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RESEARCH ARTICLE

THE LOW-TEMPERATURE STRESS TOLERANCE OF THE GRAPE VARIETIES OF ECOLOGICAL AND GEOGRAPHICAL ORIGIN

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ABSTRACT

The cold-tolerance of 'Kristal' (Euro-Amur-American origin), 'Dostoyniy' and 'Krasnostop AZOS' (interspecific hybrids of Euro-American origin) grapevine varieties in the conditions of the Russian South winter is characterized by the second (true dormancy) and third (induced dormancy) winter-resistance components. Here we used complex approach to estimate the plants adaptability to abiotic stress factors. We used gravimetric method for humidity assessment and water content; spectral analysis for detection of the protein level and pigments; capillary electrophoresis method to analyze the level of carbohydrates, Krebs cycle organic acids, phenolcarbonic, ascorbic acids, and amino acids. The obtained results allow to suppose the various mechanisms of adaptation of the studied varieties to the winter period stressors. The water content of the shoots, the level of protein, aminoacids, proline, sugars and the sum of phenolcarbonic and ascorbic acids are the most informative indicators of grape plants frost-resistance in the climate conditions of Anapa - Taman region.

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INTRODUCTION

The understanding of the mechanisms of grape plants cold-resistance to abiotic and biotic factors of the environment have a relevance for the agricultural industry and biotechnology of highly productive grape varieties (Ollat, 2017 and Nenko, 2017). The natural and climatic conditions of the South of Russia are favourable to ensure the high yields of grape, able to withstand the competitions on a worldwide basis. At the same time, producing the stable high yields is restricted by the influence of such unfavourable environmental factors, as winter frosts, especially after the long warm weather in summer period. That is why only the varieties, bringing together high productivity, good quality and adaptability to conditions of this region, may be successfully cultivated here on a sufficiently broad scale. It dictates the need for improved range of varieties through the cultivation of grape varieties, better adapted to the weather conditions of the habitats (Ferrandino, 2014 and Sha Valli Khan, 2014). A key issue of adaptation is to study the realization of the potential, genetically determined organism abilities in response to the action of unfavourable environmental factors (Nenko, 2017 and Yegorov, 2017). The complex estimate of the grape plants based on the physiological and biochemical indicators, which may be used as the indirect diagnostic techniques, permits to obtain more reliable characteristic of the genotypes resistance to the unfavourable weather and climatic conditions (Xian, 2017 and Arun-Chinnappa, 2017). In this connection, the study of physiological and biochemical mechanisms of adaptation of the grape plants, providing the orderliness and regulation of the physiological processes, is especially topical. The objective of this work was to study the physiological and biochemical mechanisms of low-temperature resistance of grape varieties of different ecological and geographical origin during winter period and identify varieties of low-temperature resistance during winter period on the basis of metabolic evaluation of genotype expression in hydrothermal conditions of the Russian South.

MATERIAL AND METHODS

The minimum temperature on the territory of the Anapa-Taman zone of the South of Russia (Fig. 1) in the winter 2014-2015 was -7°C in December 2014,- 19°C in January, -11°C in February, and precipitation quantity- 81 mm, 73 mm, 15 mm, respectively. The minimum air temperature in the winter period 2015-2016 reached -9°C in January-13°C, in February - 5 °C, with precipitation quantity to 28 mm, 118 mm, 47 mm, respectively.

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Figure 1. The location of the territory of the Anapa-Taman zone of the South of Russia

The study was done during the period from December to February of 2014 - 2016 years on the territory of Anapa. During this period there was an increase in the minimum air temperature in January, February by 6°C and in precipitation quantity– by 45 and 32 mm, respectively. During March of the same period the precipitation quantity was 13 mm, which is an evidence of gradual warming conditions. The research was conducted on the basis of ampelographic collection of the Federal state budgetary scientific institution "Anapa Zonal Pilot Station of Viniculture and Wine-making", located in Anapa city, block of the grape vine industrial varieties on southern calcareous chernozem. The research was carried out using the equipment of the Centre of collective usage "Tool and Analytical" and physiology and biochemistry laboratory of the Federal state budgetary scientific institution "North-Caucasian federal scientific centre of horticulture, viniculture and winemaking" " (Nenko, 2017 and Yegorov, 2017). The plants were of the same implantation year (1995), parent stock is Kober 5BB. Pruning for shape is a bilateral high-standard spiral cordon of Anapa Zonal Pilot Station. Cultivation of grape plants was performed in bare fallow with landing procedure 3×2.5 m. The objects of research were the grape vine varieties of different ecological and geographic origin and maturation periods, survived after extreme temperatures of the year 2012 winter (Xian, 2017). The 'Kristall'is a variety, the plant of early maturation period, was used as a control (interspecific hybrid of Euro-Amur-American origin). 'Dostoiniy' and 'Krasnostop' are medium maturation period varieties of Anapa Zonal Pilot Station (interspecific hybrids of Euro-Amur-American origin) (Yegorov, 2017). The 'Kristall' is a variety of Hungarian breeding, selected for the industrial purposes. The 'Kristall' parental pair is 'Amurskiy' X Csalloci Lojos and Villar Blan Hungarian variety. This 'Kristall' is high-productive variety characterized by very early maturation period and frost-resistance (up to the -27°C). The 'Kristall' grapes have yellowish-green color and harmonious taste. The bunch of 'Kristall' variety have medium density, weighing 180-200 g. The sugar and acidity content ranges from 17 to 18 percent, and from 6 to 7 g/l, respectively (Yegorov, 2017).

'Dostoyniy' variety was selected by the staff of Anapa Zonal Pilot Station for the industrial purposes. The parental pair is phylloxera-resistant Jemete X Muscat Hamburg. The 'Dostoyniy' variety has medium maturation period. Phylloxera-resistant, it may be grown on the own roots, but it is highly susceptible to mildew and oidium. bunches are of conical form with medium density and medium size. The taste is ordinary; its berries are used to prepare the dry wines. (Yegorov, 2017).

1. 'Krasnostop AZOS' variety was also selected by Anapa Zonal Pilot Station breeders for industrial purposes and wine production. Its parental pair is phylloxera-resistant Jemete X Krasnostop Anapsky. It has medium early maturation period. On an average the acidity of grapes juice is 8 g/l, the sugar content is 24 percent. The form of 'Krasnostop AZOS' grapevines is cylindrical. The bunches are of medium density and small, about 120-130 g in weight (Yegorov, 2017).
2. To estimate the adaptation resistance of the grape vine plants to abiotic stresses, humidity, free and bound water content we used the weight method (Kushnirenko, 1991). To determine the content of free water the specimens of shoots weighing 1g each were dried in three replications for 24 hours on open air, after which they were weighed. To determine the content of free water the weight difference was referred to the weight of total water content. To determine the total content of water in the shoots, the 1g weighed quantities were dried in three replications in thermostat until the weight becomes constant at 105°C. The water content of shoots was determined as a ratio between difference of the raw weight of shoots and its dry mass to the weight of raw biomass. The content of bound water within the specimens was found as the difference between the shoots water content and free water content and expressed as a percentage. The protein and pigments content was determined by a spectral method (Yermakov, 1982).

3. To determine the protein content in the shoots, the 1g specimens were sampled in three replications. The specimens were ground in cryogenic nitrogen followed by protein extraction. For extraction of protein we used the buffer solution, containing in 0.48 mg 0.1 M Tris, 0.1g ascorbic acid, 0.08g ethylenediaminetetraacetate, 0.18g sodium diethyldithiocarbamate, 0.145g sodium chloride, 2.0g polyethylenglycol and distilled water in a total 100ml volume. Samples were homogenized and stored in a buffer for at least for 2 hours. After that samples were centrifuged in the cold (+4°C, centrifuge 5418 R eppendorf). . The centrifugate was poured off into the separate test glasses, and the precipitate was repeatedly topped up with the buffer. The extraction procedure was repeated three times, the precipitate of each replicate was combines in 100 ml flasks with the addition of a buffer to the label. The obtained solution was poured into the 10 mm thick quartz cells. The optical density of solutions was determined by UNICO 2800 spectrophotometer at 280 nm wavelength. Finally, the protein content of specimen was determined by calibration curve (Yermakov, 1972).
4. To estimate the content of pigments (anthocyanins, chalcons) in the shoots 1g specimens were ground in cryogenic nitrogen, flooded with 10 ml of 0.1 n hydrochloric acid solution, infused in the cold for 1 hour and centrifugated. The extraction was performed three times. The supernatant was put into 100 ml flasks, completed with 0.1 n hydrochloric acid solution to the mark. The anthocyanins and chalcons content were determined on LEKI SS1207 spectrophotometer at 490 nm and 364 nm wavelength, respectively (Yermakov, 1972).
5. The starch content was determined by polarimetric method on universal polarimeter (Yermakov, 1972).
6. The level of carbohydrates (sucrose), Krebs cycle organic acids (malic, citric, succinic), phenolcarbonic acids (chlorogenic, caffeic), ascorbic and proline aminoacid was done by capillary electrophoresis. For this the 1g specimen was ground in three replications each one in liquid nitrogen, then put into fluoroplastic container of “Minotaur” microwave mineralizer, 25ml of 10 percent alcohol (ethanol 98 %) aqueous rectified alcohol were added, and the container was placed into mineralizer magnetron and processed for 10 minutes in “pressureless decomposition” mode, applying 10 percent of mineralizer magnetron capacity. After that the container was removed from microwave mineralizer, cooled in the natural conditions for 5 minutes and the obtained liquid was quantitatively taken into the 25 ml graduated flasks by adding the initial alcohol-water mixture. Further the content of these components were determined quantitatively by Kapel 105 P tool, equipped by the UV photometric detector r adding the initial alcohol-water mixture (Yakuba Yu, 2015).
7. Further the content of these components were determined quantitatively by Kapel 105 P tool, equipped by the UV photometric detector, operating at 254 nm wavelength, quartz capillary at 0.5m length at least to the detector, 50-100 μm inner diameter, positive polarity high-voltage source of variable voltage ranging from 1 to 25 kV and personal computer with the relevant software for collection and processing of data. The target values of the analyzed substances content were determined with the parameters as follows: positive voltage on capillary – 16 kV, time of analysis - 15 minutes for ascorbic, chlorogenic, caffeic acids. The individual connections were identified by the standard addition method.
8. For organic acids (malic, citric, succinic) the operating parameters were laid down as follows: the wavelength of spectrophotometric detector – 270 nm, negative voltage – 25 kV, pneumatic dosage of sample at 30 mbar for 5s at 0 kV voltage, time of analysis – 10 minutes. The terms of the samples electrophoregram registration met those of calibration solutions electrophoregram registration. The individual connections were identified by the standard addition method. The target values of the analyzed substances content were determined with the parameters as follows: positive voltage on capillary – 10 kV, time of analysis - 40 minutes.
9. For the determination of the amino acid, to 0.05 ml of extract was added 0.1 ml of a 10% aqueous solution of sodium carbonate and 0.3 ml of a solution phenylisothiocyanate in isopropyl alcohol and left in 35 minutes to undergo reaction. The resulting solution is dried dry, dissolved in 0.5 ml of distilled water, centrifuged for 5 minutes and transferred to the device under a pressure of 30 millibars for 5 seconds. The individual connections were identified by the standard addition method.
10. The analysis employed aqueous leading electrolyte, containing 0.33% of boric acid, 0.05% sodium tetraborate and 0.5% isopropylalcohol with the positive polarity of voltage and the length of detection wave – 254 nm.

The preparation of samples for morphological studies involved commonly used botanic micro methods (NCRRIH and V, 2010). The measurement data were processed with the use of conventional methods of variation statistics (Dospekhov, 1979 and Welham, 2014). The preparation of samples for morphological studies involved commonly used botanic microengineering methods (Yakuba Yu, 2015). The plants resistance to the low temperatures of winter period was studied in the natural conditions as well as in conditions of induced stress, including forced dehydration and low temperature (-25°C). The experimental data were analyzed by the commonly used methods of variation statistics (Yakuba Yu, 2015). The studies were carried out with the use of filter photometer; MBI-3, MBI-10 and Olympus microscopes; UNICO 2800 and LEKI SS1207 spectrophotometers; Kapel 105 P capillary electrophoresis tool; JW-1-3000 Acom balance and analytic balance; LE-402, Type-310, TsLN-16 centrifuges; Eppendorf 5418R LOIP LB-163 (ТБ-6/24-ВК) water bath; SESh-1 drying cupboard; Gronland refrigerating cabinet.

RESULTS

All the analyzed varieties showed the lower water content of shoots and their content of free water in December of 2015, as compared with December of 2014. For vegetative season of 2015 as compared with 2014, the grape plants accumulated the macronutrients store in the shoots. The level of water increased by 13.72% for ‘Kristall’ variety and by 6.1- 9.0% for ‘Dostoyniy’ and ‘Krasnostop’ AZOS varieties, respectively. (Fig. 2). In December of 2015 as compared with 2014, free-water contents of ‘Kristall’ and ‘Krasnostop AZOS’ varieties, along with the lesser water content of shoots, lowered (29.4 and 7.2%, respectively) and slightly increased in ‘Dostoyniy’ variety (1.2%), prompting the suggestions that its metabolic behavior is more active. During the period of 2012 – 2015 the free-water contents of shoots from ‘Kristall’ variety in December correlates with the maximum air temperature (r= 0.94) and precipitation depth (r= 0.92), and in ‘Dostoyniy’ and

'Krasnostop AZOS' varieties – both to the maximum and minimum air temperature (r=0.65 – 0.73) and precipitation depth (r=0.66 – 0.68) (Fig. 3). In the December of 2015 as compared with 2014, the contents of chalcones, protecting the cell membranes from the breakage [2], in the vine grape varieties under study grew by 63.7 – 159.9% (Fig. 4). Consequently, the increase in chalcones contents of the grape vine is a common pattern for all the studied varieties.

The determination of starch in vine, characterizing the frost resistance in the true dormancy period, showed its lower contents in 'Kristall' variety (1.67 mg/g) in the December of 2015 and higher one in 'Dostoyniy' variety (4.98 mg/g), 'Krasnostop AZOS' variety (4.2 mg/g) holds intermediate position between these. The ratios of saccharose contents to that of starch in 'Kristall' variety in December of 2015 was 3.8, 'Dostoyniy' – 0.96 and 'Krasnostop AZOS' – 0.55,

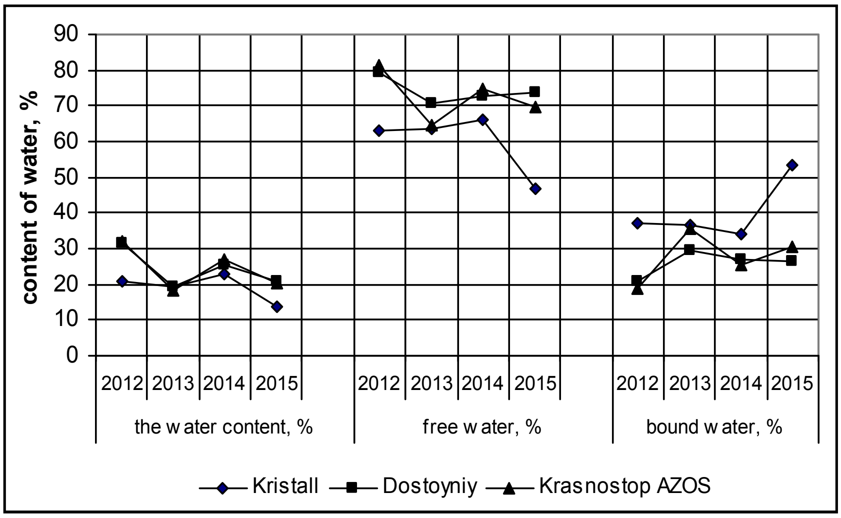

Figure 2. Moisture status of the grape shoots in December of 2012 – 2015

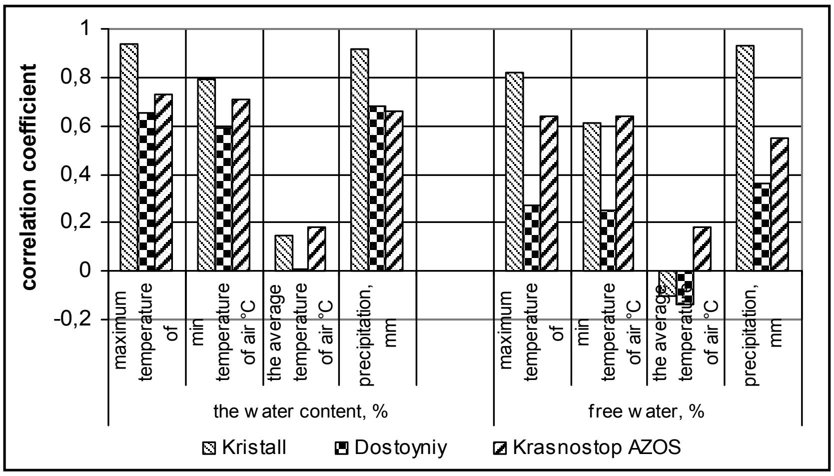

Figure 3. The dependence of the grape shoots' moisture status on the 2012-2015 December hydrothermal conditions in Anapa -Taman zone

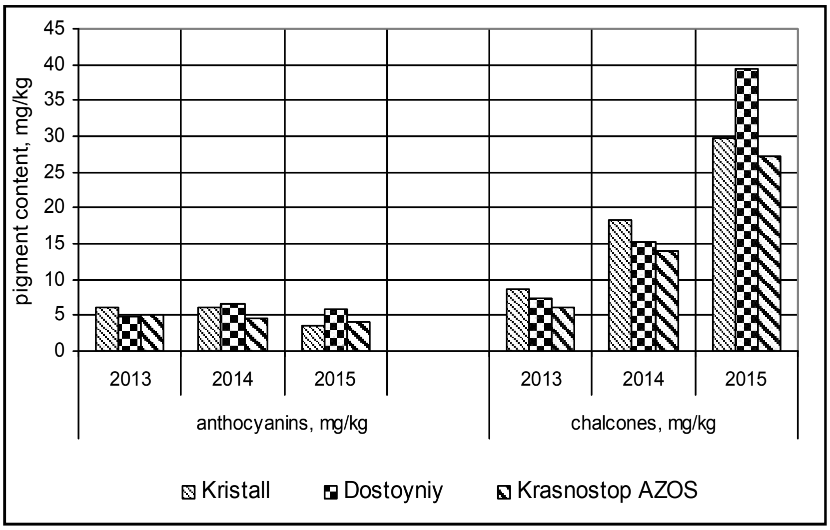

Figure 4. The pigments' contents of the grape vine in December of 2012-2015

characterizing 'Kristall' variety as a more frost-proof one. In the December of 2015 the bound water contents of the grape varieties' shoots were mostly influenced by sucrose contents (r = 0.7). In the December of 2014 the desiccation resistance of the grape plants of the studied varieties was mostly influenced by proline contents (in 2013 proline and sucrose), characterizing the impact of year conditions on specificity of protective response to the stressors in the state of true dormancy (Fig. 5).

62.5 mg/kg, respectively), protecting the cell membranes from destruction, and 'Krasnostop AZOS' variety – by the lower one (9.3 mg/kg and 25.1 mg/kg, respectively) (Fig. 7) (2, 6). The histochemical studies established the starch contents to be 5.0 points for the fine-cellular core zone of 'Kristall' and 'Dostoyniy' varieties, and 4.7 points for 'Krasnostop AZOS' variety, permitting to characterize these as the highly frost-proof ones and frost-proof one, respectively. The frost-proof grape varieties are characterized by the fast hydrolysis of

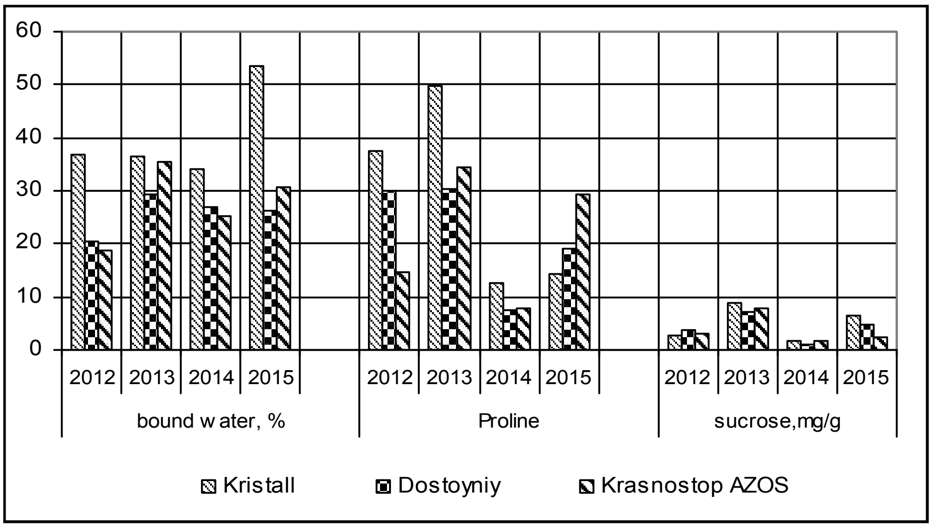

Figure 5. Water-retaining capacity of the grape vine in December of 2012-2015

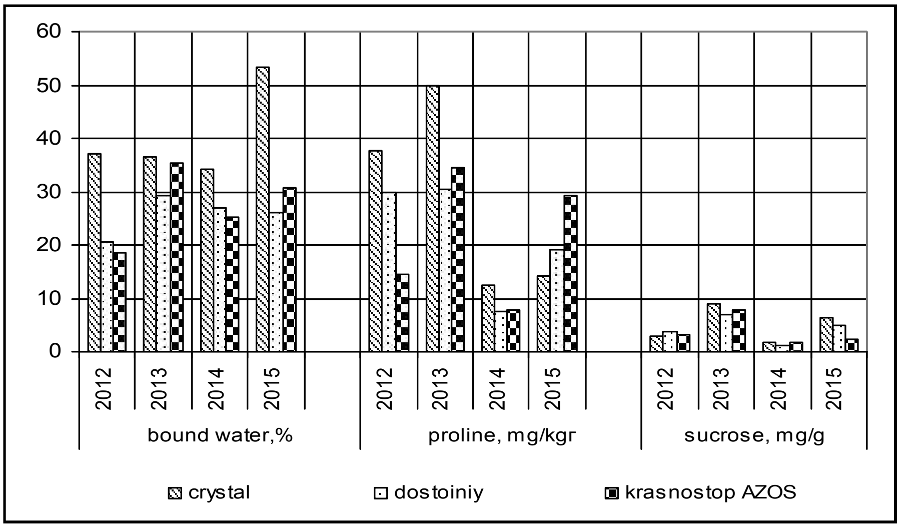

Figure 6. Biochemical characteristic of the grape shoots' water-retaining capacity in December of 2012-2015

The estimation of mechanism of the vine's desiccation resistance showed proline to have greater effect and sucrose to have lesser one on the vine's bound water contents (r= 0.5) in 'Kristall' variety (r= 0.9), in 'Krasnostop AZOS' variety – both proline and sucrose (r= 0.8), and in 'Dostoyniy' variety – proline and sucrose to have a slight impact (r= 0.3) (Fig. 6). It brings us to assumption of the different mechanisms of adaptation to the varied environment in the plants of varieties under study in the state of true dormancy. For the 2012-2015 period in December, the proline contents in the vine of 'Kristall' variety was higher (12.6 – 49.8 mg/kg), than in 'Krasnostop AZOS' variety (7.9 – 34.5 mg/kg) and 'Dostoyniy' (7.6 – 30.4 mg/kg). In the December of 2015, 'Kristall' and 'Dostoyniy' varieties are characterized by the higher contents of ascorbic acid (20.6 and 12.3 mg/kg, respectively) and the phenolcarbonic acids totality (51.2 and

starch already from the beginning of winter. The hydrolysis of starch is delayed in the insufficiently frost-proof varieties. It is found that the content of starch in the vine of 'Dostoyniy', 'Kristall' varieties was 5.0 and 4.9 points, respectively in December as compared with November of 2015. The starch contents in the shoot fine-cellular core zone did not change for 'Krasnostop AZOS' variety. Thus, 'Dostoyniy', 'Kristall' varieties showed their high winter hardiness, judging from the rates of starch consumption caused by its hydrolysis. 'Krasnostop AZOS' variety showed itself as frost-proof by the rate of starch consumption. The anatomo-morphological study of buds showed them to be in the state of winter dormancy. All the grape varieties had embryonic inflorescences, responsible for the next year yield, embedded in the winter buds (eyes). The artificial freezing of the grape shoots in December of 2015 at minus 25°C temperature increased water content of the

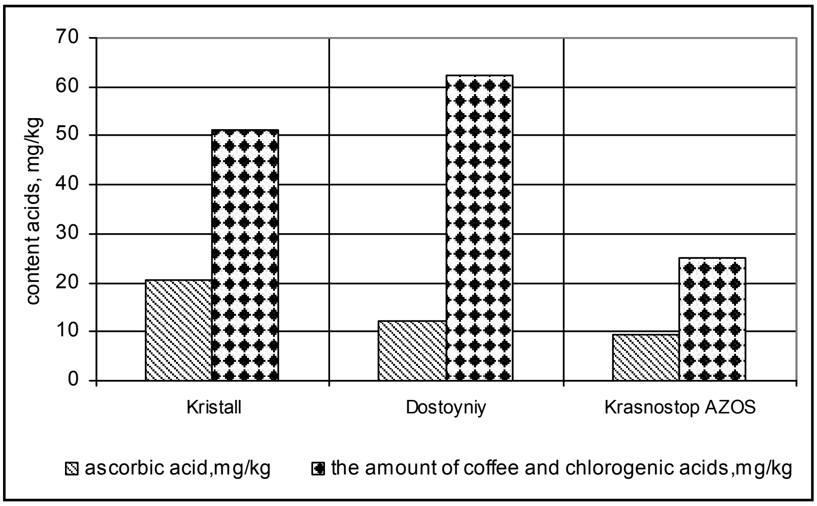

Figure 7. The ascorbic and phenol carbonic acids contents of the grape shoots in December of 2015

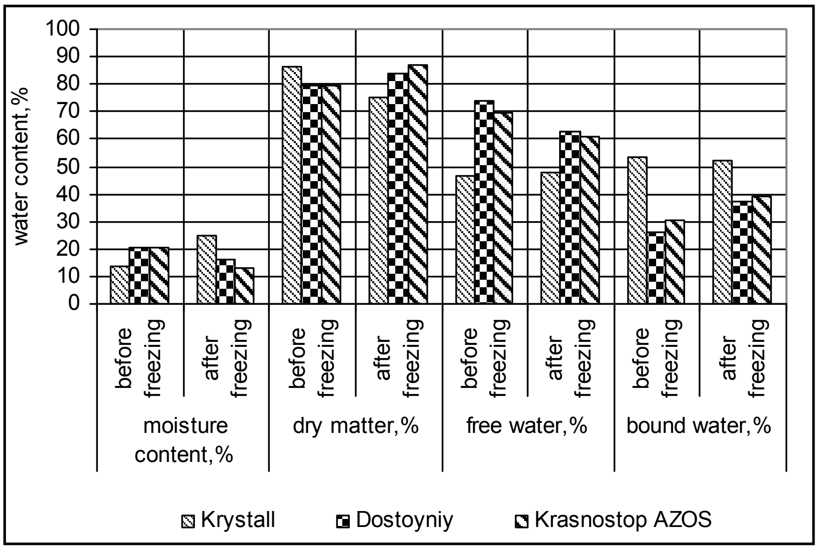

Figure 8. The impact of the low-temperature stress in model experiment on the water status of grape shoots, December of 2015

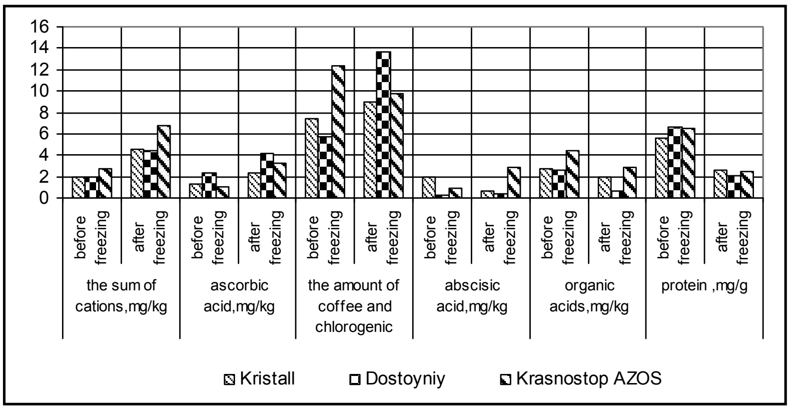

Figure 9. Biochemical characteristic of the grape vine frost resistance, December 2015

'Kristall' variety shoots by 173%, and in 'Dostoyniy' and 'Krasnostop AZOS' varieties it dropped by 29.9 and 60.3%. (Fig. 8). In such a case the free water contents of these varieties lowered by 18.2 and 13.8%, respectively. The ratio of bound water to free water content after freezing was higher by 66.7% for 'Dostoyniy' variety and by 45.5% for 'Krasnostop AZOS' variety, respectively. The same ratio changed insignificantly for 'Kristall' variety. Freezing increased protein contents of a 'Kristall' variety vine by 19.9%, it lowered in 'Dostoyniy' variety by 22.1% and 'Krasnostop AZOS' variety – by 28.6%, and starch contents – in 'Kristall' variety – by 36.5%, and as for 'Dostoyniy' and 'Krasnostop AZOS' varieties – by 175 and 96.3%, respectively (Fig. 9). This goes to prove active hydrolytic behavior that agrees with the higher proline contents of 'Krasnostop AZOS' variety by 197.6%. The contents of the Krebs cycle organic acids in 'Dostoyniy' variety was 52.2% lower that reflects the decrease in respiration intensity. Thus, we may assume, that in the December of 2015 the grape plants of this variety did not enter the state of deep dormancy.

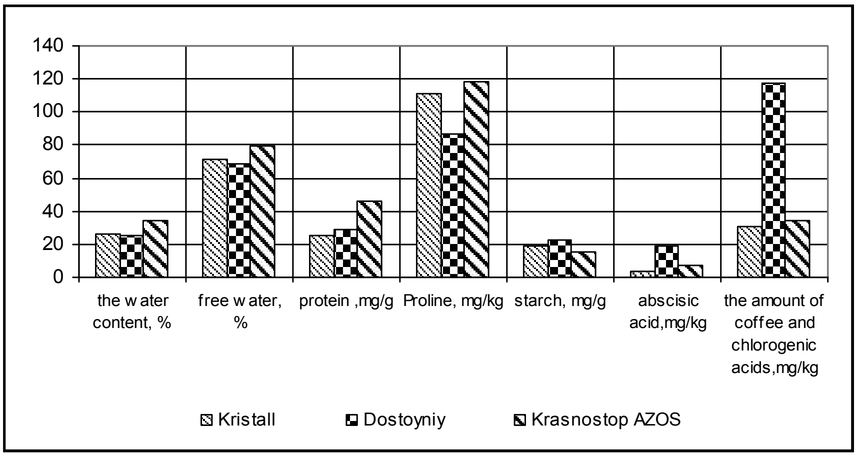

Figure 10. Biochemical characteristic of grape vine on the basis of the third winter-resistance component, February of 2016

The elevated level of organic acids was detected in ‘Kristall’ and ‘Krasnostop AZOS’ varieties, 50.0% and 17.8% higher, respectively, displaying the protective response of plants through the activation of breathing. The impact of the low-temperature on the contents of phenol carbonic acids in the grape vine of the studied varieties was also assessed. When freezing the shoots, the increase in the contents of the phenolcarbonic acids (chlorogenic, caffeic) sum is reported for ‘Kristall’ variety by 135.4%, for ‘Dostoyniy’ – by 45.6% and ‘Krasnostop AZOS’ variety – 6.4 times more. It characterizes the different mechanisms of the extremely low temperature resistance of the grape varieties varying in ecological and geographical origin in the period of true dormancy. The histochemical analysis demonstrated, that after freezing of the shoots the starch contents of the fine-cellular core zone of grape vine in ‘Dostoyniy’, ‘Krasnostop AZOS’, ‘Kristall’ varieties, noted as the highly frost-proof ones, did not change and continued to be 4.7 - 5.0 points. Thus, the influence of the low temperatures on the shoots of the studied grape varieties caused the largest changes in the vine cortex and did not damage its core. In the natural conditions of Anapa and Taman regions the air temperature in January of 2015 dropped down to minus 19°C, which affected adversely the plants of grape in the state of induced dormancy. In late February of 2016 the water content of the shoots grew, as compared with December 2015 in ‘Kristall’ variety by 12.6%, ‘Dostoyniy’ – 22.2%, ‘Krasnostop AZOS’ – 68.8%. The free water contents in ‘Kristall’ variety increased by 53.9%, in ‘Dostoyniy’ variety - 7.7%, in ‘Krasnostop AZOS’ variety – by 14.8%, which is due to activation of metabolic processes. In February of 2015 the contents of proline in vine increased by 4.1 – 7.7 times, and the level of starch diminished by 63.65 % and 49.05 % that agrees with activation of the hydrolytic processes (Fig.10).

DISCUSSION

The study was conducted in the winters of 2012-2016. During these periods, the grape plants did not enter the state of deep dormancy and lacked the tolerance to the extremely low temperatures (second winter-resistance component). This is evidenced by the high content of the free form of water in the vine of the studied varieties, which characterizes the intensity of metabolic processes in the grape plant and can be associated with hydrothermal conditions in December 2015 (Kushnirenko, 1991). It should be noted the higher content of the bound form of water in the vine of early maturing varieties of Krystall, which suggests that he had a better preparation for the winter of 2015.

In determining the content of pigments (anthocyanins and halcons) - antioxidants that protect cell membranes from destruction (Koshkin, 2010) it was found that the vine grape varieties anthocyanins contain less than halcons. The biggest difference in their content is observed in the variety Krasnostop AZOS and smaller-in the variety Krystall, which may be due to the winter hardiness of varieties. One of the indicators characterizing frost resistance in the period of organic rest is both the starch content in the vine and its ability to hydrolyze. Determination of the ratio of sucrose to starch in the variety Krystall was 3.8, in the variety Krasnostop AZOS-0.55, which also characterizes the winter hardiness of varieties. A Dostoyniy varieties occupies an intermediate position between these varieties (Nenko, 2017). Winter-hardy varieties are characterized by a high content of bound water forms and a great influence on the resistance of plant cells to dehydration have osmoprotectors, including such as Proline and sucrose. Determination of the correlation coefficients between the content of the bound form of water, Proline and sucrose allowed to establish that the more winter-hardy grade Krystall dependence of the bound water on the content of Proline is higher than that of the varieties Dostoyniy and Krasnostop AZOS. The accumulation of Proline is accompanied by the prevention of protein denaturation, preservation of the structure and activity of enzymes, which also provides winter hardiness of the variety (Koshkin, 2010 and Kuznetsov, 1999). These facts suggest a great influence of the genotype of the grape variety, its origin on winter hardiness. Consequently, the presence in the genome of the variety Krystall genetic potential of the Amur grape has a predominant effect on its frost resistance and determines the specificity of metabolic processes. In the pedigree of varieties Dostoyniy and Krasnostop AZOS present winter-hardy variety Dzhemete, which determines their winter hardiness. Physiological and biochemical patterns of resistance to low temperatures in these varieties have their own specifics. One of the elements of resistance of grape varieties to low temperatures is the resistance of the lipid phase of cell membranes to destruction. A large role in preserving the integrity of the lipid phase belongs to the water-soluble antioxidant-ascorbic acid, which is able to restore membrane-bound tocopherol – antioxidant of the lipid phase, which causes a break in the chains of free radical oxidation, interacting with peroxyl and alkoxyl radicals (Koshkin, 2010; Davey, 2000; Foyer, 1991). With artificial freezing of shoots of grapes, the content of bound water in varieties Dostoyniy and Krasnostop AZOS increased by 66.7 and 45.5%, respectively, and in the variety Krystall has not changed, which may be due to the greater stability of the latter.

At the same time, hydrolytic processes were activated (the protein and starch content decreased), the Krystall and Dostoyniy varieties increased the content of phenolcarboxylic acids, and the grade of Krasnostop AZOS decreased, which may be due to their ecological and geographical origin. Our results are consistent with the literature. For example, Beheshti Rooy et al. an increase in the concentration of sugar in response to stress caused by low temperature is shown. The authors suggested that sugars can enhance the stabilization of biomembrane by reducing the freezing temperature of intercellular water (Beheshti, 2017 and Wample, 1992). Under cold stress, the content of abscisic acid inducing cold resistance genes increased almost 5-fold in the Krasnostop AZOS variety (Koshkin, 2010). The decrease in the amount of organic acids in the vine of all three varieties after freezing characterizes the decrease in the intensity of breathing.

In February all grape varieties showed the higher contents of the sum of phenolcarbonic and ascorbic acids, preventing the damage of cell membranes. The elevated levels of proline contents suggest the enhanced water-retaining capacity of cytoplasm and were used to display their adaptability by the third winter-resistance component. According to some literature data (Yakuba Yu, 2015 and Yakuba, 2015), we may assume better resistance of 'Kristall' variety plants to desiccation and formation of proteins with the higher proline contents, strengthening the cell walls in response to stress factors. Accumulation of proline in response to various stress factors, including low-temperature conditions has been correlated with stress tolerance. Proline concentration is higher in stress-tolerant than in stress-sensitive plants (Yakuba Yu. 2015). In the present study, starch and sucrose content was increased markedly in 'Kristall' variety plants as compared to other varieties. This reflects the higher resistance adaptability of 'Kristall' variety to low temperature stress.

Our results are consistent with some literature data. For example, Beheshti Rooy et al. showed the elevation of sugar concentrations in response to stress induced by low temperature. Authors supposed that sugars can increase biomembrane stabilization through decreasing of freezing point of intercellular water (NCRRIH and V, 2010). Our data, together with the literature, indicated the better adaptation of 'Kristall' variety to environmental conditions of Anapa and Taman zone, as compared to 'Krasnostop AZOS' and 'Dostoyniy' varieties.

The anatomo-morphological and histochemical studies of the one-year-old vine of the grape varieties under study in March showed them to enter vegetation season, characterized by activity of the growth processes. The starch contents of the fine-cellular core zone in 'Dostoyniy', 'Krasnostop AZOS', 'Kristall' varieties was established to be 4.9 points. The anatomo-morphological studies of the buds showed the embryonic processes, responsible for the current year yield, to begin in those. Thus, the obtained results allow supposing the various mechanisms of adaptation of the studied grape varieties to the stress factors of winter period that are typical for Anapa and Taman zone. The most informative parameters, characterizing the resistance of the studied grape varieties to the stressors of the 2007-2015 winter periods (water content of the shoots, free and bound water contents and their ratio, protein, aminoacids, proline, sugars, the sum of phenolcarbonic and ascorbic acid) are listed in Table 1.

Table 1. Biochemical indicators of the grape interspecific hybrids adaptation to the stress factors of 2007-2015 winter period

Biochemical indicators	State of deep dormancy	State of induced dormancy
Water content of shoot,%	13.70– 31.87	16.44 – 34.60
Dry matter contents,%	68.13 – 86.28	65.40– 83.56
Free water contents,%	46.54– 81.25	47.5 – 80.43
Bound water contents,%	18.65 – 53.46	19.85 – 52.5
Ratio of bound water to free water	0.34 – 1.15	0.24 – 1.11
Saccharose contents, mg/g	1.07– 11.83	2.18 – 6.15
Starch contents, mg/g	1.67– 15.24	1.51– 8.51
Proline contents, mg/kg	7.6 – 92.0	46.2 – 190.0
Protein contents, mg/g	2.43– 6.58	2.49 – 8.21
The sum of phenolcarbonic and ascorbic acids, mg/g	0.03 – 13.4	0.01 – 0.06

These parameters, indicating physiological and biochemical changes to cold-induced stress could be applied in breeding programs of grape varieties. We assume that the above-mentioned parameters could be useful as markers of tolerance to low-temperature conditions in different climatic regions that are relevant for grape plants growing. The chapter DISCUSSIONS must contain comparative discussions between your results to those of other authors, mostly foreign authors (at least 7-8 authors, recommended more than 10), who made similar studies. Also, if such studies are presented for the first time in the world in this article, you specify this. These discussions will be made in such a way as to highlight the new / novelty of your study at global level. If is necessary, there will appear a number of new references to the literature (and in the chapter REFERENCES there must appear those bibliographic citations). At the end of this chapter (not separately, not in another chapter called *Conclusions*), in light of comparative observations that you made, you will draw the conclusions of your study, highlighting the importance, impact and scientific innovation of the presented data.

Conclusions

Here we studied the key changes of biochemical and morphological characteristics of grape plant of the Anapa and Taman regions. The plant response to the different environmental factors showed significant difference among plants of early maturation period (Kristal) as compared to varieties with medium maturation period ('Dostoyniy' and 'Krasnostop AZOS'). For the interspecific grape hybrids the optimum physiological and biochemical parameters of winter-resistance (in the state of deep and induced dormancy) and the ranges of their variation, permitting these varieties to survive in extreme conditions of Anapa -Taman zone winter period, were established. The low temperatures conditions of 2016 brought to insignificant subfreezing of grape plants that may have effect on their production performance. In the state of induced dormancy under favourable climatic conditions of February 2016 the varieties may be characterized positively by the third winter-resistance component. Thus, our study indicated significant differences between studied grape varieties by their ability to adapt to the hydrothermal conditions of the Russian South. The results of the study can be used to monitor frost-resistance of the grape plants for the agricultural and breeding purposes.

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