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Research Article

**PRO / ANTIOXIDANT STATUS IN PLANTS OF SPRING
WHEAT UNDER THE EFFECT OF DROUGHT AND
STEVIOSIDE**

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Abstract:

This paper for the first time studies the effect of diterpene glycoside stevioside (10^{-8} M) on the redox status of 15-day spring seedlings in soil drought. Two types of spring soft wheat - Omskaya 33 (selected and recommended for cultivation in the Omsk region and Tatarstan of the Russian Federation) and Tamož 2 (selected, certified and widely distributed in Iraq) were chosen as research objects. It was found that soil drought causes an increase in lipid peroxidation both in Omskaya 33 and Tamož 2. Stevioside more strongly reduced the content of malonic dialdehyde against the background of water stress in Omskaya 33 that showed more active lipid peroxidation processes during drought. To determine the effectiveness of the antioxidant system in plant cells under drought conditions, the activity of antioxidant enzymes of ascorbate peroxidase, soluble peroxidase and catalase, as well as the content of a non-enzymatic antioxidant proline were determined. Soil drought stimulated the activity of the antioxidant enzymes studied. Preliminary treatment with diterpene glycoside with stevioside reduced the effect of water deficiency on the activity of antioxidant enzymes to a greater extent in Tamož 2. Soil drought raises the proline level more in Tamož 2 than in Omskaya 33, which may be due to the greater resistance of Tamož 2 to water stress.

Keywords: *Triticum aestivum L., stevioside, drought, stability, lipid peroxidation, antioxidant enzymes, proline.*

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INTRODUCTION:

One of the main conditions for obtaining stable yields of agriculturally valuable crops is the use of growth regulators and biologically active substances that increase the yield of plants and their resistance to phytoablation and abiotic stresses. Modern science makes strict requirements to the drugs used, which must be non-toxic to both humans and the environment. In this regard, of particular interest are substances isolated from natural raw materials, which are ecologically safe and possess high biological activity in low concentrations. That is why for the past few years the department of botany and plant physiology of the Kazan Federal University have been studying the derivatives of the diterpenoid of steviol (13-hydroxy-ent-kaur-16-ene-19-oic acid) [1], which has a hydrocarbon skeleton characteristic for phytohormone gibberellins. During this time, we studied more than 20 compounds and found that the highest biological activity is characteristic of the steviol glycoside – stevioside, which has the ability to increase plant stress resistance and activate their growth [2-6].

One of the most common stress factors is drought, which, along with other unfavorable conditions, causes oxidative stress in plants and the accumulation of reactive oxygen intermediates in the cell (ROI) [7, 8]. Using the system of antioxidant enzymes, as well as low-molecular antioxidants such as carotenoids, proline, α -tocopherol and others, inactivation of ROI in cells occurs [7-9]. In this regard, our objective was to identify the effect of diterpene glycoside – stevioside on the redox status of two varieties of spring wheat Omskaya 33 and Tamož 2 under soil drought.

METHODS:

The objects of research were 15-day plants of two varieties of spring soft wheat (*Triticum aestivum* L.) Omskaya 33 and Tamož 2.

A variety of spring soft wheat (*Triticum aestivum* L.) Omskaya 33 is included in the State Register for the Western Siberian and Middle Volga regions, recommended for cultivation in the Omsk region and Tatarstan of the Russian Federation. A variety of spring soft wheat (*Triticum aestivum* L.) Tamož 2 is certified and widely distributed in Iraq [10, 11].

The experiment scheme included four options: control, drought, seeds presowing treatment of seeds with stevioside (10^{-8} M) in drought, and normal water supply of plants. Seeds were planted in pots, forty in each, containing gray forest soil. The experimental

seeds were previously aged in stevioside for 3 hours (10^{-8} M). The concentration of stevioside was determined in preliminary experiments. The plants were grown at 23°C and 12-hour photoperiod with an illumination of 100 W/m². The variants with normal water supply maintained soil humidity at 70%, while drought variants had 30% soil moisture. The soil drought was created by stopping irrigation of 10-day plants until the moisture content of the soil in the test vessels was reduced to 30% of the total moisture capacity of the soil [12].

The level of lipid peroxidation, the activity of antioxidant enzymes of ascorbate peroxidase, catalase, soluble peroxidase, the content of polynucle and carotenoids were determined by the methods previously described in [6].

The experiments were carried out in 4-5 analytical and 5-6 biological replicas. Statistical processing of data was carried out with Microsoft Excel. Reliability of the difference was determined by Student t-test at $P \leq 0.05$.

RESULTS AND DISCUSSION:

The unfavorable environmental conditions, including drought, cause activation of lipid oxidation processes in plants, including lipid peroxidation (LPO), which is a universal signaling mechanism that triggers the development of adaptive programs of the cell and the whole organism [13]. We found that soil drought causes an increase in lipid peroxidation both in Omskaya 33 and in Tamož 2 (Figure 1). Pre-treatment of wheat seeds with stevioside (10^{-8} M) reduced the effect of drought on lipid peroxidation in the two study varieties (Figure 1). Thus, water deficiency in Tamož 2 resulted in less oxidation of lipids of the plasma membrane as compared to Omskaya 33. This may indicate that this variety is more resistant to drought. At the same time, stevioside decreased LPO in Omskaya 33, which usually features more active lipid peroxidation processes.

An important role in adapting plants to various unfavorable factors of the external environment is assigned to the functioning of effective antioxidant systems able to provide protection against reactive oxygen intermediates accumulating as a result of damage to cells from stress [14, 15]. To determine the effectiveness of the antioxidant system, we determined the activity of antioxidant enzymes of ascorbate peroxidase, soluble peroxidase and catalase.

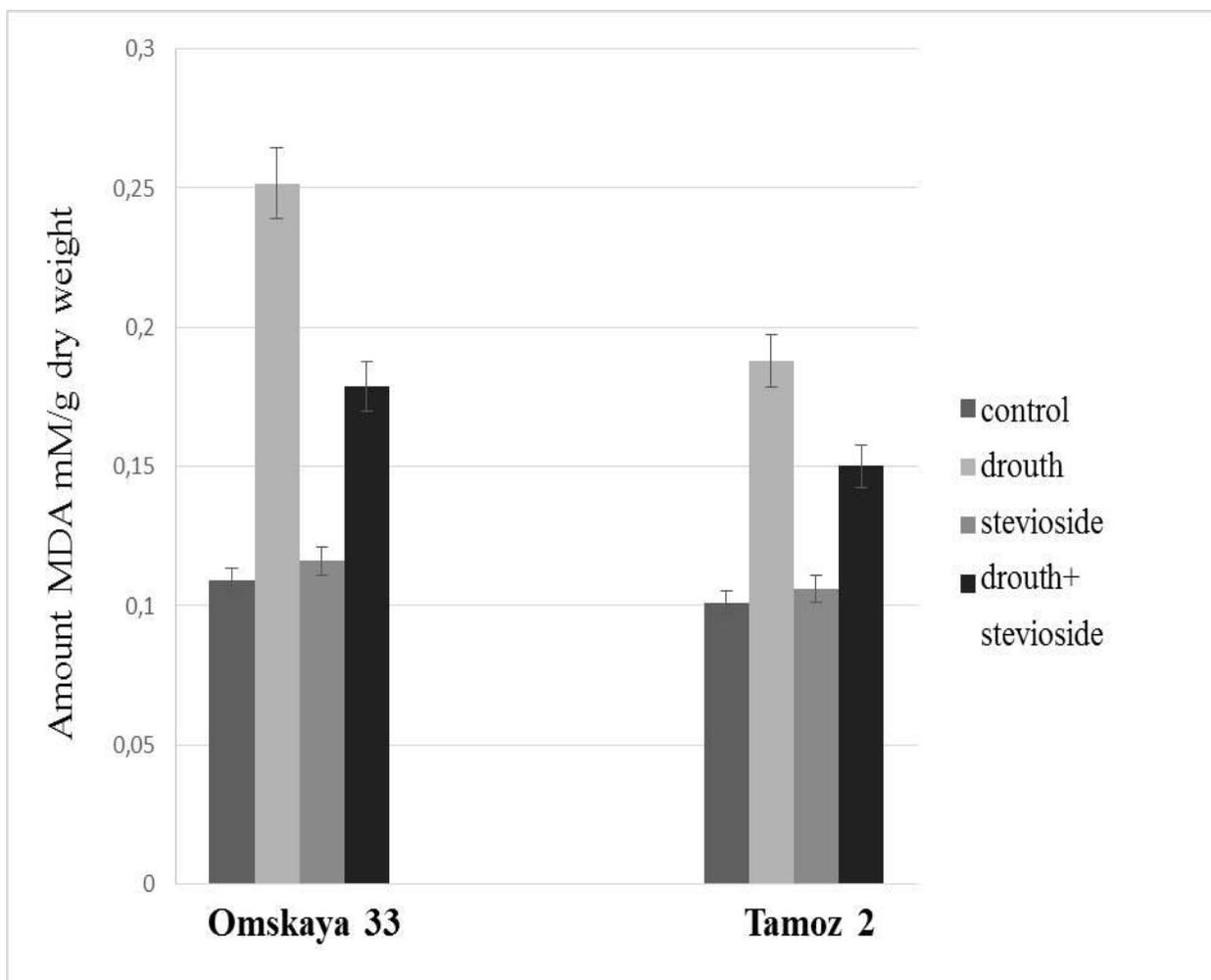


Fig 1: Influence of soil drought and stevioside (10^{-8} M) on the content of malondialdehyde in leaves of spring wheat seedlings.

Catalase is one of the leading enzymes that utilize hydrogen peroxide in a plant organism. The activity of this enzyme is one of the indicators of plant resistance to oxidative stress. The activity of catalase during the drought in Omskaya 33 increased by 94% compared to control plants, and in Tamoż 2 - by 87% (Figure 2). It is known that the activity of catalase can depend on the intensity and type of stress, the organ of the plant being studied, the time of observation and other factors. Thus, under conditions of water stress in drought-resistant genotypes of soft and hard wheat, catalase activity increases, while in sensitive ones it remains unchanged or decreases [16].

Stevioside (10^{-8} M) did not affect the activity of this enzyme in the leaves of Tamoż 2 and slightly increased its activity in Omskaya 33 sprouts (by 16%) in comparison with the control. It is known that the effect of growth regulators on plants is ambiguous and depends on the initial oxidative status of the plant.

The combined effect of pretreatment with stevioside (10^{-8} M) and soil drought led to a decrease in catalase activity compared to the single drought effect: the catalase activity in Omskaya 33 was 149%, in Tamoż 2 - 118% as compared with the control (Figure 2). Thus, stevioside largely reduced the effect of drought on catalase activity in Tamoż 2.

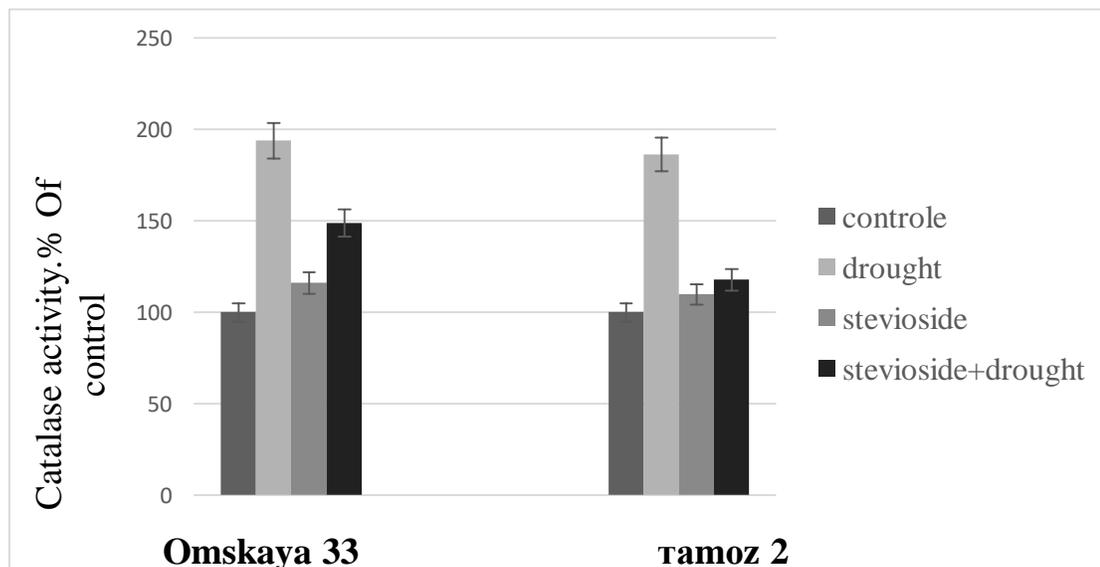


Fig 2: The activity of Catalase in the leaves of spring wheat under the effect of drought and stevioside (10-8 M).

Catalase decomposes hydrogen peroxide at a very high rate, while it has a low affinity for the substrate and starts to work only at a sufficiently high content of H₂O₂ [17]. It can be assumed that treatment with stevioside reduces the level of oxidative stress caused by soil drought.

Ascorbate peroxidase belongs to the peroxidase group and is the main enzyme utilizing hydrogen peroxide in the cell. Ascorbate peroxidase plays a key role in the compensatory mechanism of antioxidant protection [18]. The enzyme has a high affinity for the substrate and is able to neutralize peroxide in very low concentrations [19].

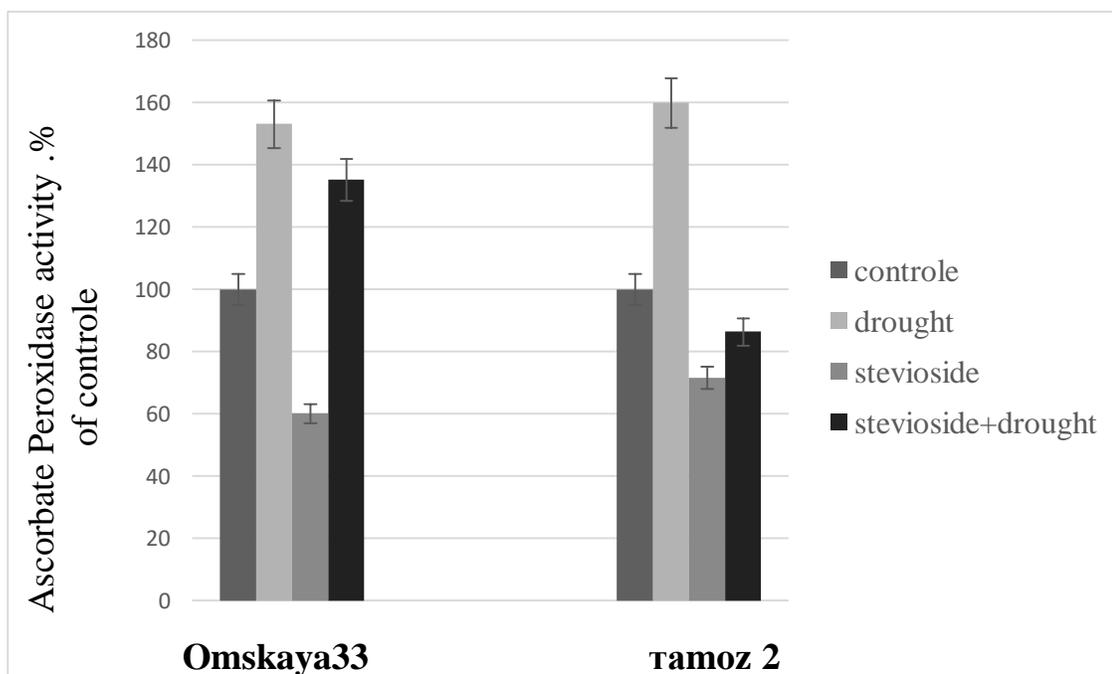


Fig 3: The activity of Ascorbate peroxidase in the leaves of spring wheat under the effect of drought and stevioside (10-8 M).

The activity of ascorbate peroxidase also increased in soil drought in spring wheat seedlings of two varieties: in Omskaya 33 by 53% compared to the control variant, and in Tamoz 2 by 60% (Figure 3).

The diterpene glycoside – stevioside (10^{-8} M) reduced the activity of ascorbate peroxidase in Omskaya 33 by 40%, in Tamoz 2 – by 28% (Figure 3).

Thus, pre-treatment with stevioside (10^{-8} M) led to a greater decrease in the effect of drought on the activity of ascorbate peroxidase in Tamoz 2.

Plants induce peroxidase in response to the effects of various physical, chemical and biological factors. Peroxidase is a functionally labile enzyme that can react to most homeostatic disorders. In this case, there are changes, both in the set of molecular forms of the enzyme, and in the manifestation of their activity [14]. The activity of soluble peroxidase, under the influence of soil drought in two varieties, also changed as the activity of catalase and ascorbate peroxidase, i.e. increased (Figure 4). Stevioside (10^{-8} M) had practically no effect on the activity of this enzyme in the investigated varieties of spring wheat. Pre-treatment with diterpene glycoside (10^{-8} M) led to a decrease in the effect of drought on the activity of soluble peroxidase in the sprouts of Tamoz 2 only (Figure 4).

It should be noted that antioxidant enzymes are not always able to completely inactivate ROI in a cell under stress conditions, and in some cases low molecular weight organic antioxidants more effectively inactivate ROI [19]. Proline is an inert compatible osmolyte [20] and in addition to antioxidant functions performs membrane-protective,

chaperone, and signal-regulatory ones under various stressors, as well as regulates the expression of some genes [21, 22]. In our experiments, the level of proline was slightly higher in Tamoz 2 control plants as compared to Omskaya 33 sprouts (Figure 5), which may also indirectly indicate greater resistance of Tamoz 2.

At present, the question of the relationship between proline content and plant resistance to stressors is debatable [9]. It was shown that there was no correlation between the proline content and the salt tolerance of barley [23], and the varieties of rice with greater drought resistance showed higher content of proline [24].

Under the influence of drought, Omskaya 33 showed proline content equal to 317.6% of control, and Tamoz 2 – 426.6% (Figure 5). As can be seen from these data, the action of soil drought in Tamoz 2 raises the proline level more than in Omskaya 33, which may be due to its greater resistance to this stress. Some plants, under the influence of adverse factors, have more than a hundredfold increase in the level of proline [25].

Stevioside lowered proline content in two varieties of spring wheat – Omskaya 33 by 53%, and Tamoz 2 by 40% of the level of control plants. Perhaps, this effect depends on the genetic characteristics of plants.

Pretreatment of seeds with stevioside (10^{-8} M) reduced the proline content in wheat plants subjected to soil drought - the level of proline in Omskaya 33 was 200%, in Tamoz 2 – 193%. The results obtained may indicate the protective effect of stevioside on plants under soil drought conditions (Figure 5).

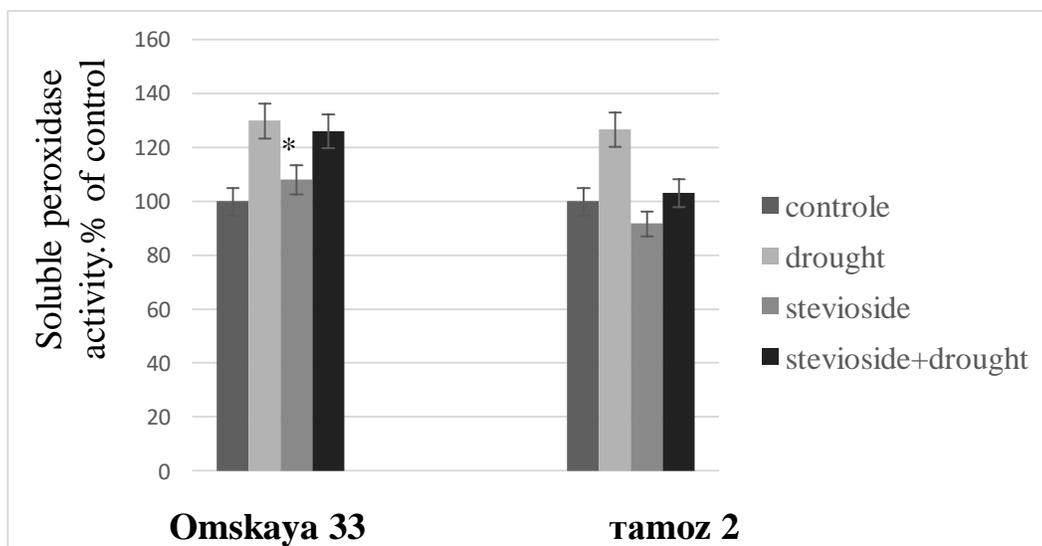


Fig 4: The activity of soluble peroxidase in the leaves of spring wheat under the effect of drought and stevioside (10^{-8} M).

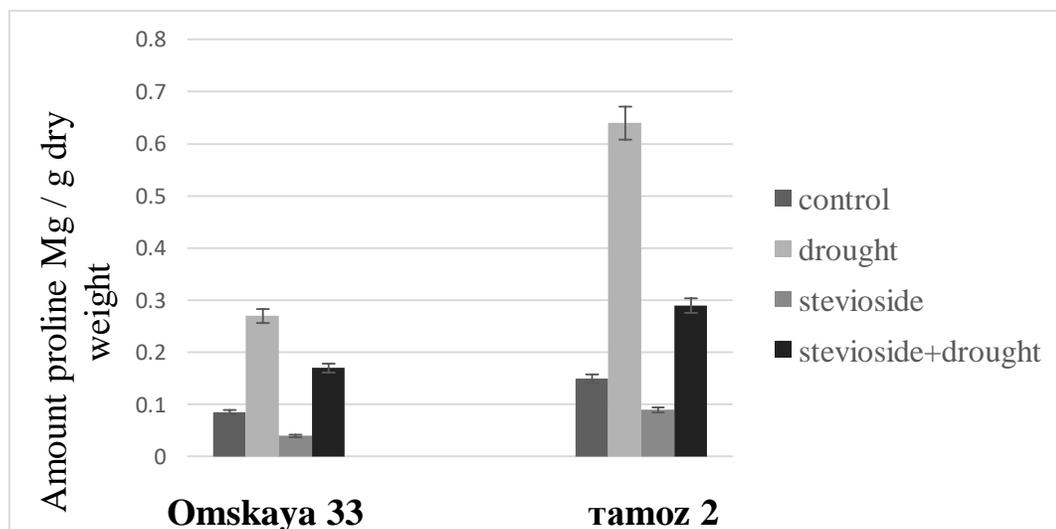


Fig 5: Effect of soil drought and stevioside (10⁻⁸ M) on proline content in leaves of spring wheat seedlings.

SUMMARY:

1. Stevioside largely reduced the level of lipid peroxidation against the background of water stress in Omskaya 33, which usually features more active lipid peroxidation processes during drought.

2. Soil drought raises the proline level more in Tamoz 2 than in Omskaya 33, which may be due to the greater resistance of Tamoz 2 to water stress. Stevioside reduced proline content during drought more in Omskaya 33 (53%) than in Tamoz 2 (40%).

3. Pre-treatment with stevioside (10⁻⁸ M) led to a greater decrease in the effect of drought on the activity of antioxidant enzymes more in Tamoz 2 varieties.

CONCLUSION:

Thus, soil drought stimulates the activity of the main antioxidant enzymes involved in the detoxification of H₂O₂: catalase, soluble peroxidase and ascorbate peroxidase in two varieties of spring wheat. Pre-treatment with diterpene glycoside – stevioside – reduces the effect of water deficiency on the activity of antioxidant enzymes to a greater extent in Tamoz 2. It can be assumed that stevioside has a greater effect on antioxidant enzymes of a more stable variety.

Despite the important role of enzymes in detoxifying ROI under stress of different etymologies, the enzymatic antioxidant system does not always provide 100% protection of the cell from death. This is due, first of all, to the fact that the enzymes are located in different tissue structures and cellular compartments, have different substrate specificity and affinity for the active forms of oxygen. In

addition, under the influence of stress on plants, rapid inactivation of the constitutive pool of antioxidant enzymes is observed.

Apparently, pre-treatment of plants with the growth regulator stevioside in order to increase drought resistance is more effective for a less stable variety. It can be assumed that external signs of damage (plant growth, water content) caused by water stress occur in Tamoz 2 varieties, as more drought-resistant, after a longer action of the stress factor than in Omskaya 33. However, cellular protective reactions, in particular, an increase in the activity of antioxidant enzymes and proline content, start earlier. Stevioside, by reducing the level of LPO, probably due to a decrease in ROI [6], leads to a decrease in the activity of antioxidant enzymes. And in the case of a stable variety of Tamoz 2, the level of LPO is less than that of Omskaya 33, and therefore, the preliminary treatment with stevioside reduces the effect of water deficiency on the activity of antioxidant enzymes more in Tamoz 2.

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