The effect of agronomic practices and terroir on the phenolic complex of autochthonous grape variety 'Kefesiya'

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Abstract. This study aimed to assess the cropping capacity, quality indicators, and phenolic compounds in various tissues of the 'Kefesiya' grape variety, influenced by cultivation terroir and agricultural practices. Results indicate that bush training methods, trunk height, and green operations significantly affect cropping capacity, quality, and phenolic content. A multi-armed fan training on a medium trunk in the Sudak terroir yielded 1.8 times higher cropping capacity compared to a one-sided Guyot on a high trunk in Alminskiy. Combining topping with defoliation increased sugar content by 15% and reduced titratable acids by 24% in Sudak, while in Alminskiy, sugar content rose by 5% and acids decreased by 9%. However, this combination also led to a decrease in phenolic compounds across both terroirs. Topping and defoliation positively correlated with phenolic compounds, especially catechins. The study highlights that grape quality and phenolic accumulation are influenced not only by soil and climate but also by specific agricultural practices tailored to crown management.

1 Introduction

Phenolic compounds play an important role in the quality of grapes and wine. Antioxidant activity of grapes is positively correlated with phenolic composition, consisting of secondary metabolites synthesized in response to biotic or abiotic stress factors in plants [1]. Their biosynthesis and accumulation in grape berries during the process of ripening is influenced by many factors, mainly by the variety, climatic and edaphic conditions of the area [2].

Experimental data obtained show that the technological stock of phenolic compounds depends on grape variety, the place of its growth, as well as favorable weather conditions during the period of ripening. The larger content of phenolic compounds was found in grapes with the place of growth having the greatest number of active temperatures [3-4].

The scientists confirm the above information and note that biosynthesis of phenolic compounds is also influenced by trellis system and rootstock variety [5]. Using of different agrotechnical practices, such as the intensity of pruning, different load of bushes with shoots as a result of different crown densities, influences plant growth and cropping capacity, pH

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values and the total amount of soluble solids during harvesting [6]. For example, defoliation of leaves impacts the color and quality of grapes: in the period between July 15 and September 1, it allows to achieve good coloring of berries [7]. A decrease in the leaf surface per bunch below a critical level delays berry growth. It is also accompanied by a decrease in the total amount of sugars and berry acidity. With a decrease in leaf surface by half in 30 days after flowering, a significant decrease in the weight of berries and an increase in berry acidity are observed. Moreover, negative effect is greater when removing upper leaves than the lower ones [8].

The experts of Roskachestvo acknowledge an increase in the proportion of grape varieties of foreign origin, mostly demanded by customers, while the trend for autochthonous varieties is also consistent, even though it is not fully implemented due to a shortage of seedlings of rare varieties.

Grape variety 'Kefesiya' is one of the leading autochthonous varieties of Crimea, interest in which is rapidly growing due to its oenological potential when cultivated in arid conditions.

Polyphenol composition of 'Kefesiya' grape variety was studied under the conditions of Ampelographic Collection of the FSBSI Institute Magarach of the RAS. It showed high values of D-catechin, non-flavonoid polyphenols of grapes - hydroxycinnamic acids, compared to other studied grape varieties [9].

For the applied viticulture, especially when placing autochthonous grape varieties in other cultivation zones, distinguished from the traditional regions of Crimean viticulture with different soil and climatic conditions, it is necessary to correctly select the elements of varietal agricultural technology for scientifically based cultivation.

Thus, the research on the practices of agricultural cultivation technology that influence productivity, quality and polyphenol composition of grapes of autochthonous variety 'Kefesiya' in various cultivation conditions is relevant.

2 Materials and Methods

2.1 Subject of research

Quality indicators of grapes, including the composition of phenolic complex of autochthonous variety 'Kefesiya' at the time of grape harvesting, depending on agrotechnical practices in various viticulture and winemaking regions during 2022-2023 are the subject of research. The plots were irrigated. The system of growth training used was a vertical trellis.

Experimental studies were carried out in two viticulture and winemaking regions of Crimea: Mountain-Valley Coastal region[1] (Sudak terroir, branch of FSUE PJSC Massandra - Morskoe), and Crimean West-Coastal Piedmont region[2] (Alminskiy terroir, Invest Plus LLC). Climatic conditions of Sudak terroir are mildly hot with dry summers and soft winters, characterized by major fluctuations in average monthly temperatures from -21.7 ºC to 24.2 ºC. The sum of active temperatures ranges from 3400 to 4200 ºC and even more. The average annual precipitation is 296 mm, mostly in summer (shower rains) and in winter. In the coastal part of the region, located at an altitude of up to 200 m above sea level, climatic conditions for growing grapes are close to optimal. Basic type of soils is a brown earth. The soils are medium rubble - low stony with dissemination of heavy rubble - low stony types 10-30%.

Climate of the Alminskiy terroir is mildly warm, soft, with thaw periods and great drops in temperature, arid. The average annual air temperature is 10-12 ºC. The average monthly air temperature of the warmest month July, is 21-23 ºC, in the coldest month February, it is from -1.2 to 2.5 ºC. Early autumn frosts are registered in October, late spring frosts - in April. The sum of active air temperatures is 3300-3500 ºC. The average annual precipitation varies between 450-480 mm, in drought years – it is 200 mm or even less. The climate is favorable for open-earth grape cultivation. Soil cover of the vineyards is represented by piedmont calcareous chernozems of medium layer thickness. Chernozems are characterized by a high content of carbonates. According to the soil profile, their percentage ranges from 5% to 25%.

2.2 Methods of experimental study performance

The studies were conducted according to the methodology reflected in the article [10]. More specifically, the crown volume of test bushes was determined by measuring the parameters of crown habitus in its three-dimensional space (length, width and height). Then all the parameters were multiplied together to obtain the crown volume [11]. The yield was recorded by direct counting and weighing of bunches of each test bush with subsequent calculation of cropping capacity per 1 ha of the vineyard. To determine yield quality, mass concentration of sugars, titratable acids, and pH using pH-meter HI08 (HANNA Industries, Germany) were defined [12].

Identification and quantitation of phenolic compounds was carried out using high-performance liquid chromatography - mass spectrometry (HPLC) on a LC20 Prominence chromatograph (Shimadzu, Japan) with a diode matrix detector of ultraviolet and visible light range. Detection was carried out at wavelengths of 280 and 320 nm. Samples were prepared for analysis by the method of extracting homogenized tissues with methanol in a mass ratio of 1:4. A Nucleosil C18 AB (Macherey-Nagel, Germany) column 250 x 2 mm, filled with a reverse-phase sorbent, grain size of 5 μm and a porosity of 100 δA was used for the analysis. Sample volume 2 μl, detection at 280 nm, 310 nm, 330 nm, 525 nm with a scanning frequency of 3 Hz. Elution was carried out in a gradient mode of increasing the proportion of solution B (a mixture of AcCN:MeOH:$HClO_4$ in the ratio 40:40:20, pH 2.5) mixed with solution A (aqueous solution of $HClO_4$, pH 1.8) for 70 minutes. Recognition was carried out based on spectral characteristics and void time in accordance with standard samples [13].

Groups of substances identified in the extract are anthocyanins (delphinidin-3-O-glycoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside, malvidin-3-O-glucoside, delphinidin -3-O-acetylglucoside, cyanidin-3-O-acetylglucoside, petunidin-3-O-acetylglucoside, peonidin-3-O-acetylglucoside, malvidin-3-O-acetylglucoside, petunidin-3-O-coumaroylglucoside, malvidin-3-O-coumaroylglucoside), low molecular weight catechins [(+)-catechins and (-)-epicatechins], dimeric procyanidins (B1, B2, B3, B4), phenolic acids (gallic, caftaric), the sum of polyphenols.

Table 1. Experiment scheme. Management of yield, quality, physicochemical indicators of 'Kefesiya' grapes

Viticulture and winemaking terroir (location)	Planting scheme, m x m	Bush training	Bush load		Length of pruning fruit canes, eyes	Element of varietal agricultural practice – green operations
			eyes	shoots		
Sudak (Morskoye village)	3 x 2 (2)	multi-armed fan on a medium trunk	15.0	12	8	topping of shoots + defoliation of leaves in grape bunches area (topping + defoliation)
						without topping (Control)
Alminskiy (Peschanoye village)	2.5 x 1.25	one-sided Guyot on a high trunk	10.0	9	8	topping of shoots + defoliation of leaves in grape bunches area (topping + defoliation Control)
						without topping

The experimental data was processed by generally accepted methods of mathematical statistics using IBM SPSS Statistics (v 17.0) and Microsoft Excel software package. All studies were performed in triplicate. Calculation of pair correlations between indicators was carried out at a significant level of 0.05. Scheme of the experiment is presented in Table 1.

3 Results and discussion

Selection of technological practices in the vineyard directly influences the formation of productivity indicators, growth vigor, organoleptic characteristics of grapes and their quality as a raw material. It is established that autochthonous grape variety ‘Kefesiya’ (Table 2), when cultivated in the Sudak terroir using multi-armed fan on a medium trunk, topping of shoots and defoliation of leaves in grape bunches area, gives a decrease in cropping capacity by 24.8% compared to the variant without shoot topping. At the same time, cropping capacity of grapes in the Sudak terroir, regardless agrotechnological practices used for growth amount, is higher than in the Alminskiy terroir (one-sided Guyot on a high trunk training) by 26.3% (without topping), and 32.9% (with topping of shoots + defoliation of leaves in in grape bunches area (C). In the Alminskiy terroir, the values of cropping capacity, despite using agrotechnological practices, did not have a significant difference.

Table 2. Experiment scheme. Management of yield, quality, physicochemical indicators of ‘Kefesiya’ grapes

Variant of experiment		**Crown volume, m^3**	**Cropping capacity, t/ha**	**Mass concentration**		**pH**	**Harvesting date**
Terroir (location)	**Element of green operations**			**sugars, g/100 cm^3**	**titratable acids, g/dm^3**		
Sudak (Morskoye village)	topping + defoliation	2.8	7.6	21.0	3.5	3.57	Dec. 3 IX
	without topping (C)	4.0	10.1	17.9	4.6	3.65	Dec. 3 IX
Alminskiy (Peschanoye village)	topping + defoliation (C)	1.9	5.1	21.6	5.4	3.60	Dec. 3 IX
	without topping	3.3	5.6	20.5	4.9	3.63	Dec. 3 IX

In the Sudak terroir, a decrease in the crown volume as a result of using topping and defoliation of leaves in grape bunches area was leading to an increase in the mass concentration of sugars by 14.8%, a decrease in the mass concentration of titratable acids by 23.9%, pH values were not significant in the experiment variants. In the Alminskiy terroir, an increase in the mass concentration of sugars by 5.4%, a decrease in the mass concentration of titratable acids by 9.3% were registered, pH values were not significant in the experiment variants. A property of low-acidity of ‘Kefesiya’ variety is controlled by the terroir of its cultivation when using shoot topping and defoliation of leaves in grape bunches area, as proven by scientists who additionally used topping at different dates of grape bush development [14].

The results of correlation analysis showed strong direct correlation between cropping capacity, pH values and crown volume r=0.77 and r=0.7; strong invert correlation between mass concentration of sugars and crown volume r=0.90; weak invert correlation between mass concentration of acids and crown volume r=0.26.

During grape processing, phenolic compounds undergo a complex transformation and serve as a source of formation new compounds to further determine the quality of wine (color, acerbity, bitterness, aroma and flavor).

Groups of phenolic compounds in grapes were studied, as well as their total composition depending on the terroir and elements of varietal agricultural technology (Table 3).

The highest values of phenolic profile of 'Kefesiya' variety are typical for grapes cultivated in the Alminskiy terroir, in both variants with or without topping + defoliation of leaves in grape bunches area, and regardless the bush training. The difference amounts: in the Alminskiy terroir, in the group of anthocyanins (28.6%), catechins (29.9%), procyanidins (14.1%), phenolic acids (61.9%), total phenols (30.0%); in the Sudak terroir, in the group of anthocyanins (14.1%), catechins (15.4%), procyanidins (30.0%), phenolic acids (decreased by 13.3%), total phenols (17.7%).

Table 3. The content of phenolic compounds in 'Kefesiya' grapes depending on the terroir and cultivation technology

Variant of experiment	Anthocyanins, mg/l^{-1}	Procyanidins		Phenolic acids, mg/l^{-1}	Total phenols, mg/l^{-1}
		Catechins, mg/l^{-1}	B1-B4, mg/l^{-1}		
Sudak terroir (Morskoye village)					
Topping of shoots + defoliation	460.4	443.8	337.4	42.6	1284.2
Without topping (C)	536.1	524.6	462.0	37.6	1560.3
Alminskiy terroir (Peschanoye village)					
Topping of shoots + defoliation of leaves (C)	689.8	468.3	196.9	36.3	1391.3
Without topping	966.4	667.9	229.3	95.4	1959.0
Coefficient of variation (Cv)	0.33	0.19	0.39	0.53	0.19
Coefficient oscillation (VR)	0.76	0.43	0.87	1.11	0.44
r	0.78	0.93	0.06	0.73	0.95

The analysis of basic components of berry structure showed that in the group of anthocyanins, the largest amount was introduced by delphinidin-3-O-acetylglucoside and malvidin-3-O-cumaroylglucoside in grape skin, regardless the terroir of cultivation. In the group of procyanidins, epicatechin was accumulated in greater amount in grape skin (from 86.8% in the variant with topping and defoliation of leaves in the area of bunches, and up to 89.3% in the variant without topping), rather than in grape seed in the Alminskiy terroir. In the Sudak terroir, its greater amount was accumulated in grape seed rather than in grape skin (from 5.0% in the variant with topping and defoliation of leaves in the area of bunches, and up to 25.0% in the variant without topping), as well as procyanidin B3 with its largest amount in grape seeds in the Alminskiy terroir (from 61.6% in the variant without topping up to 65.2 % in the variant with topping and defoliation of leaves), and procyanidin B1 in the Sudak terroir (from 66.7% in the variant with topping and defoliation of leaves to 71.8% in the variant without topping), compared to other procyanidins. According to literature sources, the variety placed in different terroirs is capable of accumulating phenolic complex components in different ways [15]. Defoliation and shoot topping also impact the accumulation of phenolic substances [16, 17].

By means of variation analysis, different variability degree of phenolic compounds of 'Kefesiya' grape variety was identified depending on the elements of agricultural technology and cultivation terroir used, which showed that the greatest variability was typical for procyanidins B1-B4 (Cv=0.39), as well as phenolic acids (Cv=0.53).

The use of topping and defoliation of leaves in grape bunches area or only rectification or tying of shoots was positively correlated with all phenolic compounds, and to the greatest extent with catechins (r=0.93), total phenols (r=0.95), anthocyanins (r=0.78) and phenolic acids (r=0.73).

4 Conclusion

Cropping capacity and quality characteristics, including the phenolic composition of ‘Kefesiya’ grape variety, depend both on the terroir of grape cultivation and the elements of varietal agricultural technology used (bush training, tying or rectification of shoots, topping and defoliation of leaves in grape bunches area). Training the bush with a multi-armed fan on a medium trunk in the Sudak terroir provides cropping capacity 1.8 times higher than when training a one-sided Guyot on a high trunk in the Alminskiy terroir. The complex of green operations (tying, topping shoots and liberation of bunches) is aimed at formation of an optimal leaf surface and control of photosynthetic activity, providing the given enological potential and regulating the duration of grape ripening, in particular, an increase in the mass concentration of sugars by 14.8%, a decrease in the mass concentration of titratable acids by 23.9% in the Sudak terroir; an increase in the mass concentration of sugars by 5.4%, and a decrease in the mass concentration of titratable acids by 9.3% in the Alminskiy terroir.

The use of topping and defoliation of leaves in grape bunches area under the lack of soil and air humidity in 2022-2023 has led to a decrease in the phenolic complex of grapes.

Cropping capacity and quality characteristics, including the phenolic composition of ‘Kefesiya’ grape variety, depend both on the terroir of grape cultivation and the elements of varietal agricultural technology used.

It is established that autochthonous grape variety ‘Kefesiya’, cultivated in the Sudak terroir using multi-armed fan on a medium trunk bush training, and topping with defoliation of leaves in grape bunches area, shows a decrease in cropping capacity by 24.8% compared to the variant without topping.

Biological properties of ‘Kefesiya’ grape variety change when its cultivation moves from the zone close to historical origin to more western regions of Crimea. Cropping capacity in the Sudak terroir, regardless of the applied agricultural practices, is higher than in the Alminskiy terroir with training a one-sided Guyot on a high trunk, respectively, by 26.3% (without topping) and 32.9% (topping of shoots + defoliation of leaves in grape bunches area (control)).

Cropping capacity in the Alminskiy terroir, despite using of agrotechological practices, did not have a significant difference. The values of least significant difference were 0.4 kg.

A decrease in the crown volume as a result of using topping and defoliation of leaves in grape bunches area caused an increase in the mass concentration of sugars by 14.8%, a decrease in the mass concentration of titratable acids by 23.9% in the Sudak terroir; and in the Alminskiy terroir –an increase in the mass concentration of sugars by 5.4%, a decrease in the mass concentration of titratable acids by 9.3%. The values of pH were not significant in both cultivation terroirs.

A property of low-acidity of ‘Kefesiya’ grape variety is restrained by the terroir of its cultivation. Mass concentration of titratable acids is higher in the Alminskiy terroir, regardless of the applied elements of varietal agricultural technology.

The results of correlation analysis showed strong direct correlation between cropping capacity, pH values and crown volume r=0.77 and r=0.7; strong invert correlation between mass concentration of sugars and crown volume r=0.90; weak invert correlation between mass concentration of acids and crown volume r=0.26.

The highest values of phenolic profile of ‘Kefesiya’ variety were identified for grapes cultivated in the terroirs in general, and without topping and defoliation of leaves in grape bunches area: in the Alminskiy terroir, in the group of anthocyanins (28.6%), catechins (29.9%), procyanidins (14.1%), phenolic acids (61.9%), total phenols (30.0%); in the Sudak terroir, in the group of anthocyanins (14.1%), catechins (15.4%), procyanidins (30.0%), phenolic acids (on the contrary, decreased by 13.3%), total phenols (17.7%).

It was found that in the group of anthocyanins, the largest amount was introduced by delphinidin-3-O-acetylglucoside and malvidin-3-O-cumaroylglucoside in grape skin, regardless the terroir of cultivation. In the group of procyanidins, epicatechin was accumulated in greater amount in grape skin in the Alminskiy terroir. In the Sudak terroir, its greater amount was accumulated in grape seed, as well as procyanidin B3 with its largest amount in the Alminskiy terroir, and procyanidin B1 - in the Sudak terroir, compared to other procyanidins.

By means of variation analysis, the greatest variability of procyanidins B1-B4 (Cv=0.39), as well as phenolic acids (Cv=0.53) was revealed depending on the elements of varietal agricultural technology and terroir of cultivation.

The use of topping and defoliation of leaves in grape bunches area or only rectification, or tying of shoots was positively correlated with all phenolic compounds, and to the greatest extend with catechins (r=0.93), total phenols (r=0.95), anthocyanins (r=0.78) and phenolic acids (r=0.73).

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