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### Physiological response of pear cultivars to short-term high temperature stress

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**Abstract.** To optimize and improve fruit crop breeding, it is essential to identify cultivars with high adaptive and productive potential. In summer, the climatic conditions of the Krasnodar Krai are characterized by insufficient and uneven precipitation, along with high air temperatures, which have been observed almost annually in recent years. In this study, our objective was to detect differences among four pear (*Pyrus sp.* L.) cultivars under moderate heat stress by analyzing physiological parameters. Pear trees were planted under identical cultivation practices and environmental conditions at the experimental orchard in the Krasnodar Krai (45°16'N, 38°93'E). Pear leaves were exposed to 40°C for 3 hours in a climatic chamber, after which physiological parameters were assessed. It was found that the studied cultivars are resistant to short-term heat treatment, as indicated by low levels of malondialdehyde (16.2–37.5 nmol g<sup>-1</sup> fresh weight), a marker of lipid peroxidation in plant cells. However, the cultivars exhibited different stress responses. The Russian cultivar Krasnodarskaya Letnyaya showed the highest levels of peroxidase activity (25.5–41.8 unit mg<sup>-1</sup> protein min<sup>-1</sup>) and polyphenol oxidase activity (3.9 unit mg<sup>-1</sup> protein) under stress conditions, as well as the greatest number of peroxidase isoforms (9–10 bands) compared with the Russian cultivar Leven and the American cultivars Kieffer and Devo. The total phenol content increased on average from 20.7 to 22.5 mg g<sup>-1</sup> fresh weight across all pear cultivars, with the highest values observed in Krasnodarskaya Letnyaya under stress. Based on these results, we conclude that Krasnodarskaya Letnyaya possesses a broader range of protective physiological features, indicating its high adaptive potential. It is also likely that under prolonged high-temperature stress conditions, this cultivar will maintain a high level of resistance.

*The article contains 41 References.*

**Keywords:** *Pyrus*, heat stress, photosynthetic pigments, antioxidant system defence, oxidative stress

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## ФИЗИОЛОГИЯ И БИОХИМИЯ РАСТЕНИЙ

Научная статья

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### Физиологическая реакция сортов груши на кратковременный высокотемпературный стресс

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**Аннотация.** Для оптимизации и усовершенствования процессов селекции плодовых культур необходим поиск сортов с высоким адаптационным и продукционным потенциалом. Летом погодные условия Краснодарского края характеризуются недостаточным и неравномерным выпадением осадков, а также экстремально высокими температурами воздуха, которые в последнее время наблюдаются практически ежегодно. В настоящем исследовании наша задача заключалась в выявлении различий между четырьмя сортами груши (*Pyrus sp.* L.) по физиологическим параметрам в условиях моделируемого высокотемпературного стресса. Деревья груши произрастали в одинаковых экологических условиях при использовании однотипных технологий выращивания на опытном садовом участке на территории Краснодарского края (45°16' с. ш., 38°93' в. д.). Побеги груши выдерживали при температуре 40°C в течение 3 ч в климатической камере. Затем оценивали содержание физиологических параметров в листьях. По данным статистического анализа, взаимодействие между сортами, термической обработкой и годом исследования оказало существенное влияние на содержание общих фенолов и ферментативную активность в листьях груши. Установлено, что изучаемые сорта устойчивы к кратковременной термообработке, о чем свидетельствовали низкие показатели малонового диальдегида (16,2–37,5 нмоль г<sup>-1</sup> сырого веса), который является одним из маркеров перекисного окисления липидов в растительных клетках. В то же время сорта характеризовались различной ответной реакцией на стресс. Выявлено, что отечественный сорт Краснодарская летняя имеет наиболее высокий уровень пероксидазной (25,5–41,8 у. ед. мг<sup>-1</sup> белка мин<sup>-1</sup>) и полифенолоксидазной активности

(3,9 у. ед.  $\text{мг}^{-1}$  белка) в условиях стресса, а также имеет наибольшее количество изоформ пероксидаз (9–10 изоформ) при сравнении с отечественным сортом Левен и американскими сортами Киффер и Дево. Содержание общих фенолов увеличилось в среднем с 20,7 до 22,5  $\text{мг г}^{-1}$  сырого веса у всех исследованных сортов груши. Наибольшие значения этого показателя в условиях стресса обнаружены у сорта Краснодарская летняя. На основании полученных результатов можно заключить, что сорт Краснодарская летняя обладает более широким спектром защитных физиологических особенностей, что может определять его высокий адаптационный потенциал. Можно также предположить, что в условиях длительного высокотемпературного стресса этот сорт сможет проявить высокий уровень устойчивости.

**Ключевые слова:** *Pyrus*, высокотемпературный стресс, фотосинтетические пигменты, антиоксидантная система защиты, окислительный стресс

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## Introduction

Currently, one of the primary objectives in agriculture is to develop highly adaptive and productive cultivars. Climate change significantly impacts the sustainable development of agricultural communities and profoundly affects the growth and productivity of crop plants. Therefore, continuous improvement of cultivars is essential, taking environmental factors into account. Heat stress has emerged as a major limiting factor for crop growth and development [1]. The global mean temperature of the land and ocean surface has increased by 0.85°C from the 20th to the 21st century [2]. Climate projections indicate that temperatures are expected to rise by 0.2°C every decade. Elevated air temperatures adversely affect plant growth and development by inducing morphological, physiological, and biochemical changes. Temperatures exceeding 30°C have been associated with increased pollen sterility [3]. Moreover, heat stress reduces crop yields, as reported for wheat, maize, rice, and tomato [4, 5]. A 1% decrease in grain number per degree day was observed in maize and barley following exposure to temperatures above 30°C [6].

Understanding the mechanisms of plant adaptation during the reproductive stage is a critical step in maintaining stable crop yields under elevated air temperatures. This type of stress results in a wide range of plant responses at the organ, tissue, cellular, and molecular levels. Water loss in leaves and reduced root conductance hinder the normal development of physiological processes in plant tissues [4]. Additionally, heat stress has been reported to reduce photosynthetic

activity, chlorophyll content, photosynthetic rate, and the activities of key enzymes [7, 8].

High-temperature stress induces oxidative damage through the formation of reactive oxygen species (ROS). Under optimal conditions, ROS levels remain balanced; however, their production increases under stress. Free radicals impair cellular function by damaging lipids, proteins, and nucleic acids. Plants possess two types of antioxidant defense mechanisms to scavenge and detoxify ROS: enzymatic and non-enzymatic [9, 10]. Enzymatic antioxidants include superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, and peroxidase. Non-enzymatic antioxidants comprise ascorbic acid, glutathione, carotenoids, flavonoids, and phenolic acids. Consequently, a robust antioxidant defense system protects plant cells from oxidative damage and provides an effective strategy for growth under heat stress. These mechanisms enable crop plants to acclimate while maintaining high adaptive and productive potential.

Pear is one of the most important fruit crops worldwide, ranking second only to another pome fruit - the apple [11]. Pear cultivation requires specific growing conditions. In Russia, this crop is primarily grown in the southern regions, where over 90% of pear plantations are located [12]. In Krasnodar Krai, pears account for up to 7% of all fruit crops [13]. Although most cultivars listed in the state register are of Russian origin (94.8%), the pear assortment is predominantly composed of European cultivars [14, 15]. It is important to note that these cultivars were developed under the mild climatic conditions of European countries, whereas the Prikubanskaya horticultural zone in Krasnodar Krai experiences different air temperatures and moisture levels.

Summer abiotic stressors can lead to a decrease in photosynthetic activity and relative water content in pear leaves and an increase in the accumulation of peroxidation products and ROS content [16, 17]. The authors also noted that in case of the launch of protective mechanisms, responsible for the plant's resistance to the stress factors, there was an increase in the content of carotenoids and osmolytes, as well as in the activity of antioxidant enzymes. In addition, high temperature and strong light had negative effects leading to an inhibition of photosynthetic activity, a decrease in stomatal conductance and degradation of photosystem II proteins that were recorded in pear leaves [18]. One-year-old seedlings of two pear cultivars were characterized by changes in the activity of antioxidant enzymes and its gene expression level under heat stress [19]. In general, different physiological traits are used as markers of heat tolerance in better performing cultivars.

The objective of the present study is to identify differences among pear cultivars under heat stress by analyzing physiological parameters.

### **Material and methods**

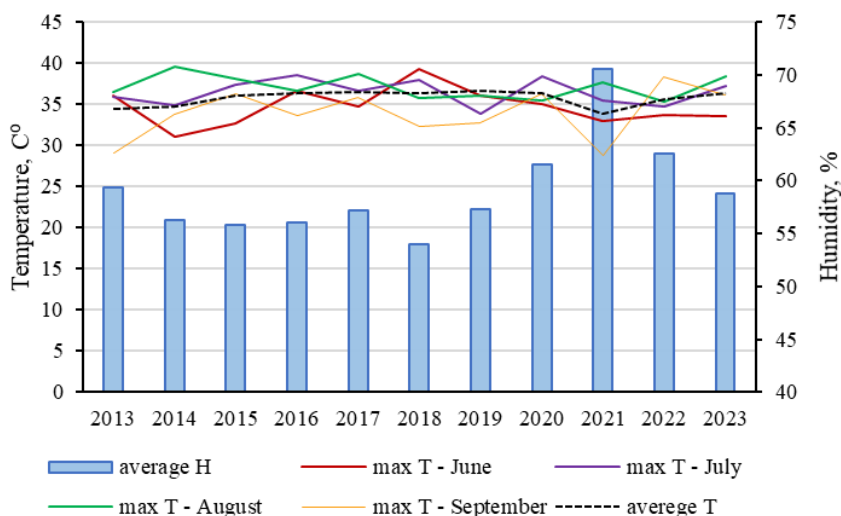
Pear trees were planted under identical cultivation practices and environmental conditions at the experimental orchard of the North Caucasian Federal Scientific Center of Horticulture, Viticulture, and Winemaking (NCFSCHVW), located in Krasnodar Krai (45°16'N, 38°93'E). Four pear cultivars were used in this

study. Leven and Krasnodarskaya Letnyaya are European pear cultivars (*Pyrus communis* L.) developed in Russia. Devo and Kieffer are American cultivars; the former is related to *P. communis*, while the latter is an interspecific hybrid (*P. communis* L. × *P. pyrifolia* (Burm.F.) Nakai). Kieffer was used as a more resistant cultivar to various stress factors [20]. These cultivars are included in the collection of genetic resources of NCFSCHVW.

Pear annual branches with 5-6 undamaged leaves were collected in August 2022 and 2023. In total, approximately 20-30 mature leaves from 3-5 trees of each cultivar were included in the experiment. The experiment was conducted in a climatic chamber, the BPC500D/CVSI-Spector (Fujian Jiupo Biotechnology Co., China). Under short-term stress conditions, pear leaves were exposed to 40°C for 3 hours, while control leaves were kept at room temperature.

It was revealed that the average air humidity during the four months from June to September fluctuated between 54% and 70.5%, while the average air temperature ranged from 33.8 to 36.7°C (Fig. 1). August is the hottest and driest month in Krasnodar Krai. A 10-year period of air temperature observations showed an increase in the number of years with extremely high temperatures, as well as an expansion in the temperature range, varying from 35 to 39°C (Fig. 1). The highest temperatures were recorded in 2014, 2015, 2017, 2021, and 2023. Therefore, high-temperature treatment simulates a short, sudden heat stress in the field. A similar experiment was conducted on annual apple branches and leaves [21].

Leaf samples were rapidly frozen in liquid nitrogen and then stored at -80°C prior to measuring various physiological and biochemical parameters. Chlorophyll and carotenoid contents were analyzed using a spectrophotometric method [22]. Total phenols were quantified with the Folin-Ciocalteu reagent [23, 24].



**Fig. 1.** Weather conditions over the hot period 2013-2023 in the Krasnodar region: average H - average air humidity from June to September, average T - average air temperature from June to September, max T - maximum air temperature for month

Soluble proteins were extracted following the protocol of Z. Wei et al. [25]. For enzyme activity assays, proteins were extracted accordingly. Superoxide dismutase (SOD) activity was measured by inhibiting the photochemical reduction of nitroblue tetrazolium [26]. Peroxidase (POX) activity was determined by the rate of color change in a solution containing benzidine and hydrogen peroxide [27]. Polyphenol oxidase (PPO) activity was assessed after incubation for 1 hour at 37°C in a catechol solution [28]. Protein content was measured using the Bradford method with bovine serum albumin as the standard [29]. Malondialdehyde (MDA) content was estimated spectrophotometrically through its reaction with thiobarbituric acid [30, 31]. The isozyme profile of peroxidase was analyzed by polyacrylamide gradient gel electrophoresis under nondenaturing conditions [26]. The Spectra Multicolor High Range Protein Ladder (Thermo Scientific, USA) was used as a protein marker.

The data were obtained during the experimental study performed on the equipment of the Center for Collective Use of NCFSCHVW.

The results were represented as the mean  $\pm$  standard error from 3-4 independent replicates, analyzed using Statistica 12 software. The data were subjected to one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range test at a significance level of  $p \leq 0.05$ .

## Results and discussion

Although the physiological responses of plants to heat stress was studied on many crops, there are insufficient studies on *Pyrus* species. The mechanisms of heat resistance in Asian pear species and cultivars have been more extensively investigated [19, 32, 33]. In our research, temperature treatment affected the content of total phenols, MDA and the activities of SOD and PPO in pear cultivars (table). Additionally, field conditions across different years also affected the physiological responses of the cultivars. For example, changes were observed in the content of photosynthetic pigments, MDA and the activities of SOD, POX, and PPO. As noted earlier, the maximum temperature in August was recorded in 2023 (See Fig. 1). The interaction among cultivar, treatment, and year significantly influenced the content of total phenols and enzymatic activities, as demonstrated by the three-way ANOVA (Table 1).

Phenolic substances, including non-enzymatic antioxidants, are secondary metabolites that perform numerous functions in plant cells [34]. Due to their polyphenolic structure, they can scavenge oxygen radicals and thereby inhibit cascade oxidative reactions within plant cells. An increase in phenolic content is often associated with enhanced plant tolerance to stress conditions [34]. In the studied pear cultivars, the highest total phenol content was observed under heat stress in 2023, averaging 23.1 mg g<sup>-1</sup> FW (Fig. 2). The greatest values were recorded in the Krasnodarskaya Letnyaya and Devo cultivars, measuring 25.7 mg g<sup>-1</sup> FW, respectively. Although the increase in phenolic content under treatment was more pronounced in 2022, the phenolic content under control conditions was higher for all pear cultivars in 2023.

Table 1

Summary of variance analysis for the main effects of cultivars (C), high temperature stress (T), and year (Y), and their interactions

	DF	Total phenols	Total chlorophylls	Carotenoids	MDA	SOD	POX	PPO
Cultivars	3	*	**	**	**	**	**	**
Treatment	1	**	*	**	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
Year	1	**	<i>ns</i>	<i>ns</i>	**	**	<i>ns</i>	**
C×T	3	*	<i>ns</i>	<i>ns</i>	**	**	<i>ns</i>	**
C×Y	3	<i>ns</i>	**	**	**	**	**	**
T×Y	1	**	<i>ns</i>	<i>ns</i>	<i>ns</i>	**	**	**
C×T×Y	3	**	<i>ns</i>	<i>ns</i>	<i>ns</i>	**	**	**

Note. DF - degree of freedom, \* significant at  $p \leq 0.05$ , \*\* significant at  $p \leq 0.01$ , *ns* - non-significant.

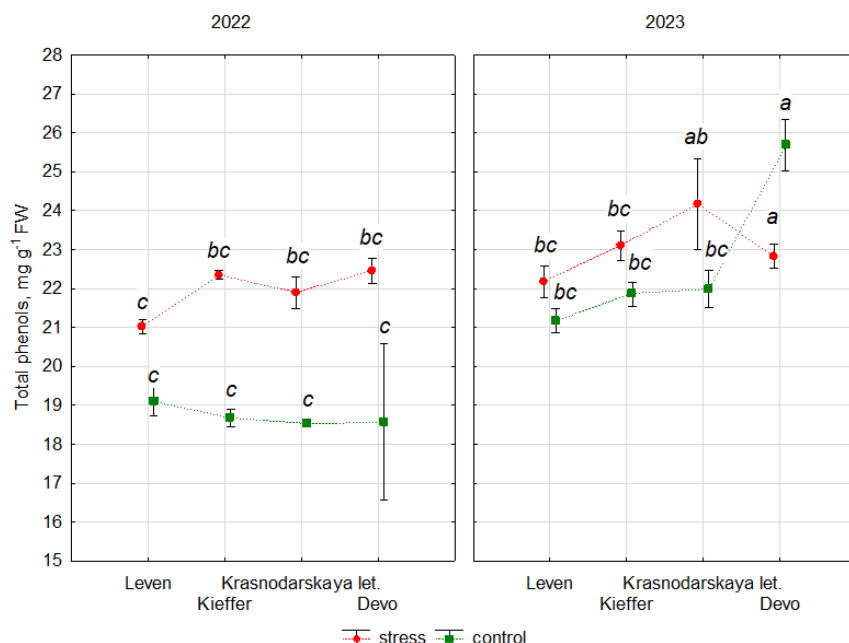


Fig. 2. The content of total phenols in the leaves of pear cultivars under control and heat stress conditions in 2022 and 2023

Abiotic stress often leads to a decrease in the content of photosynthetic pigments [35]. In pear cultivars, short-term heat stress did not have a significant effect on total chlorophylls and carotenoids. Differences were observed only between field conditions in 2022 and 2023 (Table 1). The concentration of total chlorophylls under thermal treatment was comparable to that under non-stress conditions for each pear cultivar (Fig. 3). The Krasnodarskaya Letnyaya cultivar exhibited the highest total chlorophyll content in 2022, measuring 2.0 mg g<sup>-1</sup> FW. The lowest chlorophyll was observed in the Leven cultivar in 2022, at just over 0.9 mg g<sup>-1</sup> FW.

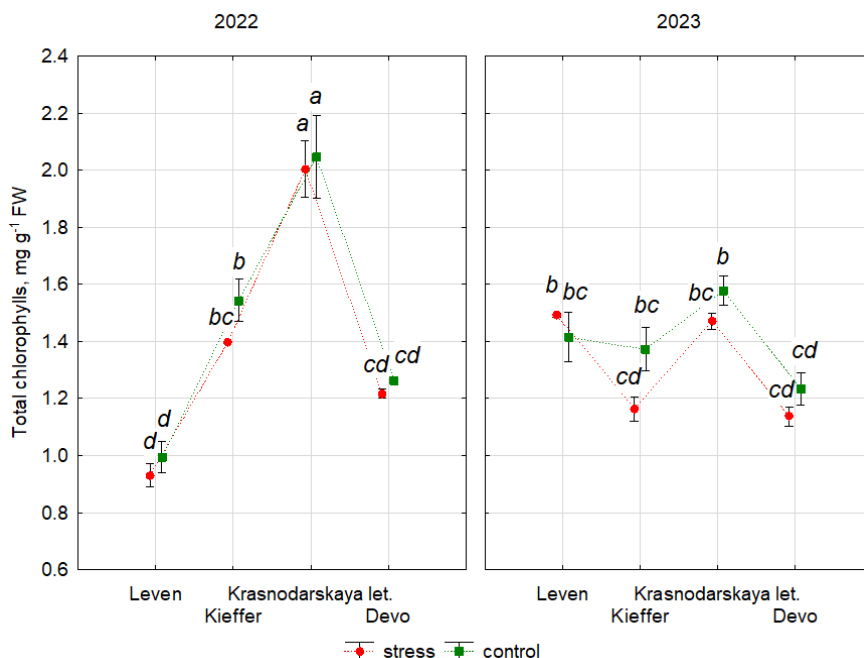


Fig. 3. The content of total chlorophylls in the leaves of pear cultivars under control and heat stress conditions in 2022 and 2023

Carotenoids, along with phenolic compounds, contribute to the antioxidant defense of plants against stress factors [10]. It has been suggested that high temperatures (35–45°C) cause damage to the membrane stability of chloroplast thylakoids [36]. Carotenoids help counteract the increased leakage in the thylakoid membrane induced by heat stress. In the studied pear cultivars, carotenoid content in leaves did not differ significantly between control and stress conditions (Fig. 4). The highest values were observed in Krasnodarskaya Letnyaya, reaching 0.52 mg g<sup>-1</sup> FW in 2022 and 0.47 mg g<sup>-1</sup> FW in 2023.

Malondialdehyde (MDA) is one of the end products of lipid peroxidation [30]. Typically, an increase in MDA levels under stress indicates enhanced secondary oxidative damage in plant cells. In the present study, MDA content did not increase significantly following thermal treatment, with mean values of 26.5 nmol g<sup>-1</sup> FW in 2022 and 21.4 nmol g<sup>-1</sup> FW in 2023 (Fig. 5). The exception was the Leven, which exhibited the highest MDA content in the control group in 2022, amounting to 37.5 nmol g<sup>-1</sup> FW.

Superoxide dismutase (SOD) plays a crucial role as the first line of defense against reactive oxygen species (ROS) by converting superoxide radicals into hydrogen peroxide within the chloroplast stroma, cytosol, peroxisomes, mitochondria, and apoplast [10]. Generally, increased SOD activity correlates with enhanced plant tolerance to stress. The SOD activity under stress conditions was significantly higher than control values, with a 23% increase observed in Leven in 2022 and an 11% increase in Kieffer in 2023 (Fig. 6).



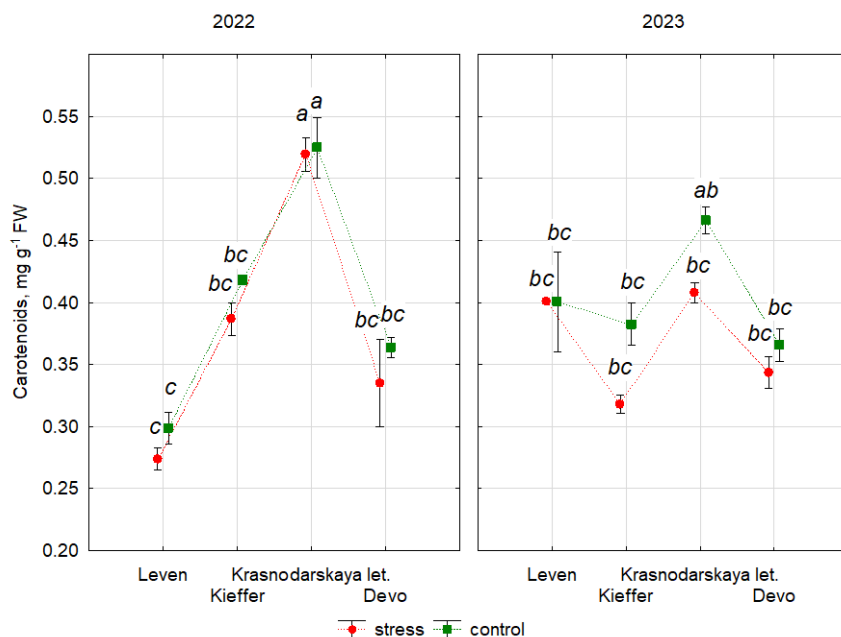


Fig. 4. Carotenoid content in the leaves of pear cultivars under control and heat stress conditions in 2022 and 2023

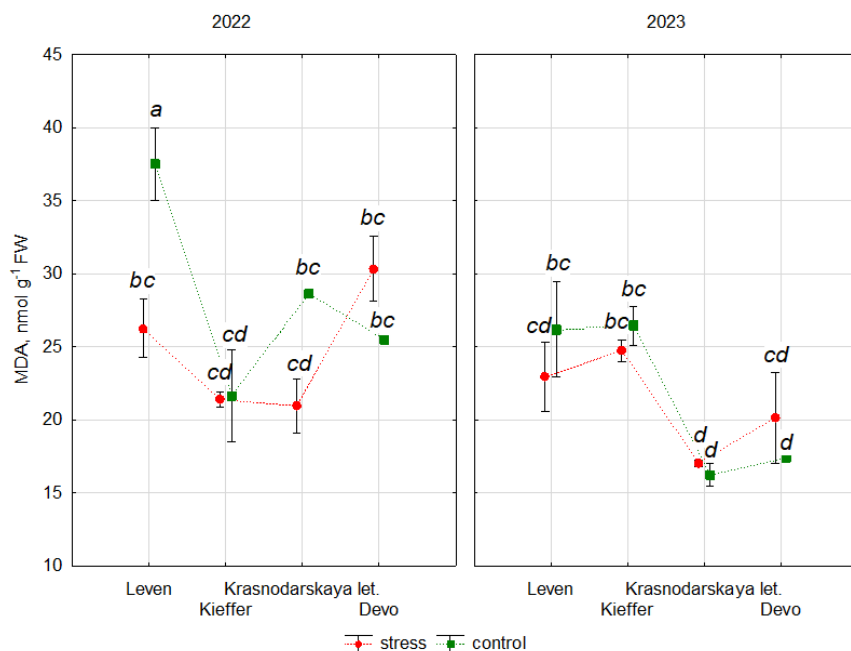


Fig. 5. Malondialdehyde content in the leaves of pear cultivars under control and heat stress conditions in 2022 and 2023

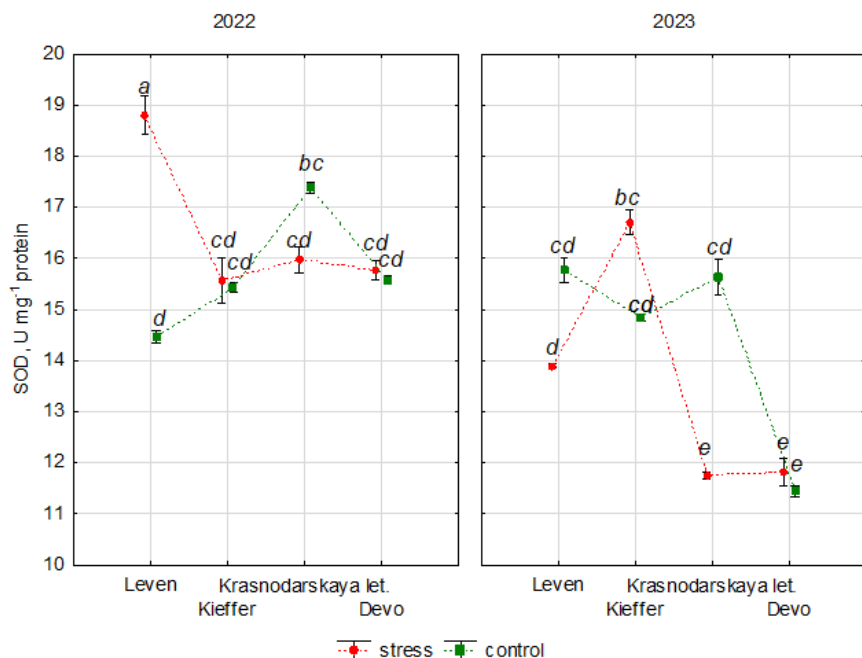


Fig. 6. Superoxide dismutase activity in the leaves of pear cultivars under control and heat stress conditions in 2022 and 2023

The maximum SOD activity in pear was 18.8-unit  $\text{mg}^{-1}$  protein for Leven. Notably, Krasnodarskaya Letnyaya exhibited higher SOD activity under control conditions compared to stress conditions, with values ranging from 15.7 to 17.4-unit  $\text{mg}^{-1}$  protein.

The intracellular level of hydrogen peroxide is regulated by class III peroxidase, which primarily oxidizes phenolic compounds to generate phenolic radicals and converts hydrogen peroxide into water [10]. During high-temperature treatment, a rapid increase in peroxidase activity was observed in the Krasnodarskaya Letnyaya cultivar, rising from 23.0 to 41.8-unit  $\text{mg}^{-1}$  protein  $\text{min}^{-1}$  in 2022 (Fig. 7). For the same cultivar in 2023, under control conditions, peroxidase activity measured 41.0-unit  $\text{mg}^{-1}$  protein  $\text{min}^{-1}$ , but after stress, it decreased to 25.5-unit  $\text{mg}^{-1}$  protein  $\text{min}^{-1}$ . In other cultivars, the increase in POX activity was not significant, ranging from 3.8 to 15.3-unit  $\text{mg}^{-1}$  protein  $\text{min}^{-1}$ .

Polyphenol oxidase (PPO) is an important enzyme that interacts with peroxidase, classifying it as a component of the antioxidant defense system that protects plant cells from damage [37]. PPO oxidizes phenolic compounds to quinones in the thylakoid membranes of chloroplasts. Therefore, this enzyme can directly influence photosynthesis. Short-term stress did not cause a significant increase in PPO activity in the pear leaves of the studied cultivars in 2022 (Fig. 8). Only the values for Leven increased to 3.6-unit  $\text{mg}^{-1}$  FW under thermal treatment. The highest PPO activity in 2022 was detected in Krasnodarskaya Letnyaya (3.9-unit  $\text{mg}^{-1}$  FW).

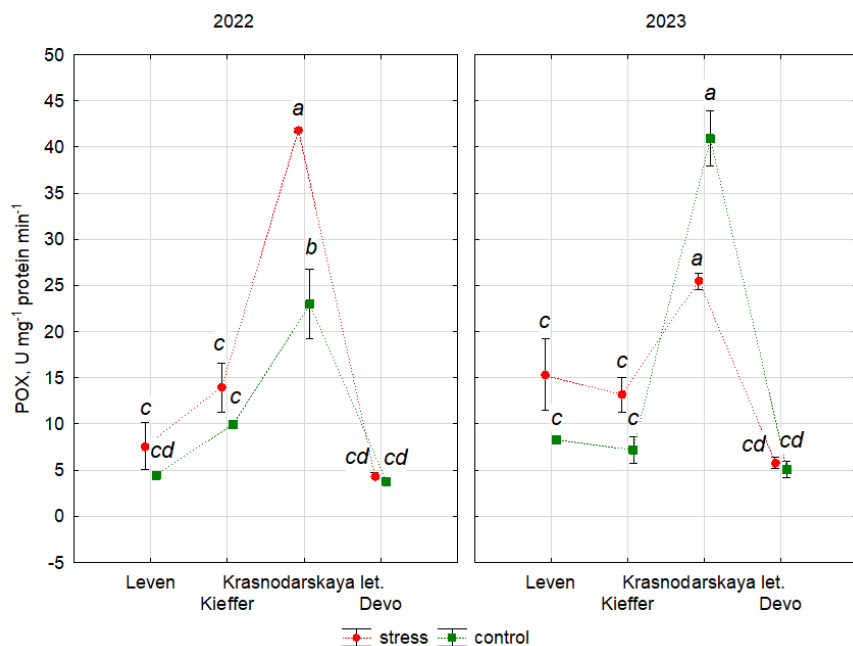


Fig. 7. Peroxidase activity in the leaves of pear cultivars under control and heat stress conditions in 2022 and 2023

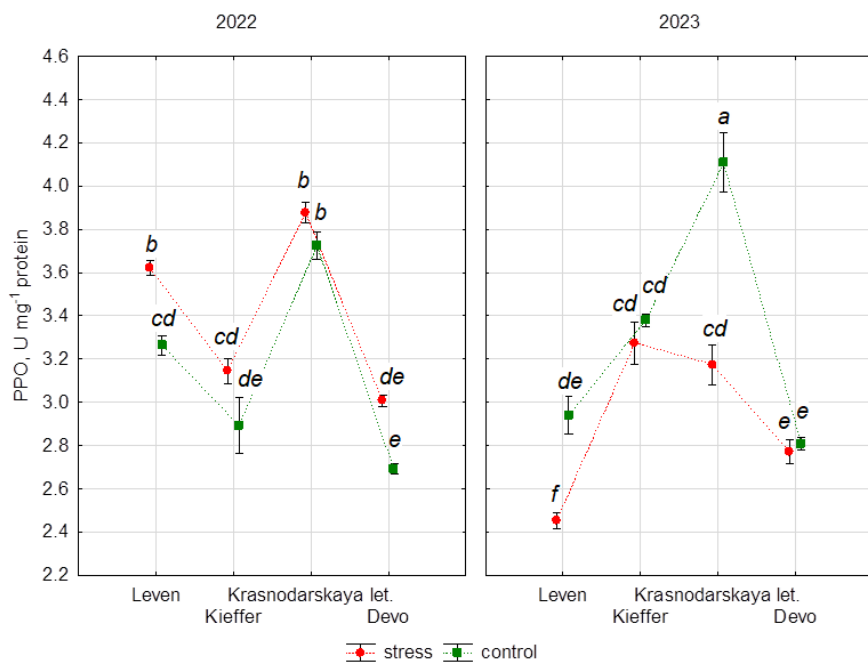
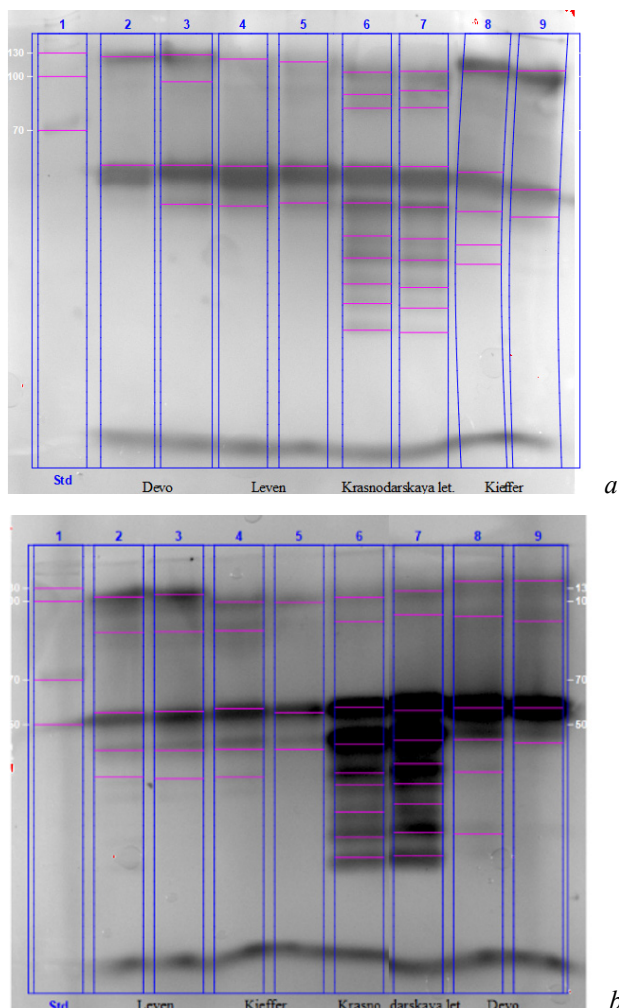


Fig. 8. Polyphenol oxidase activity in the leaves of pear cultivars under control and heat stress conditions in 2022 and 2023

Additionally, Leven had the lowest level of PPO activity after stress in 2023 (2.5-unit  $\text{mg}^{-1}$  FW), and the maximum values were found in the leaves of Krasnodarskaya Letnyaya under control conditions (4.1-unit  $\text{mg}^{-1}$  FW).

The isozyme profiles of peroxidase (POX) varied among pear cultivars (Fig. 9). All cultivars exhibited several bands with differing intensities. Under stress conditions, both the intensity and the number of bands were reduced compared to control conditions. Additionally, isoenzyme profiles differed between 2022 and 2023 for the studied cultivars, except for Krasnodarskaya Letnyaya. Four main POX isoforms were identified in all pear cultivars, with molecular weights of 110, 90, 55, and 45 kDa. The greatest number of isoforms was observed in Krasnodarskaya Letnyaya, with 9-10 bands, which correlated with the highest POX activity in this cultivar (Fig. 7).



**Fig. 9.** Isozyme profiles of peroxidase in the leaves of pear cultivars under control (line 2, 4, 6, 8) and heat stress (line 3, 5, 7, 9) conditions in 2022 (a) and 2023 (b)

Thus, short-term high-temperature stress did not cause significant disturbances in the leaf cells of the four pear cultivars. This is indicated by the absence of a rapid increase in MDA levels. Changes in MDA accumulation not only serve as indicators of oxidative stress development in cells but also play a signaling role [38]. Such effects are possible when external stress factors have only minor impacts on the plant. At the same time, differences in cultivar responses to stress were observed. Notably, significant changes were detected in the content of total phenols and the activity of antioxidant enzymes. Higher maximum air temperatures under field conditions in 2023 apparently contributed to elevated values of these parameters compared to those recorded in 2022. Therefore, the moderate stress in 2022 led to increases in total phenols, SOD activity in Leven, and POX activity in Krasnodarskaya Letnyaya. However, in 2023, under thermal stress, pear cultivars exhibited lower values compared to the control, especially Leven and Krasnodarskaya Letnyaya. The accumulation of phenolic compounds and the increase in antioxidant enzyme activity are characteristic features of pear, as noted by researchers studying various types of stress [19, 39, 40].

An interesting feature was discovered in Krasnodarskaya Letnyaya: a larger number of fast-migrating, high-intensity POX isoenzymes. This distinctive profile correlated with the elevated activity of the enzyme. A similar pattern was observed when comparing different apple cultivars [41].

## Conclusion

Short-term high-temperature stress in pear cultivars caused significant changes in enzymatic activities, including SOD, POD, and PPO, and led to an increase in the total phenol content in leaves. Intensive oxidative stress in plant cells was not detected, as indicated by low levels of membrane lipid peroxidation (MDA). The pear cultivar Krasnodarskaya Letnyaya differed from the other studied cultivars by exhibiting higher POX and PPO activities, as well as greater polymorphism in the POX isozyme profile. We suggest that this cultivar may be the most resistant to heat stress during prolonged exposure.

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