity (TA, as tartaric acid) by neutralizing acids with NaOH 0.1 N. TSS/TA ratio was also calculated. The other 20 berries of the sub-sample were used to assess, after skin peeling, the indices of skin phenol content for total polyphenol, flavonoids, anthocyanins and proanthocyanidins; these indices were spectrophotometrically determined according to the method described by Di Stefano and Cravero (1991).

The remaining berries of each cultivar, after washing, were used by 12 researcher and students of the laboratory team to annotate their visual color and their taste.

On grapes produced by deficit-irrigated Autumn Royal and Crimson Seedless, the same measurements of cluster and berry weight, berry length and width, berry juice composition and berry phenol content were performed.

All data from carpological measurements, skin color measurements and chemical analyses were statistically processed by means of ANOVA and Duncan test.

RESULTS AND DISCUSSION

Under the experimental conditions, the grapes reached 18 °Brix SST concentration as follows: end of July for cv Black Emerald (07/28), before mid September for Autumn Royal (09/12), late September for Crimson Seedless (09/22) and Black Pearl (09/30). Carpological traits of the four varieties respected their own standards, according to previous studies conducted in the same growing area (Colapietra *et al.*, 1995; Tarricone and de Palma, 2003; de Palma *et al.*, 2005) and in other countries (Ramming, 1999; Dokoozlian *et al.*, 2000; El-Baz *et al.*, 2002). In particular, Black Emerald showed a small berry and a medium sized bunch, Crimson Seedless had a medium size of both bunch and berry, Autumn Royal had large size of both bunch and berry, that was close to that showed by Black Pearl (Tab. 1).

When grape juice attained 18 °Brix, Black Emerald and Autumn Royal showed low titratable acidity (3.7-4.3 g L⁻¹), while Crimson seedless maintained a medium acid level (6.5

g L⁻¹); the TSS/TA ratio was 43 and 48 for the first two cultivars and 28 for the third one (Tab. 2). Nevertheless, the grape taste was described as sweet-acidulous for either Black Emerald or Crimson Seedless, and as sweet-sapid for Autumn Royal. Black Pearl showed titratable acidity as high as 8 g L⁻¹, thus its TSS/TA ratio was 23.

The berry skin color, objectively individuated by the chromatic coordinates (Tab. 3), was visually described as black-dull violet in Black Emerald and Black Pearl, dark blue-violet in Autumn Royal, and purple-violet in Crimson Seedless. As it was expected, the first two cultivars showed very close values of their chromatic coordinates.

It is known that berry skin color has a non-linear correlation with the anthocyanin content (Peppi *et al.*, 2007). However in the present study, after analyzing the berry skin phenol content (Tab. 4), the two cultivars with darker skin color, that are, Black Emerald and Black Pearl, resulted in a higher amount of total anthocyanins per kilogram of grape, that is, 1300 and 1050 mg kg⁻¹ respectively, while the cultivar with red skin color, that is, Crimson Seedless, performed at the opposite (140 mg kg⁻¹), and the cultivar with dark-blue skin color, that is, Autumn Royal, performed intermediately (408 mg kg⁻¹). Nevertheless, this pattern of difference was not limited to total anthocyanins, but concerned also total polyphenols, that ranged from 610 mg kg⁻¹ in Crimson Seedless to 2150 mg kg⁻¹ in Black Pearl (+252%), and total flavonoids, that showed the largest range of difference, ranging from 615 mg kg⁻¹ in Crimson Seedless to 2500 mg kg⁻¹ in Black Emerald (+307%). As for total proanthocyanidins, they varied from 1100 mg kg⁻¹ in Crimson Seedless to 2450 mg kg⁻¹ in Black Pearl (+123%); Black Emerald and Autumn Royal showed a not different amount.

Generally speaking, except for Crimson Seedless, the amount of berry skin phenols of these tablegrape grapevine varieties was quite comparable with that of many winegrape varieties. If we consider a red wine having 2000 mg L⁻¹ total polyphenol content, one glass of 150 ml contains about 300 mg of polyphenols. It is well-known that a very high correlation exists between total polyphenol content and antioxidant activity in grapevine products (Burin *et al.*, 2010). From our data, to attain the same polyphenol supply provided by one red wine glass, it is sufficient to eat about 175 g of Black Emerald fresh grape, or 270 g of Autumn Royal, or 140 g of Black Pearl. On the other hand, with Crimson Seedless 450 g of fresh grape are required.

As concern the effects of the irrigation water supply on Autumn Royal and Crimson Seedless grapes, it was evident that the 50% lower irrigation impacted the berry growth of both cultivars reducing berry weight by -23% and -33%, respectively, and the bunch weight by -33% and -39% respectively (Tab. 1).

As expected, these grapes had a precocious maturation: the reaching of 18 °Brix was advanced by one week in Autumn Royal (08/05) and by two weeks in Crimson Seedless (09/07). This kind of response was likely due to juice concentration; Crimson Seedless seemed the most sensitive to this effect (Tab. 2). As for titratable acidity, under our experimental conditions it was increased by deficit-irrigation in Autumn Royal (+35%), but was decreased in Crimson Seedless (-23%), hence this treatment modify the TSS/TA ratio in opposite way.

Analyzing the berry skin phenol accumulation as related to the vine irrigation level (Tab. 4), grape of Crimson Seedless from deficit-irrigated vines showed a moderate rise in polyphenols (+15%) and proanthocyanidins (+19%) and a moderate lowering in flavonoids (-16%) and anthocyanins (-7%); however, all these differences were not statistically significant. Moreover, we verified that phenol content per single fresh berry did not increase in this cultivar. On the contrary, Autumn Royal deficit-irrigation induced a great and significant increase of total berry skin phenols, that is, +50% polyphenols, +86% flavonoids, +111% anthocyanins and

+53% proanthocyanidins per kilogram of fresh grape. We calculated that, as a consequence, 190 g of Autumn Royal fresh grape produced by low-irrigated vines had about the same polyphenol content of one red wine glass. It is known that, according to the dietary guideline, a healthy nutrition requires to consume 5 “servings” per day of fruits and vegetables different in color and that, as for fruits, one serving corresponds to 150 g (Fondazione Veronesi, 2012). The concept of a proper “serving” is necessary to avoid other nutritional problems such, for example, as the intake of sugar in excess from fruits.

CONCLUSIONS

Among the black/red seedless tablegrape varieties analyzed in this study, Black Emerald reached the highest berry skin phenol content followed by Autumn Royal and then by Crimson Seedless. All the analyzed phenol classes showed significant differences among cultivars, pointing out the genotype effect.

The amount of berry skin phenols found in the seeded cv Black Pearl was closer to that of Black Emerald than that of Autumn Royal. It seemed that, under our trial conditions, grapes with darker berry skin showed a tendency to accumulate a higher amount of skin phenols, differently from what found in some other studies. It may be supposed that our finding is related to the quite large dissimilarity of berry skin color among the tested cultivars. Since the above mentioned tendency was not limited to anthocyanins (notoriously responsible for skin color), but concerned also other classes of phenols, such as polyphenols and flavonoids, berry skin color could be considered as an indicator of the genotype suitability to accumulate these types of phenol compounds; however, a wider range of red/blue/black fresh grapes should be analyzed to confirm this behavior.

The effect exerted by a low vine irrigation regime on Autumn Royal and Crimson Seedless grapes was not univocal, hence, a genotype-irrigation interaction was pointed out. Grapes produced by deficit-irrigated Autumn Royal vines showed a moderate decrease in berry weight but a high increase in phenol amount per kilogram of fresh grape, while Crimson Seedless, in this trial, lost 1/3 of its berry weight but did not show a relevant difference in berry skin phenol content related to the lower irrigation. With Autumn Royal, and other tablegrape cultivars having a same type of response, the choice of a low watering regime may be useful to improve the grape healthy properties.

Under the experimental conditions of this study, Black Emerald, Black Pearl and deficit-irrigated Autumn Royal gave grapes that, when harvested at 18 °Brix total soluble solid concentration, were able to provide, per single serving, a total polyphenol amount comparable with that of a polyphenol-rich glass of red wine.

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Table 1 – Carpological traits of four tablegrapes.

CULTIVAR	PARAMETER														
	Bunch weight			Berry weight			Berry length			Berry width			Berry length/width ratio		
	I	DI	sig. ⁽¹⁾	I	DI	sig. ⁽¹⁾	I	DI	sig. ⁽¹⁾	I	DI	sig. ⁽¹⁾	I	DI	sig. ⁽¹⁾
Black Emerald	445 d	-	-	3.5 c	-	-	17.9 c	-	-	16.5 c	-	-	1.1 b	-	-
Autumn Royal	900 a	600	*	8.2 a	6.3	*	27.0 c	24.8	*	21.7 a	19.0	*	1.3 a	1.3	n.s.
Crimson Seedless	660 c	400	*	4.5 b	3.0	*	22.2 b	14.9	*	17.6 b	11.7	*	1.3 a	1.3	n.s.
Black Pearl	800 b	-	-	8.4 a	-	-	23.5 b	-	-	18.1 b	-	-	1.3 a	-	-
sig. ⁽²⁾	-	*	-	-	*	-	-	*	-	-	*	-	-	n.s.	-

I = normal irrigation; DI = deficit-irrigation.

Within the I column, values followed by different letters are significantly different at $P \leq 0.05$ (Duncan test).

⁽¹⁾ Within line: * = significant difference at $P \leq 0.05$, n.s. = not significant difference.

⁽²⁾ Within column: * = significant difference at $P \leq 0.05$, n.s. = not significant difference.

Table 2 - Berry juice composition of four tablegrapes.

CULTIVAR	PARAMETER											
	TSS (°Brix)			TA (g L ⁻¹)			pH			TSS/TA		
	I	DI	sig. ⁽¹⁾	I	DI	sig. ⁽¹⁾	I	DI	sig. ⁽¹⁾	I	DI	sig. ⁽¹⁾
Black Emerald	18.3 a	-	-	4.3 c	-	-	4.0 a	-	-	43.1 a	-	-
Autumn Royal	18.0 a	18.1	n.s.	3.7 d	5.0	*	3.8 b	3.2	*	48.5 a	39.6	*
Crimson Seedless	18.2 a	18.3	n.s.	6.5 b	5.0	*	3.4 c	3.5	n.s.	27.8 b	36.4	*
Black Pearl	18.1 a	-	-	8.0 a	-	-	3.4 c	-	-	22.5 b	-	-
sig. ⁽¹⁾	-	n.s.	-	-	n.s.	-	-	*	-	-	n.s.	-

I = normal irrigation; DI = deficit-irrigation.

Within the I column, values followed by different letters are significantly different at $P \leq 0.05$ (Duncan test).

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⁽²⁾ Within column: * = significant difference at $P \leq 0.05$, n.s. = not significant difference.

Table 3 – Berry skin chromatic coordinates of four tablegrapes.

PARAMETER	CULTIVAR			
	Black Emerald	Autumn Royal	Crimson Seedless	Black Pearl
L*	30.4 a	25.2 b	35.8 c	29.1 a
a*	0.7 c	1.18 a	6.4 b	0.5 c
b*	-0.2 b	-1.4 d	4.2 a	-0.5 c

Within line, values followed by different letters are significantly different at $P \leq 0.05$ (Duncan test).

Table 4 – Indices of total content of the main phenol compounds of the berry skin of four tablegrapes.

CULTIVAR	Total phenol compounds (mg kg ⁻¹ fresh grape)											
	Polyphenols			Flavonoids			Anthocyanins			Proanthocyanins		
	I	DI	sig. ⁽¹⁾	I	DI	sig. ⁽¹⁾	I	DI	sig. ⁽¹⁾	I	DI	sig. ⁽¹⁾
Black Emerald	1650 b	-	-	2500 a	-	-	1300 a	-	-	1880 b	-	-
Autumn Royal	1130 c	1700	*	1380 b	2560	*	408 c	860	*	1700 b	2600	*
Crimson Seedless	610 d	700	n.s.	615 c	518	n.s.	140 d	130	n.s.	900 c	1070	n.s.
Black Pearl	2150 a	-	-	2240 a	-	-	1055 b	-	-	2450 a	-	-
sig. ⁽²⁾	-	*	-	*	*	-	-	*	-	-	*	-

I = normal irrigation; DI = deficit-irrigation.

Polyphenols and flavonoids are expressed as (+)-catechin; anthocyanins are expressed as malvidin mono-glucoside, proanthocyanidins are expressed as cyanidin chloride.

Within the I column, values followed by different letters are significantly different at $P \leq 0.05$ (Duncan test).

⁽¹⁾Within line: * = significant difference at $P \leq 0.05$, n.s. = not significant difference.

⁽²⁾Within column: * = significant difference at $P \leq 0.05$, n.s. = not significant difference.