Crimean autochthonous grape varieties as a factor of high-quality winemaking in a changing climate

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Abstract. An increase in ambient temperature affects the quality of wines, which is largely due to a change in the metabolism of phenolic substances in grapes. A possible solution to the problem is the use of autochthonous varieties adapted to unfavorable conditions. The influence of agroecological conditions of 4 vineyards on the phenolic complex of the Crimean grape variety 'Kokur Belyi' has been studied. Methods of geoinformation and mathematical modeling, HPLC were used. It is established: vineyards differ in heat resources in the row: Vilino < Privetnoe < Solnechnaya Dolina < Morskoe. An increase in the heat supply of territories caused the accumulation of phenolic acids, flavonols, (+)-D-catechine and procyanidins B4 in grape seeds and a decrease in the content of phenolic acids, (-)-epicatechine, procyanidins B3 in skin and pulp. The highest content of procyanidins in seeds and the lowest content of monomeric phenolic components in skin and pulp was determined in grapes from the village of Morskoye. Grapes from Vilino contain 3.7 times less procyanidins in the seeds, 6.5 and 3.6 times more monomeric components in the skin and pulp. Conclusion: the heat supply of vineyards contributes to both the biosynthesis of monomeric phenolic components and their polymerization during the ripening of grapes, leads to a significant differentiation of the phenolic complex of grapes 'Kokur Belyi'.

1 Introduction

The observed climate changes, such as an increase in the average ambient temperature, an increase in the shortage of fresh water, make adjustments to the metabolism of the grape, which affects the quality of berries and wine products, up to the transformation of the style of wines [1-4].

The response of the grape to the influence of climatic factors is manifested, first of all, in changes in the content of primary metabolites of the plant cell in berries – sugars, organic acids, enzymes, and among the secondary metabolites – phenolic components [5-8]. Numerous studies show a significant variation in the phenolic profile of grapes depending on natural and agrotechnical growing conditions, which is associated with the protective

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function of phenolic compounds [8-11]. A high level of plant insolation, average daily ambient temperatures, a lack of moisture in the soil lead to an increase in the concentration of flavonoids in grapes, in particular flavonols; air temperature above 30 °C inhibits the biosynthesis of anthocyanins [6, 12-15]. The variability of the component composition and biochemical properties of grapes under the influence of natural and anthropogenic factors is determined by its species and varietal affiliation [7, 10, 11, 16]. Phenolic compounds play an important role in the formation of wine quality as components involved in redox processes and in the formation of the color scheme and taste of wines, their antioxidant activity [17-19]. Flavonols, flavan-3-ols, tannins can cause coarseness, excessive tannin of wines, a shift in the color scheme towards brown shades [20-21].

The negative consequences of climate change from the standpoint of the quality of wine products make it necessary to revise the classical technologies of viticulture and winemaking: from the choice of new territories for planting vineyards and agrotechnologies, grape varieties to methods, modes and conditions of grape processing and wine production. One of the ways to solve this problem is the use of autochthonous grape varieties adapted to unfavorable soil and climatic conditions of the historical area of growth. Among them are autochthonous varieties of the Crimea, which in the process of evolution have developed the ability to grow and yield a good quality crop in a hot arid climate on poor soils with a high level of salinity and liming [22-23]. In the last decade, there has been an expansion of industrial plantings of autochthonous varieties outside the historically established ampelocenosis (the village of Solnechnaya Dolina, eastern part of the South-Coastal zone of Crimea) [24]. The successful choice of the territory for planting a vineyard requires the disclosure of the relationship between the natural conditions of grape cultivation and the quantitative, qualitative characteristics of berries and wine products. The All-Russian National Research Institute of Viticulture and Winemaking "Magarach" of the RAS conducts a systematic study of the climatic plasticity of Crimean autochthonous grape varieties, the component composition and biochemical properties of berries, the potential of varieties in winemaking in conditions of various agrocenoses and in the breeding process [23, 25-26]. This article is devoted to assessing the influence of agroecological conditions on the formation of a phenolic complex in the berries of the Crimean autochthonous variety 'Kokur Belyi'.

2 Materials and methods

2.1 Characteristics of the variety, growing conditions, methods of assessing agroecological resources

'Kokur Belyi' (Vitis vinifera L.) is the most common among white autochthonous varieties of the Crimea: the area of cultivation is 659 hectares [24]. According to morphological features and biological properties, 'Kokur Belyi' belongs to the ecological and geographical group of grape varieties of the Black Sea basin; the growing season is 160-170 days with the sum of active temperatures (above 10 °C) 3300-3400 °C; the average yield is 49 centners per hectare [23].

The research was carried out on industrial vineyards of the variety 'Kokur Belyi' located in the mountain-valley seaside region of the South-Coastal zone of the Crimean Peninsula - near the village of Morskoye (N44.8371, E34.8268), the village of Privetnoe (N44.8247, E34.7116), the village of Solnechnaya Dolina (N44.8663, E35.0414) and in western foothill- seaside region – the village of Vilino (N44.8507, E33.6440). Vineyards differ in morphometric parameters of the relief, proximity to the sea. The scheme of planting is 3 × 1.5 m; the formation of bushes is a 2-sided cordon; agrotechnologies correspond to the technological map of the region.

Agroecological resources of vineyards were evaluated according to the following parameters: the sum of temperatures above 10 °C (∑T°C10), above 20 ºC (∑T°C20) for the growing season, the Huglin index (HI), the Winkler index (WI), the average temperature for September (T°09) and the growing season (T°grow), total precipitation for the year (Pyear), the growing season (Pgrow) and September (P09), Selyaninov hydrothermal coefficient (HTC) [27]. The calculation of agroecological parameters at the location of the vineyards was carried out by the method of geoinformation modeling using data from the network of stationary weather stations of the Crimean Peninsula for 2016-2021, digital relief models SRTM-3 and ASTER GDEM, global climate model Worldclim ver. 2.0 and developed mathematical models reflecting the patterns of spatial variation of climatic indicators under the influence of orographic, hydrological and geographical parameters of the analyzed territory [28-29].

2.2 Preparation of grape samples and methods of analysis

Grape sampling was carried out in the vineyards during the industrial harvest period in an amount of at least 10 kg in accordance with [30]. For analytical studies, 1000 g of berries of each sample were randomly selected.

The analysis of the phenolic complex by HPLC was carried out separately for seeds, skins and pulp. Extraction of phenolic substances from homogenized seeds, skins, and pulp was carried out with an extractant (1 ml HCL / 100 ml CH_3OH) in a ratio of 1:3 for 3 months [31]. The separation of phenolic compounds was carried out on a Shimadzu LC 20 Prominence chromatograph with a diode-matrix detector of the ultraviolet and visible range: a Nucleosil C18 AB column (Macherey-Nagel, Germany), 250 mm long, 2 mm in diameter, with a pore size of 100 Å. Detection of procyanidins was carried out at 280 nm, flavonols (quercetins) – at 360 nm; the scanning frequency was 3 Hz. Identification was carried out by spectral characteristics and exit times in accordance with standard samples.

2.3 Statistical data processing

Experimental data were processed by methods of variance (ANOVA), discriminant, cluster and correlation analysis (Statistica 10 program). Differences in quantitative features in independent subgroups were assessed by the Mann-Whitney U-criterion, the informativeness of discriminant variables was assessed by Wilks L for the significance point $\alpha < 0.05$. The number of grape samples was 18. The analyses were carried out in 2-3–fold repetition. The tables and the text of the article show the arithmetic mean of the indicators ± standard deviation of a single result.

3 Results and discussion

Using the methods of geoinformation and mathematical modeling, the climatic parameters of the studied vineyards 'Kokur Belyi' for 2019-2021 were determined. Statistical analysis of the data presented in Table 1 revealed that the vineyards differed in heat resources, while the best way (Wilks L.= 0.00009 at $\alpha < 0.00001$) were discriminated with the combined consideration of the parameters ∑T°C10, HI, T°grow and T°09. The Vilino vineyard was characterized by the lowest heat supply, the Morskoe vineyard was the largest: according to the results of the hierarchical classification, the Euclidean distance (Ed) between them was 705 (Fig.). The heat resources of the Solnechnaya Dolina vineyard were close to those of the Morskoe vineyard: the territories were combined into one cluster with an Ed between them equal to 100. According to the parameters of heat supply, the Privetnoe vineyard occupied an intermediate position and was almost equally removed from the vineyards of Vilino (Ed=371) and Solnechnaya Dolina (Ed=396). There were no significant differences between vineyards in terms of moisture availability during the years of research.

Table 1. Climatic parameters (means±SD) of 'Kokur Belyi' vineyards located in different geographical objects

Parameters	Values			
	Vilino	Morskoe	Privetnoe	Solnechnaya Dolina
∑T°C10, °C	3825±180	4372±123	4018±123	4280±123
∑T°C20, °C	2215±261	2508±158	2348±158	2383±158
HI	2649±79	2645±133	2414±133	2683±133
WI	1734±88	2068±141	1898±141	2020±141
T°09, °C	18.1±2.0	20.3±2.2	19.9±2.2	20.0±2.2
T°grow, °C	18.4±0.2	20.0±0.5	19.7±0.5	20.7±0.5
HTC	0.66±0.12	0.71±0.37	0.81±0.37	0.75±0.37
Pyear, mm	446±30	376±92	393±96	386±95
Pgrow, mm	239±40	219±90	231±94	226±93
P09, mm	40.6±19.0	27.0±10.4	27.7±10.7	29.1±11.2

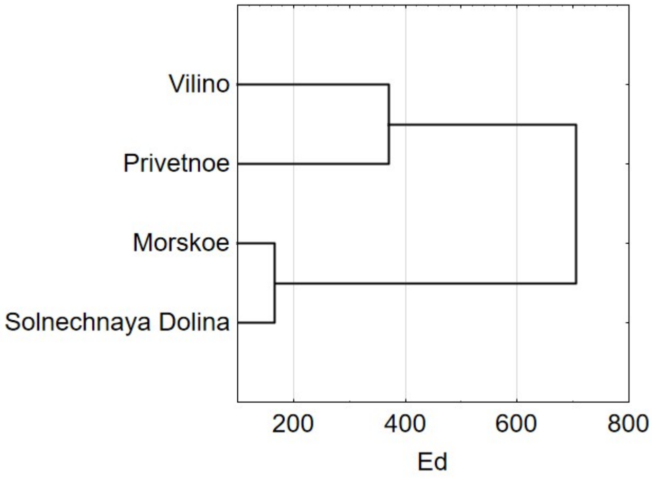

Fig. 1. The result of clustering of vineyards 'Kokur Belyi' by heat supply.

The information available in the literature [6, 12-15] indicates that the temperature conditions of grape growth affect the formation of the phenolic complex of berries. In this regard, using the HPLC method, we analyzed the content of monomeric phenolic components and procyanidins in the structural parts of the 'Kokur Belyi' berries of the 2020 – 2021 vintages obtained in the studied vineyards. Hydroxybenzoic and hydroxycinnamic acids, flavonols, flavan-3-ols and procyanidins B1-B4 were identified (Table 2).

Table 2. The content (arithmetic mean value*, mg kg^{-1}) of phenolic compounds in the skin, seeds and pulp of berries 'Kokur Belyi' from different vineyards

Phenolic compounds	Morskoe			Privetnoe			Solnechnaya Dolina			Vilino		
	skin	seed	pulp	skin	seed	pulp	skin	seed	pulp	skin	seed	pulp
Hydroxybenzoic and hydroxycinnamic acids												
Gallic acid	0.8	28.9	0.5	5.1	28.1	3.3	9.3	44.6	4.4	12.7	16.8	6.9
Caftaric acid	16.1	9.6	8.2	18.1	8.6	7.3	11.8	9.7	4.1	22.5	4.7	14.2
Flavonols												
Quercetin	5.7	14.0	0.3	10.3	8.4	5.9	12.7	12.9	4.7	8.7	0.7	4.0
Quercetin -3-O-b-D-glycosides	0.1	13.6	0.9	3.7	11.3	4.2	28.5	12.2	4.4	23.3	1.1	2.0
Flavan-3-ols												
(+)-D-Catechine	10.2	229.8	2.5	10.3	153.1	1.1	7.5	141.2	1.4	10.9	27.8	2.0
(-)-Epicatechine	13.3	579.5	8.0	47.8	598.6	19.8	96.8	809.2	10.7	221.5	421.6	45.7
Procyanidins												
B1: epicatechine-4→8-catechine	15.5	49.5	4.8	9.3	53.0	3.5	11.2	77.2	2.2	16.6	23.9	5.1
B2: epicatechine-4→8-epicatechine	13.3	241.1	2.5	16.4	300.5	1.8	2.8	116.1	4.6	8.9	28.2	4.9
B3: catechine -4→8-catechine	13.0	220.0	2.9	17.0	217.8	2.0	8.6	251.1	1.1	25.3	94.2	1.8
B4: catechine -4→8-epicatechine	7.3	362.2	2.9	11.4	224.3	4.3	4.7	224.9	4.5	8.0	92.0	2.2

*SD-values were lower than 9 % for all vineyards in each year of the studies. When taking into account 2-year data, SD-values were lower than 17%

Data from Table. 2 showed that the seeds are the structural element of the berry most enriched with monomeric and dimeric phenolic compounds: the content of the components ranged from 711.0± 113.8 mg kg^{-1} in grapes from Vilino to 1748.2±201.4 mg kg^{-1} in grapes from Morskoe. The identified phenolic complex of seeds was dominated by flavan-3-ols and procyanidins B1-B4: in grapes from the Privetnoe and Morskoe components were represented in parity proportions - 46-47 % and 50 %, respectively; in grapes from Solnechnaya Dolina, the proportion of flavan-3-ols exceeded the proportion of procyanidins B1-B4 by 1.4 times, from Vilino – by 1.9 times. The content of flavan-3-ols in the seeds of grapes from Solnechnaya Dolina was 933.7-967.1 mg kg^{-1} and exceeded (α=0.0002) that in grapes from Privetnoe and Morskoe by 1.2 times, in grapes from Vilino – by 2.1 times. The content of procyanidins B1-B4 in grape seeds in the territories of its growth increased in a row: Vilino< Solnechnaya Dolina< Privetnoe< Morskoe. Grape seeds from Solnechnaya Dolina contained 1.7 times more (α=0.000014) phenolic acids – 52.7-55.4 mg kg^{-1}. Grapes from Vilino differed by an order of magnitude in the content of flavonols in seeds compared to grapes from other territories.

The total content of monomeric phenolic components in the pulp (74.8±8.7 mg kg^{-1}) and the skin (299.6±17.7 mg kg^{-1}) of grapes from Vilino was the highest and exceeded the values of the indicator in grapes from the vineyards of the mountain-valley seaside region by 1.9-3.8 and 1.8-6.5 times, respectively. The content of monomeric phenolic components in the skin of grapes in the territories of its growth decreased in a row: Vilino>Solnechnaya Dolina> Privetnoe>Morskoe, in the pulp – Vilino>Privetnoe>Solnechnaya Dolina>Morskoe. The phenolic complex of grapes from Vilino was distinguished by the highest proportion of flavan-3-ols: in the pulp – 54 % (in other samples – 29-39 %), in the skin – 65 % (in others – 25-54 %). The content of flavan-3-ols, with a significant predominance of (-)-epicatechin, in berries from Vilino exceeded ($\alpha<0.05$) that in grapes from other vineyards: in pulp – by 2.3-4.5 times, in the skin – by 2.2-9.8 times. The lowest content of flavan-3-ols in the pulp (10.5±1.6 mg kg^{-1}) and the skin (23.6±1.9 mg kg^{-1}) was characterized by grapes from Morskoe. The content of flavonols in the skin of grapes from Vilino and Solnechnaya Dolina (32.0-41.2 mg kg^{-1}) was on average 3.7 times higher than the value of the indicator in berries from other territories. The lowest content of flavonols in the pulp was characterized by grapes from Morskoe (1.2 ± 0.3 mg kg^{-1}), in other cases, the values of the indicator were 6.0-10.1 mg kg^{-1} and did not differ by vineyards. At the same time, grapes from Solnechnaya Dolina differed in the highest proportion of flavonols in the skin – 21 % (in others – 6-9 %), and grapes from Morskoe and Vilino – the lowest proportion of components in the pulp – 3-7 % (in others – 19-22 %). The proportion of phenolic acids in the phenolic complex of grape pulp was 20-26 %, in the skin – 10-18 % with an average content in the yield from each vineyard of 8.8-21.1 mg kg^{-1} and 17.0-35.2 mg kg^{-1}, respectively. The highest content of phenolic acids in the pulp and skin was recorded in grapes from Vilino. The content of dimeric flavonols – procyanidins B1-B4 – in grape pulp was 11.6-14.1 mg kg^{-1}: there was no significant difference in the values of the indicator for the territories of grape growth. Grapes from Solnechnaya Dolina were characterized by the lowest content of procyanidins B1-B4 in the skin, which was 27.3 mg kg^{-1} and was less than the values of the indicator in grapes from other vineyards by an average of 2 times. Grapes from Morskoe were distinguished by the highest proportion of procyanidins in the phenolic complex of pulp and skin – 39 % and 52 %, respectively.

Statistical analysis of the research results revealed a number of correlations ($\alpha<0.05$) between the content of monomeric and dimeric phenolic components in grapes and the parameters of vineyard heat supply: the correlation coefficients significant at $\alpha<0.05$ were /0.83-0.98/. The amount of phenolic acids in grape seeds directly correlates with ∑T°C10 and T°grow. The content of components in the skin and pulp is negatively correlated with the parameters of the heat supply of vineyards (∑T°C20, WI and T°09). At the same time, the quantitative content of gallic acid in the berry elements was more dependent on the agro-climatic resources of vineyards than the content of caftaric acid. The presence of a positive relationship between most (excluding HI) parameters of the heat supply of vineyards and the content of flavonols in seeds (the closest – with quercetin) was revealed; on the contrary, there is no relationship in the case of skin and pulp. The available data demonstrate a direct correlation between the amount of (+)-D-catechine and procyanidins B4 (catechine -4→8-epicatechine) in grape seeds and ∑T°C20, ∑T°C10, WI, T°09, the reverse – between the content of (-)-epicatechine in the skin and pulp, procyanidins B3 (catechine -4→8-catechine) in the skin and the same parameters of heat supply of territories, as well as T°grow.

Generalization of the presented relationships between the content of monomeric and dimeric phenolic compounds in the structural elements of grape berries and agro-climatic resources of vineyards allows us to comment on them as follows. The high level of heat supply of vineyards according to the parameters ∑T°C10, ∑T°C20, WI, T°grow and T°09 caused the accumulation of monomeric and dimeric phenolic components in the berries of the grape 'Kokur Belyi'. In the presented data, this is evidenced by the content of phenolic acids, flavonols, (+)-D-catechine and procyanidins B4 in grape seeds. On the other hand, according to modern concepts, the biosynthesis of flavan-3-ols, phenolic acids and procyanidins in the berry is mostly completed by the beginning of its maturation and subsequently their oxidative polymerization occurs [6, 32]. Our previous studies [25] have shown that Crimean autochthonous varieties, compared with classical varieties, are characterized by more intensive dynamics of flavan-3-ols, phenolic acids and procyanidins during the ripening of grapes. The results of these studies demonstrate the influence of thermal resources of the grape growing area on the intensity of the process of transformation of phenolic components in berries during ripening. The greater the heat supply of vineyards, the more active is the enzymatic transformation of monomeric and dimeric forms of components, leading to their polymerization, and the lower is the quantitative content of phenolic acids, quercetin, (-)-epicatechine, procyanidins B3 in the skin and pulp of grapes 'Kokur Belyi' by the time of technical maturity of the crop. The multidirectional nature of the thermal effect on the formation of the phenolic complex of berries led to the fact that in the skin and pulp of grapes from Morskoe, the most heat–supplied in the years of observation, the content of monomeric phenolic components was the lowest, and the content of procyanidins in seeds was the highest compared to grapes from other territories. On the contrary, grapes from Vilino – the least heat–supplied territory – were characterized by the lowest content of procyanidins in seeds and the highest content of monomeric components in the skin and pulp.

In the process of winemaking – during the processing of grapes, the infusion of pulp (a technique used in the production of white wines to enrich them with aromatic components of grapes), the clarification of must – monomeric fractions of phenolic compounds easily pass from the solid elements of the berry into the must and wine. Derivatives of oxycoric acids, flavan-3-ols are initiators and agents of redox processes occurring at the first stages of wine preparation by the enzymatic pathway, and then by the chain free radical mechanism, involving almost all components of must and wine in the process of conjugated oxidation [17, 33]. The high content of phenolic acids and flavan-3-ols in the skin can lead to a loss of the quality of the finished wine, especially in the case of white wines, manifested in the loss of varietal aroma and taste harmony, the appearance of oxidation tones. The results of the presented work show the need for a differentiated approach to the technology of wines from the grape 'Kokur Belyi', depending on the heat supply of the territories of its growth and the desired style of wine.

4 Conclusion

One of the solutions to the problems of winemaking caused by global climate change may be the use of autochthonous grape varieties adapted to the unfavorable climatic conditions of the historical growing area.

From these positions, we assessed the influence of agroecological growing conditions on the formation of a phenolic complex in the berries of the Crimean autochthonous variety 'Kokur Belyi' (Vitis vinifera L.). The basis for research (2019-2021) were four industrial vineyards located in two natural areas of the Crimean peninsula. Using the methods of geoinformation and mathematical modeling, HPLC, statistical analysis, the following conclusions has been established.

Vineyards in the years of research (2019 – 2021) differed (Wilks L.= 0.00009 at $\alpha < 0.00001$) in terms of heat resources in the row: Vilino<Privetnoe<Solnechnaya Dolina<Morskoe.

Increased heat supply of territories, including by WI, ∑T°C10, ∑T°C20, T°grow and T°09, caused (r=/0.83-0.98/; $\alpha < 0.05$), on the one hand, the accumulation of phenolic acids, flavonols, (+)-D-catechine and procyanidins B4 in grape seeds; on the other hand, the intensity of oxidative polymerization of monomeric phenolic components during grape ripening, leading to a decrease in the content of phenolic acids, (–)-epicatechine, procyanidins B3 in the skin and pulp.

The highest content of procyanidins (872.8±114.7 mg kg-1) in seeds and the lowest – phenolic acids, flavonols, flavan-3-ols in the skin (46.4±3.9 mg kg-1) and pulp (20.5±2.8 mg kg-1) was determined in grapes from Morskoe. Grape seeds from Vilino contained 3.7 times less procyanidins, and monomeric components in the skin and pulp – 6.5 times and

3.6 times more. Grapes from Solnechnaya Dolina differed from grapes from other territories with a higher content of flavan-3-ols (1.4 times) and phenolic acids (1.7 times).

Thus, the heat supply of the growing area, contributing both to the biosynthesis of monomeric phenolic components in berries and their polymerization during ripening, leads to a significant differentiation of the phenolic complex of the grape ‘Kokur Belyi’. This should be taken into account when producing wines from the grape ‘Kokur Belyi’, which is the subject of further research.

Conflict of interest

The authors declare that there is no conflict of interest.

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