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The Stability of Grapes Varieties to Drought.

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ABSTRACT

The resistance of the grape varieties Crystal (Euro-Amur-American origin), Dostoynyy and Krasnostop AZOS (interspecific hybrids of Euro-American origin) to drought was studied. The aim of the research was to study the physiological and biochemical patterns of the resistance of grape varieties of various ecological and geographical origin to high temperatures and low moisture availability in the summer period of the south of Russia. To assess the adaptive resistance of grapes to high temperature and low moisture content in the leaves of varieties, we used the following indicators: the total water content, free and bound water content by weight method, protein content by spectral method, phenol carbonic (chlorogenic, coffee), ascorbic acids, proline, malondialdehyde by capillary electrophoresis, electrophoretic spectra of the water-soluble fraction of the protein with peroxidase activity using vertical electrophoresis, an estimate of the molecular weight of proteins x components (using taps, cations coming out of cells) by the conductometric method in a model experiment at 55 °C. In model experiments, it was established that under the action of high temperature, as well as low moisture availability, the presence of water-soluble proteins (250, 130, 100, 70, 60, 40 kDa) with peroxidase activity, as well as specific proteins associated with the origin of the variety. More than that, we determined the peroxidase activity, the content of phenol carbonic and ascorbic acids, malonic dialdehyde, the damage rate of cell membranes and the release of cations from cells characterizing the heat resistance and drought resistance Grape plants in the south of Russia.
Keywords: grapes, drought resistance, heat resistance, protein, membrane damage.

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INTRODUCTION

The issues of increasing the resistance of grape plants to abiotic and biotic environmental factors occupy one of the main places in solving the problem of designing highly productive ampelocenoses. They have acquired particular urgency in recent years due to local changes in climatic conditions [1, 2]. Hydro-thermal conditions of the south of Russia favor obtaining high yields of grapes that can withstand competition in the international market. At the same time, obtaining stable high yields of grapes is limited by the impact of such adverse environmental factors as winter frosts, especially after long warm weather, extremely high temperatures, and very low moisture availability in summer. Therefore, only varieties that combine high productivity, good quality with adaptability to the conditions of this region can be successfully cultivated here on a fairly wide scale. This necessitates the improvement of the grape assortment by growing varieties that are better adapted to the weather conditions of the growing areas [3, 4]. The key issue of adaptation is the study of the realization of the potential, genetically determined capabilities of the organism in response to exposure to adverse environmental factors [2, 5].

Comprehensive assessment of grape plants according to physiological and biochemical parameters, which can be used as indirect diagnostic methods, allows us to obtain a more reliable characteristic of the resistance of genotypes to adverse weather and climatic conditions [6, 7]. In this regard, it is especially important to study the physiological and biochemical patterns of adaptation of grape plants, ensuring the orderliness and regulation of physiological processes.

The aim of the work is to study the physiological and biochemical patterns of the resistance of grape varieties of various ecological and geographical origin to high temperatures and low moisture availability in the summer period of the south of Russia.

MATERIALS AND METHODS

The research was conducted on the basis of the ampelographic collection of the Anapa Zonal Experimental Station of Viticulture and Winemaking, located in the city of Anapa, the quarter of technical grape varieties on the southern carbonate black soil, as well as the collective center “Instrument-Analytical” and the Laboratory of Plant Physiology and Biochemistry of the North Caucasian Federal Scientific Center of Horticulture, Viticulture and Winemaking. Plants of one year (q995), stock Kober 5BB. Formation is a bilateral,high stump cordon of the AZOS. Landing layout 3 x 2.5 m. The objects of research are grape varieties of different ecological and geographical origin and ripening periods: control - Crystal variety of early ripening (interspecific hybrid of euro - Amur - American origin), varieties Dostoynyy and Krasnostop AZOS of average maturity (interspecific hybrids of Euro-American origin).

Crystal grade. Variety of Hungarian selection. Technical purpose. The parent pair:“Amur” X Challotsi Loiosh and Hungarian variety Villar Blanc. A variety of very early ripening, frost-resistant (up to -27 °C). High yield. The color of the berries is yellowish green. The taste is harmonious. The cluster is medium dense, weighing 180-200 g. Berries are distinguished by tender and sweet flesh. Sugar content is from 17 to 18%, and acidity is from 6 to 7 g / l. The clusters are distinguished by an almost conical shape of a standard size. The weight of each bunch can reach up to 180-200 g. Most of the clusters are dense and only a few are slightly friable.

Dostoynyy variety. Variety selection: AZOSViV. Technical purpose. The parent pair: the phylloxero-resistant Dzhemete X Muscat of Hamburg. The variety of medium ripening period, phylloxero-resistant, can be grown on its own rootsbut is affected by mildew and oidium. The cluster is large, weighing 254 g. Brushes are conical, differ in average density, their size is average. Sugar content – 18.2%, acidity – 8.5 g / l. The taste is simple, used to prepare dry wines. Tasting assessment of dry wine is 8.3 points.

Krasnostop AZOSvariety. Variety selection: AZOSViV. Technical purpose. Parent couple phylloxero-resistant Dzhemete X Krasnostop Anapsky. Maturation period is medium early. The acidity of the juice of berries is 8 g / l, the sugar content is 24%. The shape of the brush is cylindrical medium density. The cluster is small, weighing 120-130 g. The pubescence of the underside of the leaf is weak. Flowers:hermaphroditic.

To assess the adaptive resistance of grape varieties to abiotic stresses, we determined the humidity, the content of free and bound water by the gravimetric method [8]. To determine the content of free water, samples of leaves weighing 1 g. were triply dried for 24 hours in the open air, after which they were weighed. To determine the content of free water, the difference in weight was attributed to the mass of the total water content. To determine the total water content, the leaves of the sample weighing 1 g in triplicate were dried in a thermostat to constant weight at 105 °C. The difference between the wet weight and the dry weight was attributed to the weight of the wet weight, and the water content of the shoots was determined as a percentage. The difference between the water content of the shoots and the content of free water was used to find the content of the bound form of water in the samples and expressed as a percentage.

The protein and pigment content was determined by the spectral method [9, 10]. To determine the protein content in the samples of the shoots, weighed samples weighing 1 g in triplicate were used. Samples were put in liquid nitrogen. For protein extraction, a buffer was used, 100 ml of which contained 0.48 mg of 0.1 M Tris, 0.1 g of ascorbic acid, 0.08 g of EDTA, 0.18 g of sodium diethyldithiocarbomate, 0.145 g of sodium chloride, 2.0 g of polyethylene glycol, and the rest was bidistillate to the mark.

After homogenization, the samples were infused for 2 hours in the above buffer and then centrifuged cold. The centrifugal was poured into separate tubes, and the pellet was re-filled with buffer. The extraction was performed three times, the centrifugate for each sample was combined in flasks per 100 cm^3 and adjusted to the mark with buffer. The resulting solution was poured into quartz cuvettes with a thickness of 10 mm. The optical density of the solutions was determined on a UNICO 2800 spectrophotometer at 280 nm. The calibration curve was used to determine the protein content (mg / g) in the sample. The content of phenol-carboxylic (chlorogenic, caffeic), ascorbic acids, and the amino acid Proline was determined by capillary electrophoresis [11]. For this plant material weighing 1 g in triplicate, each was crushed in liquid nitrogen, then placed in a fluoroplastic container of the microwave mineralizer "Minotaur," added 25 cm^3 of 10% aqueous alcohol-rectified "Extra" and set the container in the magnetron of the mineralizer. It was done in the "decomposition without pressure" mode, using 10% of the power of the mineralizer magnetron for 10 minutes. In this case, phenolcarboxylic and free amino acids were extracted.

After the specified time, the container was removed from the microwave mineralizer, cooled under natural conditions for 5 minutes, and the resulting liquid was quantitatively transferred to 25 cm^3 volumetric flasks using an additive of the initial water-alcohol mixture. Further, the quantitative determination of the content of these components was carried out on a Capel 105 R instrument equipped with an ultraviolet photometric detector operating at a wavelength of 254 nm. A quartz capillary with a length of at least 0.5 m Joe detector, an internal diameter of 50-100 mkm, a source of high voltage of positive polarity with adjustable voltage from 1 to 25 kV and a personal computer with appropriate software for collecting and processing information were used. The determination of the desired values of the content of the analyzed substances was carried out with the following parameters: positive voltage on the capillary – 16 kV, analysis time – 15 minutes for ascorbic, chlorogenic, caffeic acids. Individual compounds were identified by the additive method. For malonic dialdehyde, the following operating parameters were established: the SF detector wavelength was 270 nm, a negative voltage – 25 kV, the sample dosing was pneumatic at 30 mbar for 5 s at a voltage of 0 kV, the analysis time was 10 minutes [12].

The conditions for registration of electrophoregram samples corresponded to the conditions for registration of electrophoregrams of calibration solutions. Individual compounds were identified by the additive method. The analyzes were performed on a capillary electrophoresis system in a quartz capillary with an effective length of 0.5 m and an internal diameter of 75 mkm. An aqueous lead electrolyte containing 0.33% boric acid, 0.05% sodium tetraborate and 0.5% isopropanol was used for the analysis with positive voltage polarity and detection wavelength – 254 nm. The heat resistance of grape plants was determined by the conductometric method by measuring the electrical conductivity of an aqueous solution of electrolytes exiting distilled water from leaf cuttings (a sample of 20 leaves) of the control sample (exposure time: 3 hours at 20 °C), the sample subjected to temperature stress (exposure time: 1 hour at 55 °C), and the sample subjected to a temperature of 100 °C (exposure time: 15 minutes at 100 °C). Assigning the difference between the electrical conductivity of samples obtained when exposed to temperatures of 55 °C and 20 °C to the difference of electrical conductivity of samples obtained when exposed to temperatures of 100 °C and 20 °C allows one to calculate the damage factor of the membranes, which characterizes the heat resistance of grape varieties [13]. Plant resistance to high temperatures and low moisture availability in the summer period was studied in natural conditions and in modeling stress (forced dehydration; temperature of 55 °C). Experimental data were processed using conventional methods of variation statistics. [14]. For physiological and biochemical studies, a UNICO 2800 spectrophotometer, an AGAT-2 conductometer, a Kapel 105 R capillary electrophoresis instrument, an Acom JW-1-3000 scale and an analytical scale, LE-402 centrifuge, Type-310, TsLN-16, LOIP LB-163 water bath (TB-6/24-VK), SESH-1drying cabinet were used.

RESULTS AND DISCUSSION

For the analyzed period 2015-2017, in the city of Anapa, the summer of 2017, in contrast to the preceding 2015 and 2016, was more favorable for grape plants (Figure 1).

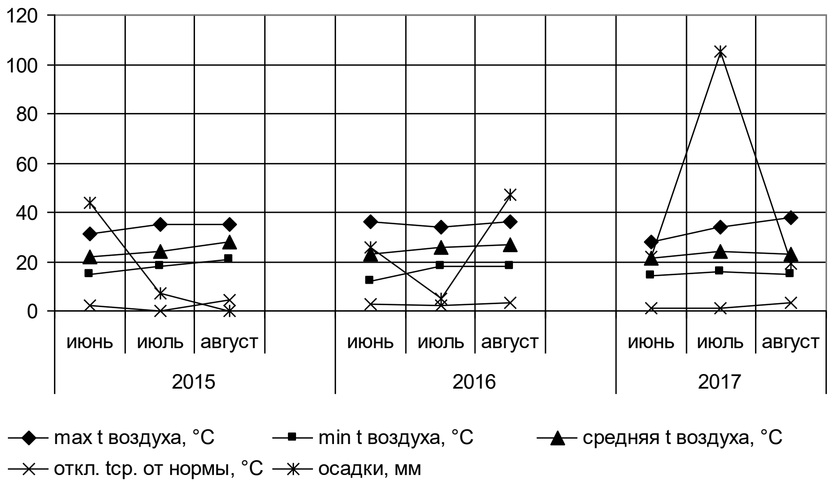


Fig 1: Hydrothermal conditions of the summer period, 2015-2017, Anapa-Taman zone (Russia).

In July, a large amount of precipitation fell, but in August there was a drought and the air temperature reached 38 °C.

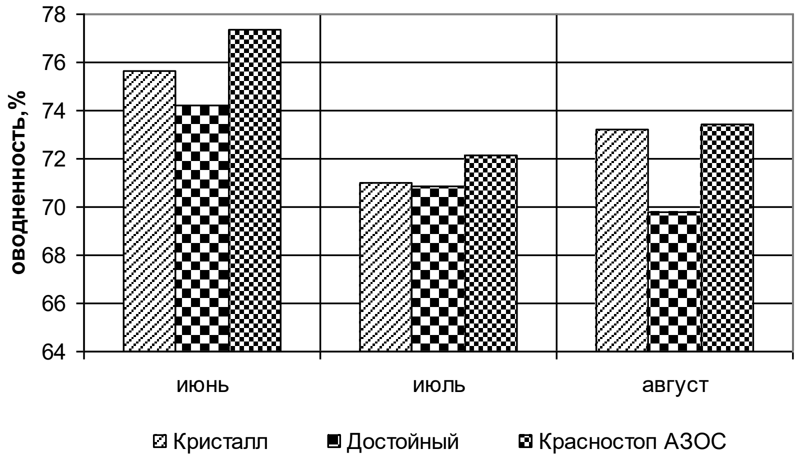


Fig 2: Hydration of leaves of grape varieties in conditions of the summer period of 2017, Anapa-Taman zone (Russia).

The study of the dynamics of the water regime of grape varieties in the summer period of 2017 made it possible to establish a decrease in the water content of the leaves in July and August compared to June. In the Krasnostop AZOS variety, the decrease in precipitation did not have a significant effect on the water content of the leaves (Kkorrel. -0.25); in the Kristall variety, their influence was more significant (Kkorrel. -0.84); the Dostoynyy variety (Kkorrel. -0.67) occupied between them an intermediate position. The increase in maximum air temperature in July and August in comparison with June had a more significant effect (Korrel. -0.8 - -0.99) on the water content of leaves of varieties of the Euro-American origin and less influence on the Kristall variety (Kkorrel. 0.61). The change in the minimum air temperature had a greater effect on the water content of the leaves of the Kristall (Kkorrel. -1) and Krasnostop AZOS (-0.96) varieties. Thus, the greatest influence on the water content of the grape leaves of the Dostoynyy variety has an extremely high air temperature, and the Crystal and Krasnostop varieties of the AES (Kkorrel - 1 / -0.96) experienced the minimum air temperature.

Therefore, the resistant variety that is more resistant to the extremely high temperature is Dostoynyy; the minimum air temperature and the low moisture content are favorable for the Crystal and Krasnostop AZOSvarieties.

The study on dynamics of the content of the bound water share in the leaves of grapes for the period from June to August allowed to establish that the content of the bound form of water in the leaves of the grapes correlated with the content of proline (Figure 3) [15].

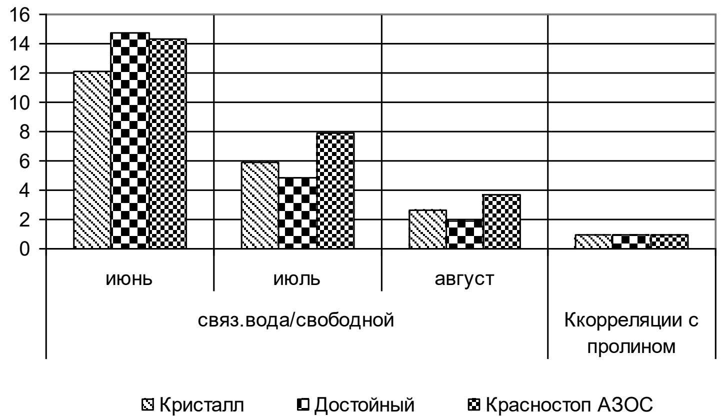
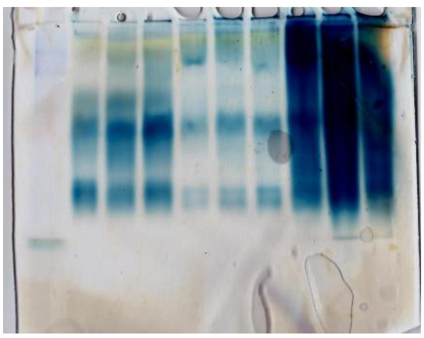


Fig 3: Dynamics of the ratio of bound to free water and its dependence on the content of proline in the leaves of grape varieties in the summer period of 2017.

The manifestation of drought in August 2017 contributed to the activation of oxidative processes in grape plants of the studied varieties [16].

To characterize the expressiveness of genetic systems of adaptation of the studied grape varieties to extremely high temperatures and low moisture supply in the summer period of 2017, heat and water stress proteins were studied using the example of proteins with peroxidase activity (Figure 4) [17, 18].



M 1 2 3 4 5 6 7 8 9 10.

1 – marker; 2 – Dostoynyy; 3 – Dostoynyy drying; 4 – Dostoynyy heating; 5 – Krasnostop AZOS;
6 – Krasnostop AZOS drying; 7 – Krasnostop AZOS heating; 8 – Crystal; 9 – Crystal drying; 10 – Crystal heating

Fig 4: Spectrum of water-soluble proteins (K, Na-phosphate buffer (pH 7.8) with the addition of 4% Triton X-100, with peroxidase activity in the leaves of grape varieties in August 2017.

It was established that the protein complex (water-, salt-soluble protein fraction) of grape varieties when compared with taps of proteins with peroxidase activity, in the studied grape varieties is represented by proteins with a molecular weight of 250, 130, 100, 70, 60, 40 kDa.

The Kristall variety of the Euro-Amur-American origin is distinguished by a high content of proteins with peroxidase activity, followed by the Dostoynyy varieties and the Krasnostop variety of the AES American Euro grapes, with a reduced content of proteins with a molecular weight of 250 and 130 kDa, which may be related to their origin (parental the Dzhemete X Muscat Hamburg and Dzhemete X Krasnostop Anapsky form, respectively).

The Krasnostop AZOS variety's(the parent form: Krasnostop Anapsky), in comparison with the Dostoynyy variety (the parent form Muscat Hamburg), leaves contain less proteins with a molecular weight of 70 and 40 kDa, which is probably due to different parental forms.

Peroxidase activity in the studied varieties ranges from 0.03 to 0.09 sec-1, which is associated with differences in the spectra of proteins with peroxidase activity.

When warming and drying the grapes leaves in a model experiment in the Dostoynyy and Krasnostop AZOS varieties, an increase in the protein content with a molecular weight of 130 kDa and 100 kDa was found. It can be assumed that this was due to the presence of both of their parent form, Dzhemete.

Thus, it can be assumed that, in the studied grape varieties, Dostoynyy and Krasnostop AZOS, under the influence of high-temperature stress, heat stress proteins are the proteins with a molecular weight of 130, 100, and 70 kDa.In the Kristall variety, additionally, proteins with a molecular weight of 250 kDa have peroxidase activity.

It is known that most heat shock proteins are shoperones with a molecular weight of 70, 60, and 40 kDa, which protect proteins from denaturation, adhesion under high temperatures and help them adopt the correct conformation [17.18].

As is well known, when exposed to stress factors, endogenous phenol carbonic acids increase plant resistance to oxidative stress, exhibit antiradical properties [17, 19].

The high activity of peroxidase in the vine of the Crystal variety characterizes its increased resistance to oxidative stress and is consistent with a lower content of phenolcarboxylic and ascorbic acids than the Dostoynyy and Krasnostop AZOS varieties, which have lower peroxidase activity and higher phenolcarboxylic acids (Figure 5).

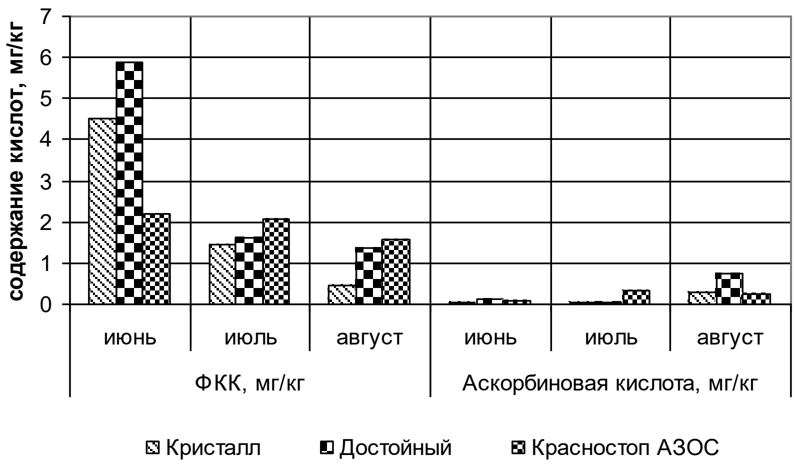


Fig 5: The content of the sum of phenol carboxylic acids and ascorbic acid in the grape leaves in the summer period of 2017.

The increased content of ascorbic acid reduces the intensity of lipid oxidation of cell membranes, while maintaining their fluidity. Under the extreme conditions of August 2017, a higher content of ascorbic acid was noted in the Kristall, Dostoynyyvarieties, and the Krasnostop AZOS variety.

The content of malonic dialdehyde (MDA), a degradation product of polyunsaturated fatty acids, serves as an indicator of lipid oxidation (Figure 6) [17].

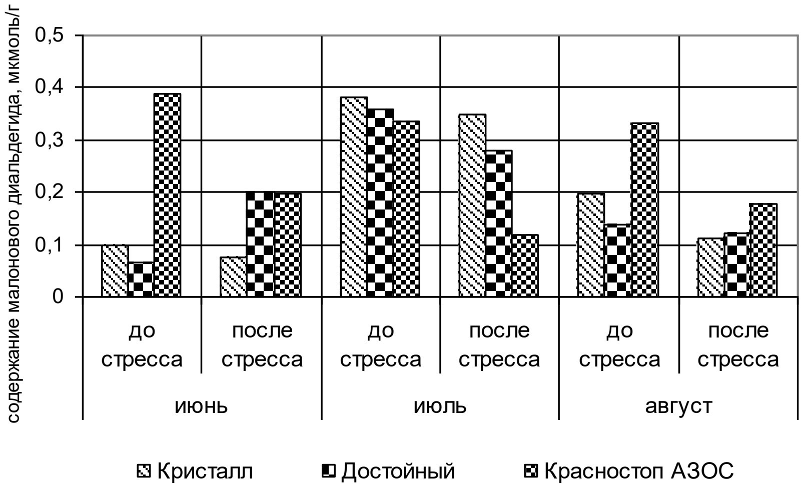


Fig 6: The content of malondialdehyde in the leaves of grapes in the summer of 2017.

In June 2017, in the Crystal, Dostoynyy varieties, the MDA content was lower (0.099 - 0.065 µmol / g) than it was in the Krasnostop AZOS variety (0.19 µmol / g).

In August, with an increase in water and high temperature stress, MDA is less found in the leaves of the grape leaves Crystal and Decent (0.138 and 0.199 µmol / g) and more in Krasnostop AZOS (0.331 µmol / g). Consequently, the Krasnostop AZOS variety is less resistant to oxidative stress, high temperature and low moisture supply (the Anapo-Taman zone).

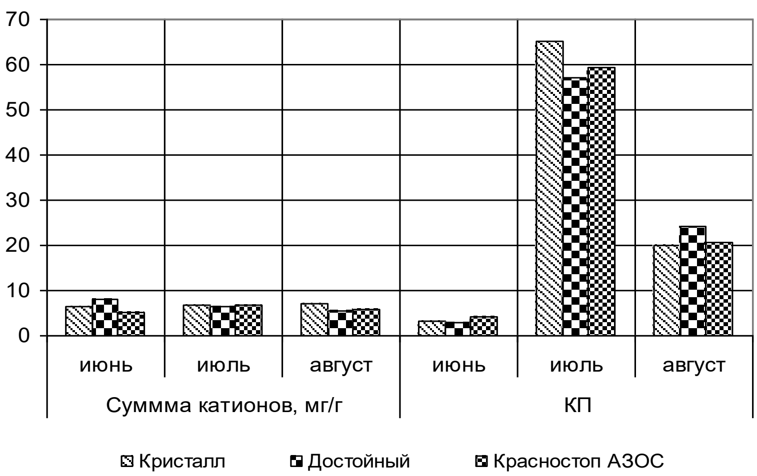


Fig 7: Permeability of cell membranes in the leaves of grapes in the summer of 2017.

The change in the content of MDA characterizes the destruction of the lipids of the cell membranes, hence, their permeability, as evidenced by the change in the coefficient of damage to the cell membranes and the amount of cations in cells (figure 7).

Consequently, the mechanism of protective action to oxidative stress in the studied varieties is different in accordance with their ecological and geographical origin.

CONCLUSION

Physiological and biochemical methods have revealed adaptation mechanisms of resistance of Euro-Amur-American (Crystal) and Euro-American (Dostoynyy and Krasnostop varieties) grape varieties to stress factors of the summer period (heat and drought). The most significant physiological and biochemical indicators of metabolic processes involved in the mechanisms of adaptive resistance of grape varieties to summer stressors (free and bound water content, phenol carbonic (chlorogenic and coffee) and ascorbic acids, malonic dialdehyde, protein, proline, peroxidase activity). A different reaction of the water-, salt-soluble protein complex to high stress temperatures of the summer vegetation period was revealed. Biochemical adaptation of grape plants to the abiotic factors of the summer period is achieved by increasing the content of the bound form of water, proline.

The heat-resistant grape variety Dignified and drought-resistant varieties (Crystal, Krasnostop AZOS) are promising for cultivation in the southern region of Russia, which are highly resistant to heat and drought.

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