Adaptation mechanisms of grape varieties in unstable climatic conditions of the autumn-winter period

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Abstract. Despite the increase in the average annual temperature observed in recent decades, the urgency of the problem of cold resistance of plants not only does not decrease, but also increases. The purpose of the work is to identify the mechanisms of adaptation of grape varieties of various origins to low temperatures in the autumn-winter period in terms of physiological and biochemical indicators, to identify varieties with a high adaptive potential for cultivation in the Anapo-Taman zone of the Krasnodar Region. Objects of the study: interspecific hybrids of grapes of various ecological and geographical origin: Crystal (control) - Euro-Amur-American; Krasnostop AZOS, Dostoyny - Euro-American; Vostorg - Amur-American; Zarif - Eastern European; Aligote - Western European origin. The implementation of the mechanisms of resistance of grapes to low temperatures was achieved by reducing the water content of tissues, increasing the proportion of bound water, the content of ascorbic acid, and changing the activity of peroxidase. Varieties Crystal, Krasnostop AZOS, Vostorg were distinguished by an increased ratio of bound and free water - 3.8-6.8; maximum accumulation of ascorbic acid (19.3-21.3 µg/g fresh weight), reduced peroxidase activity in comparison with other studied varieties. Varieties such as Crystal, Krasnostop AZOS, Vostorg proved to be more adaptive in comparison with other studied varieties and are recommended for the usage in the breeding process.

1 Introduction

Despite the increase in the average annual temperature observed in recent decades, for most countries of the globe, the urgency of the problem of cold and frost resistance of plants not only does not decrease, but also increases. [1]. In the current climatic conditions, some varieties of cultivated plants do not have time to adapt to changing environmental conditions, including winter stresses, their adaptive resistance decreases, and, ultimately, yields as well.

In the near future, abnormally cold winters with sudden temperature changes are predicted, and the vine will be more susceptible to hypothermia [2]. This necessitates physiological and biochemical studies in order to elucidate the mechanisms of adaptation of

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grape varieties in unstable environmental conditions, to identify varieties with high adaptive potential.

For successful wintering, plants have developed appropriate mechanisms of frost resistance, including autumn hardening associated with changes in the metabolism of carbohydrates, lipids and phospholipids, the accumulation of cryoprotectants, in particular, soluble sugars, free amino acids, which prevent the formation of ice crystals in cells, the consequences of dehydration and damage to cellular structures [3].

Under conditions of low temperature stress, plants attempt to restore metabolic homeostasis and continuously produce various metabolites to achieve biological balance. These metabolites can play the role of osmoregulators, cryoprotectants, antifreezes, membrane stabilizers, and antioxidants [4].

The low temperatures of the winter period cause numerous disturbances in the functional activity, primarily of cell membranes. This can increase levels of reactive oxygen species (ROS) and subsequently lead to oxidative stress. To protect against oxidative damage caused by low temperatures, plants have evolved ROS scavenging mechanisms with antioxidant systems such as peroxidase [5].

It was found that grape varieties resistant to low temperatures compared with susceptible ones initially have higher peroxidase activity [6].

Among the numerous metabolites involved in the protective response during wintering, ascorbic acid plays an important role. A number of plants showed an increase in ascorbic acid in a protective response to low temperatures [7, 8].

Nowadays it has been established that, in response to low-temperature stress, the synthesis of specific stress proteins occurs in plants. Among them are antifreeze proteins that regulate and protect plant cells from damage by ice crystals, molecular shaperones and dehydrins [9].

Scientists are also successfully using the achievements of molecular biology and biotechnology in the creation of mutant and transformed plants to study the role of individual genes and identify key proteins that can increase resistance to low temperatures. It was found that the VaPUB U-box protein gene responded to cold stress in the vine and also affected the accumulation of resistance-associated proteins [10]. The application of genomic approaches has made it possible to identify several genes associated with resistance to low temperatures in 25 wild grape varieties [11].

The purpose of this work is to identify the mechanisms of adaptation of grape varieties of various origins to low temperatures of the autumn-winter period according to some physiological and biochemical indicators, to identify varieties with high adaptive potential.

2 Materials and methods

The studies were carried out in 2019-2021 on the basis of the Anapa ampelographic collection, the laboratory of plant physiology and biochemistry of the Federal State Budgetary Scientific Institution NCFSCHVW, the Center for the Collective Use of Technological Equipment in the following areas: genomic and post-genomic technologies; physiological, biochemical and microbiological studies; soil, agrochemical and ecotoxicological studies; food safety.

Plants of one year of planting, stock Kober 5BB. Formation - double-sided high-standard spiral cordon AZOS. Cultivation of grape plants - on a black fallow with a planting pattern of 3 × 2.5 m. The objects of research were interspecific hybrids of grapes of various ecological and geographical origin:

Crystal (control) - Euro-Amur-American origin of the Hungarian selection. Very early maturity. Resistant to mildew and gray mold. Frost resistance up to -30 °C.

Dostoyny - Euro-American origin of Russian selection, medium-late. Mildew is
affected. It has increased resistance to oidium, gray mold and phylloxera. Frost resistance - 19-22 °C.

*Krasnostop AZOS* - Euro-American origin of Russian selection. Mid-late maturity. It is affected by mildew, oidium, phylloxera-resistant. Frost and drought resistant.

*Vostorg* - Amur-American origin of Russian selection, very early ripening. Resistant to mildew, gray mold, resistant to phylloxera. Frost resistance up to -25 °C.

*Aligote* - Western European origin of French selection. In wet weather susceptible to gray mold, less susceptible to oidium, affected by mildew. The berries are severely damaged by the grapevine leafworm. Relatively winter-hardy.

*Zarif* - East European origin of Tajik selection. Very early maturity. Resistant to anthracnose, gray mold and other fungal diseases. Withstands significant temperature drops.

Physiological and biochemical parameters were determined in the leaves, which were taken from 8-12 nodes of the vine. The studies were carried out in triplicate on five leaves of each variety. The parameters of the water regime were determined by the gravimetric method after the samples were dried in a thermostat at 105°C to constant weight [12]. The content of ascorbic acid was determined according to the method used by Janmohammadi [7]. Determination of peroxidase activity was carried out according to the method used by Kaya [12]. The obtained data were processed by the methods of variation statistics [13].

### 3 Results and Discussion

The formation of winter hardness of grapes is a complex, multifactorial process that develops in stages in the annual cycle of growth and development. The formation of a winter-hardy state consists of three stages: entry into dormancy, hardening with low positive temperatures (the first phase of hardening) and hardening with negative temperatures (the second phase of hardening). During hardening, complex physiological and biochemical transformations are observed in the grape plant, contributing to the formation of winter hardness.

Important features that characterize the preparation of the vine for the winter period are the timely ripening of shoots with a well-developed periderm, a high accumulation of reserve substances in them, mainly carbohydrates, and an optimal moisture content. By the beginning of the winter dormancy period, the water content in the shoots of grapes decreases, which is about 50% [14].

In our studies, by the beginning of the winter period, the water content of the tissues of the vegetative organs of grapes was different. Wood and heartwood tissues had the highest water content - 48.02-51.64%; the smallest - bark 46.17-48.96% The water content in the kidneys was 47.62-50.23% (table). During the winter, the water content of various organs of the grape bush changed slightly.

Table. Water content of the vegetative organs of grapes by the beginning of the winter period (average values for 2019-2021, % of fresh weight).

| Variety            | Water content, % |
|--------------------|------------------|
|                    | wood, core       | bark             | buds             |
| Crystal (c)        | 48.02±3.32       | 46.23±1.34       | 47.62±1.94       |
| Dostoyny           | 50.87±1.43       | 48.07±3.31       | 48.55±0.91       |
| Krasnostop AZOS    | 49.82±1.87       | 47.21±1.81       | 47.82±2.07       |
| Vostorg            | 51.64±1.71       | 47.29±1.34       | 50.23±1.62       |
| Aligote            | 50.58±3.44       | 48.96±0.25       | 49.05±1.71       |
| Zarif              | 48.96±2.51       | 46.17±1.46       | 49.93±2.30       |
It follows from the above table that the most significant differences in terms of water content of wood and shoot core were found in varieties such as Dostoyny, Vostorg, Aligote. The index of water content of the bark and buds in all varieties of grapes significantly exceeds this indicator in the control variety Crystal.

The hardening process ensures the transition of the free form of water into the bound one. At the same time, the ratio between various forms of water changes in the direction of a decrease in free and an increase in the proportion of bound water. More frost-resistant varieties are characterized, as a rule, by a lower total water content of mature shoots and an increased content of the bound water fraction in them. [14].

The increase in the percentage of bound water in various grape organs correlates with an increase in their water-holding capacity and frost resistance. A high coefficient of the quantitative ratio of bound and free water indicates a high degree of resistance of the variety to adverse winter conditions [14].

In November, the lowest ratio of bound water to free water was noted in the Aligote variety - 0.9, and the highest in the Crystal variety - 3.1 (Fig. 1).

![Fig. 1. The ratio of bound and free water in the shoots of grapes in the autumn-winter period of 2019-2021. LSD 0.5: November - 0.25; January - 1.24; February - 1.03.](image)

In January, during the period of manifestation of maximum frost resistance, the ratio of bound water to free water in all varieties increased, and had values of 2.1-6.6; in February this ratio was 2.1-6.8.

Varieties such as Crystal, Krasnostop AZOS, Vostorg in the autumn-winter period were distinguished by an increased ratio of bound and free water in comparison with other varieties, which indicates their increased winter hardiness.

An increase in peroxidase activity in winter is associated with a change in redox reactions in cells and the accumulation of reactive oxygen species. In olive, rye, and apple plants, an increase in peroxidase activity was noted during acclimatization to low temperatures [5, 7, 8]. It has been shown that the activity of peroxidase in grape shoots changes during the winter period and is due to varietal specificity [12]. Often, peroxidase activity increased in non-frost-resistant varieties of apples and grapes [8, 14].

In our studies, the maximum activity of peroxidase was noted in November - 6.51-8.23 c.u. per mg of protein (Fig. 2).
Peroxidase activity in grape shoots in the autumn-winter period of 2019-2021. LSD 0.5: November - 1.41; January - 0.74; February - 0.98.

In January, for all varieties, it decreased by 1.07-1.25 times. In February, peroxidase activity changed differently. In varieties Crystal, Krasnostop AZOS, Vostorg almost did not change in comparison with January. In varieties Dostoyny, Aligote, Zarif increased by 1.29-1.38 times.

Ascorbic acid plays an important role in the defense reactions of plants, including exposure to low temperatures. Its role in the formation of the winter hardiness of the apple tree is shown, the winter-hardy varieties of which in the autumn-winter period accumulated large amounts of ascorbic acid in comparison with the non-winter-hardy ones [8]. At low temperatures during wintering, the content of ascorbic acid in rye increased [7].

In our studies, the content of ascorbic acid increased during the autumn-winter period. In November, after hardening, its content in shoot tissues was 1.5-4.7 µg/g fresh weight. In January, the content of ascorbic acid increased by 2.6-4.0 times depending on the variety, in February it continued to increase and reached maximum values - 19.3-21.3 µg/g wet weight in the varieties Crystal, Krasnostop AZOS, Vostorg (Fig. 3).
4 Conclusion

During the autumn-winter period of 2019-2021, differences in grape varieties of various ecological and geographical origins were revealed in terms of physiological and biochemical parameters. The implementation of the mechanisms of resistance of grapes to low temperatures was achieved by reducing the water content of tissues, increasing the proportion of bound water, the content of ascorbic acid, and changing the activity of peroxidase. It was established that by the beginning of the winter period, the water content of various organs of the grape bush was different. Shoot tissues had the highest water content - 48.02-51.64%; the smallest - bark 46.17-48.96%. Varieties Crystal, Krasnostop AZOS, Vostorg were distinguished by an increased ratio of bound and free water - 3.8-6.8 in comparison with other studied varieties. It was found that in winter the peroxidase activity increased by 1.29-1.38 times in varieties Dostoyny, Aligote, Zarif, which indicates their instability to low temperatures. The maximum accumulation of ascorbic acid (19.3-21.3 µg/g fresh weight), which has a protective-regulating effect under adverse conditions, was noted in the varieties Crystal, Krasnostop AZOS, Vostorg. Varieties such as Crystal, Krasnostop AZOS, Vostorg proved to be more adaptive to winter stresses in comparison with varieties Dostoyny, Aligote and Zarif and are recommended for the usage in the breeding process for winter hardiness.

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