Studii şi Cercetări Ştiinţifice. Biologie 2026, 35 (1), 9-15
Scientific Study & Research. Biology
Print ISSN 1224-919X Online ISSN 2457-5178

VARIATION IN THE ACTIVITY OF PEROXIDASE AND CATALASE ENZYMES IN PLUM TREES LEAVES, DEPENDING ON GROWING CONDITIONS AND BIOLOGICAL PARTICULARITIES OF THE VARIETY

Alina GÎSCĂ*, Valentina SVETLICENCO, Alexandru NICUȚĂ

Moldova State University, Institute of Genetics, Physiology and Plant Protection, Chisinau, Republic of Moldova, alina.gisca@sti.usm.md

KEYWORDS

Plum trees
Leaves
Peroxidase
Catalase
Humidity
Water stress

ABSTRACT

Our study examines the enzymatic antioxidant defense of plum plants, through the assessment of the activity of the enzymes peroxidase and catalase in the leaves of different plum tree varieties cultivated under irrigated and non-irrigated conditions during the growing season. The importance of these enzymes lies in their participation, with differentiated roles, in the adaptation and resistance of trees to unfavorable environmental conditions, as well as in the processes of growth, development and fruiting.
The results of the investigations revealed an intensification of peroxidase activity in the leaves of trees grown under non-irrigated conditions. Compared to irrigated trees, the values were higher by 24.20 % in the Stanley variety, by 32 % in the Udlinionnaia, by 23.53 % in the President and by 3.17 % in the Super Prezident. During the vegetation period, the highest activity of the catalase enzyme in the leaves was observed in trees grown under non-irrigated conditions. Compared to trees grown under optimal humidity conditions (with irrigation), the enzyme activity was higher by 16.59 % in the Stanley variety, by 19.74 % in the Udlinionnaia variety, by 10.58% in the President variety and by 10.10 % in the Super Prezident variety.
The research contributes to the completion of knowledge about the mechanisms of plant tolerance to stress. Understanding these mechanisms could serve as a basis for improving antioxidant defense systems, by further developing procedures for targeted management of the functional state and optimization of the production process in fruit trees in the context of climate change, such as the application of various biologically active substances. The dynamics of the activity of the studied enzymes varied during vegetation, being influenced by the developmental stages of the trees, environmental factors, growth conditions and genotype.

INTRODUCTION

Plums from the Republic of Moldova have recently enjoyed a growing demand, both on the domestic and foreign markets. In the Republic of Moldova, plum is one of the main fruit crops, ranking second after the apple and first among stone fruit species (Popa et. al., 2014). The largest areas of plum orchards in the Republic of Moldova are occupied by the Stanley (foreign selection) and Udlinionnaia (autochthonous) varieties.
The severity of climate change has a significant effect on plants ecosystems, with an increasing susceptibility of plants to multiple abiotic stresses, which influences their survival and productivity (Kumar et al., 2018; Yang et al., 2024; Yuan et al., 2024). Abiotic stress includes temperature stress (heat and cold), drought, nutrient deficiency, soil salinity and heavy metal toxicity (Nawaz et al., 2023; Liaqat et al., 2024; Rane et al., 2024). Drought, being the most important environmental stress, seriously affects plant growth and development, limiting their productivity more than any other environmental factor (Shao et al., 2009).

* Corresponding author: Gîscă A.
E-mail address: alina.gisca@sti.usm.md
https://doi.org/10.29081/SCSB.2026.35.1.02

Plant adaptation to water deficit is the result of different processes that lead to changes in plant growth and physiological-biochemical processes, such as changes in antioxidant protection (Duan et al., 2007). Abiotic stress factors limit plant development by triggering imbalances in physiological and biochemical processes. A critical aspect is the occurrence of oxidative stress, a process caused by the excessive accumulation of reactive oxygen species (ROS) (Hernandez et al., 2004; Diaz-Vivancos et al., 2013). ROS serve a dual function in plant systems—while potentially harmful during stress, they also act as crucial signaling molecules that transmit stress signals to the nucleus via redox-mediated mitogenactivated protein kinase (MAPK) cascades (Mitler, 2017; Choudhury et al., 2017; Su et. al., 2018; Sachdev et. al., 2021). These ROS molecules are involved in plant acclimation to environmental stress and function as key signal transducers that interact with several pathways (Choudhury et al., 2017). Hydrogen peroxide (H_2O_2), a type of ROS, plays a central role in stress response across crop species, including fruits (Tanou et al., 2012; Sun et. al., 2016; Guler et. al., 2016; Hasanuzzaman et. al., 2017; Zhang et. al., 2024; Basal et. al., 2024). To compensate for the production of ROS, plants activate key antioxidant defense enzymes (Zhang et. al., 2013; Wang et. al., 2021). These enzymes, such as peroxidase (POD) and catalase (CAT), are the main active systems for preventing oxidative damage in plants caused by excessive H_2O_2 production (Niu et. al., 2023). They convert H_2O_2 into H_2O and O_2, preventing the harmful effects of the molecule (Ding et al., 2022).

A distinctive feature of the plum is its resistance to adverse environmental factors. However, in the context of climate change in recent years, under stress conditions (drought, heat, etc.) the adaptability of the crop in general, and of the varieties in particular, decreases. Under these conditions, to ensure constant and qualitative harvests, it is necessary to implement techniques for regulating plant metabolism, thus stimulating their growth rate.

Thus, the purpose of this study was to determine the effect of water stress on the activity of the antioxidant enzymes catalase and peroxidase in the leaves of plum varieties with different levels of tolerance to soil moisture deficiency.

MATERIALS AND METHODS

Scientific research was carried out during the growing season, in the lysimeters of the Institute of Genetics, Physiology and Plant Protection of the State University of Moldova on trees of 2 plum varieties of foreign selection – Stanley and President and 2 plum varieties of local origin – Udlinionnaia and Super Prezident, according to the scheme:

1. Experimental variant: plants grown under non-irrigated conditions;
2. Control variant: plants grown under irrigated conditions (soil moisture during the growing season dynamics was maintained at approximately 70% of the soil field capacity (SFC)).

Soil moisture in the dynamics of the growing season was monitored using field devices (AQUAMETER ECO T tensiometer). To maintain the soil moisture level within the planned limits, a film was installed around the tree trunk. The experiments were performed in three repetitions. In each repetition 7 plants were used.

Catalase enzyme activity in plum leaves was determined spectrophotometrically according to the method of Chance and Maehly (1955), at a wavelength of 240 nm of the H2O2 decomposition rate. Peroxidase activity was determined according to the method proposed by Ermakov et. al. (1987).

The data obtained are presented as the mean of 3 repetitions ± standard deviation. To establish the veracity and authenticity of the data, the results obtained were subjected to statistical analysis by bifactorial dispersion analysis based on the ANOVA test, using the STATGRAPHICS Plus2 software package. The processing of experimental data was carried out using a table editor Microsoft Excel 2019.

RESULTS AND DISCUSSIONS

The weather conditions during the vegetation period were characterized by high temperatures, low humidity and a deficit of precipitation. Between May and August, average daily temperatures of 21.7°C and relative air humidity of 49.9 % were recorded, with a major deficit of precipitation (4.31 mm). The maximum thermal values reached an average of 24.2°C in June, while the lowest precipitation (4 mm) and relative air humidity of 39.9 % were recorded in July.

The research conducted indicates that the analyzed plum varieties react differently to soil water deficit. The results obtained demonstrate that the activity of redox enzymes reflects the metabolic state of the leaves, both under irrigation and drought conditions.

The peroxidase enzyme plays an important role in the activity of the antioxidant system, reacting to the most diverse disturbances in the homeostasis of the plant organism (Foyer and Noctor, 2005a, 2005b; Zoz et. al., 2013). It is important to highlight the role of this enzyme as an antioxidant in plant adaptation reactions to unfavorable environmental factors.

The researches conducted showed that at the beginning of the vegetation period of the four plum varieties the activity of peroxidase in the leaves was characterized by low values (Figure 1). In the first decade of June, in the phenophase of intensive growth of shoots, when the activity of metabolic processes was more intense, the activity of this enzyme increased. Thus, in the Stanley variety, the enzymatic activity in the leaves, in the variant with plants grown under irrigation conditions, recorded values of 0.057 opt. dens. un. s•g-1, respectively in the variant without irrigation - 0.067 opt. dens. un. s•g^{-1}. At the same time, this indicator was within similar limits for the other plum varieties, as follows: Udlinionnaia variety - 0.057-0.043, President variety - 0.055-0.050 and Super Prezident variety with 0.059-0.053 opt. dens. un. s•g^{-1} (Figure 1). In the Stanley variety, peroxidase activity in leaves increased 2.7 times in the control variant, respectively 2.5 times in the variant with plants grown under non-irrigated conditions, compared to the previous term. This trend was also maintained in the Udlinionnaia variety, with approximately a 1.5-fold increase in both variants. This pattern was not valid in the case of the President and Super Prezident varieties, the enzyme activity remaining practically at the same level. A more pronounced increase in the peroxidase activity in the leaves was manifested in the subsequent periods. Towards the end of the vegetation period, a significant increase in the peroxidase activity was recorded in the President variety, by 1.5 times in the control variant, respectively by 2.4 times in the variant with plants grown under non-irrigated conditions. This growth was conditioned by the biological particularities of the variety, as well as the drought conditions recorded in July - August.

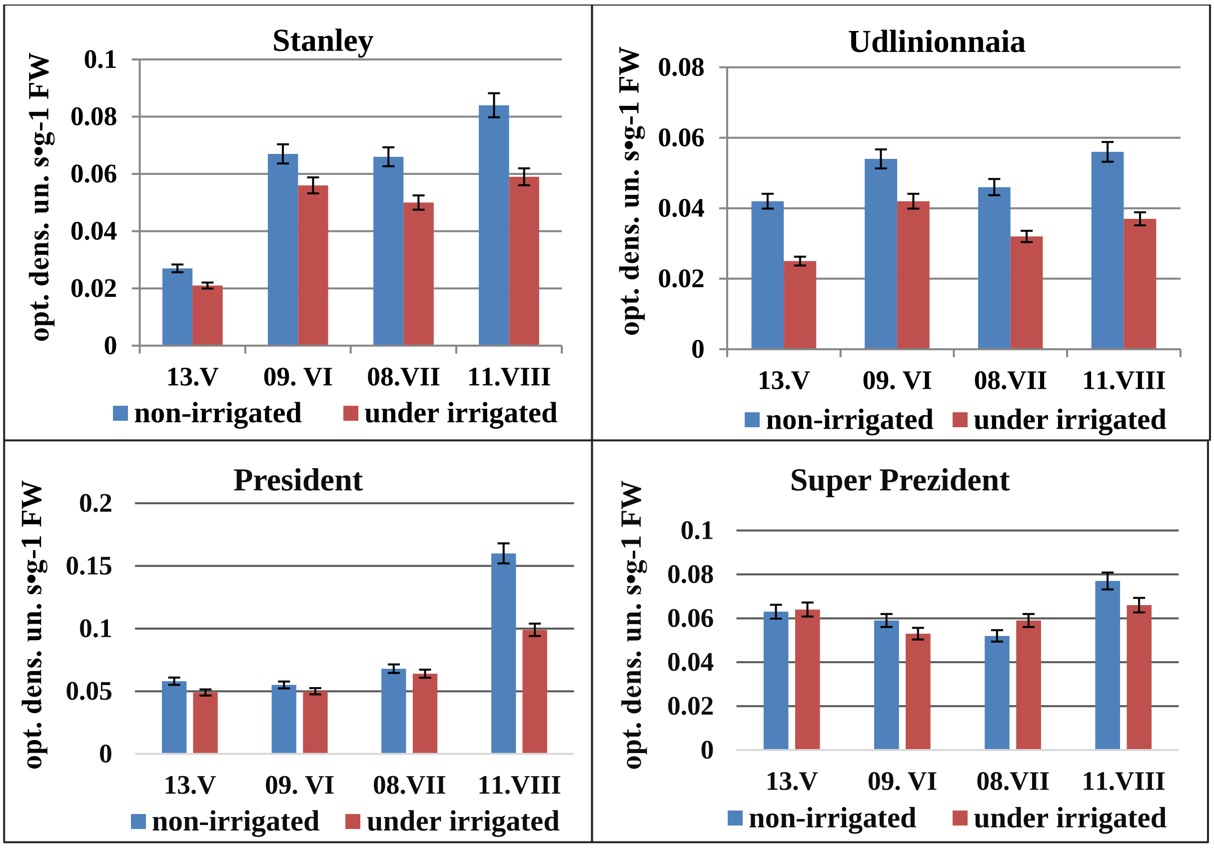

Figure 1. Peroxidase activity in plum leaves, depending on growth conditions and genotype

Thus, the investigations carried out recorded a higher peroxidase activity in the leaves of trees from the non-irrigated variant, in the Stanley variety by 24.20%, Udlinionnaia by 32%, President by 23.53%, and Super Prezident by 3.17%, respectively, in relation to those on trees grown under irrigated conditions. These results indicate an intensification of physiological-biochemical processes and a greater accumulation of oxidative substances under conditions of insufficient humidity. Under optimal growth conditions (with irrigation, 70% SFC) metabolic processes proceed normally, without excessive accumulation of oxidizing compounds.

The results of statistical data processing (ANOVA test) show that all sources of variation analyzed have a significant impact for $p \leq 0.001$ (Table 1), highlighting that the priority role in modifying the peroxidase activity in leaves is played by humidity (dependent on weather conditions during the vegetation period) with a percentage contribution of 29.40%, followed by the variety - humidity interaction with a share of 3.29%. It should be noted that at a great distance in the direction of decreasing the contribution (only 0.49%, for $p \leq 0.01$) the impact of the variety is highlighted.

Table 1. Analysis of variance of peroxidase (ANOVA test)

Source of variation	Degrees of freedom	Sum of squares	F-ratio	PI,%
Variety (A)	1	0,000408333	6,62**	0.49
Humidity (B)	1	0,0243675	39,53***	29.40
Interaction AB	1	0,00273008	44,29***	3.29
Total	47	0,00828767		

*, ** ,*** - significant differences for $p \leq 0{,}05$; 0,01; 0,001

Another important enzyme is catalase. Due to its kinetic properties, it maintains H2O2 levels below certain limits and, consequently, is considered one of the main antioxidant enzymes with a key role in plant growth, development and stress conditions (Mhamdi et al., 2012; Palma et. al., 2013; Anjum et. al., 2016). In plants, other enzymes, mainly displaying peroxidase activity, help catalase to modulate the hydrogen peroxide concentration within cells (Palma et. al., 2013; Sofo et. al., 2015). In most cases, catalase, due to its high KM, scavenges the major part of H2O2 which is driven to peroxisomes, thus protecting cells from potential damage (Sofo et. al., 2015; Anjum et. al., 2016; Tuzet et. al., 2019). The catalase enzymatic system has been also associated to fruit ripening and postharvest events (Huan et. al., 2016; Palma et. al., 2018).

The analyses performed revealed that during the vegetation period, the leaves of the plum varieties studied, grown under conditions of insufficient moisture, recorded the highest activity of the catalase enzyme, compared to those grown under optimal growth conditions. In the phenophase of intensive shoot growth, higher values of catalase activity were recorded, compared to the peroxidase enzyme. According to the data presented in Figure 2, the Stanley variety recorded the highest catalase activity (0.515 µM H_2O_2/min^{-1} • g^{-1}) under water stress conditions, considerably exceeding the control (0.40 µM H_2O_2/min^{-1} • g^{-1}). A lower enzymatic activity was recorded in the trees of the President variety – 0.131 µM H_2O_2/min^{-1} • g^{-1} grown under the same conditions (non-irrigation conditions). The results obtained confirm the increased intensity of the physiological-biochemical processes occurring in this phase of tree development. Subsequently, in the phenophase of slowing down and cessation of shoot growth, a significantly lower enzyme activity was recorded, in the Stanley variety by 26.75-29.5%, respectively by 47.60-49.02% in the case of the Udlinionnaia variety, depending on the applied variant.

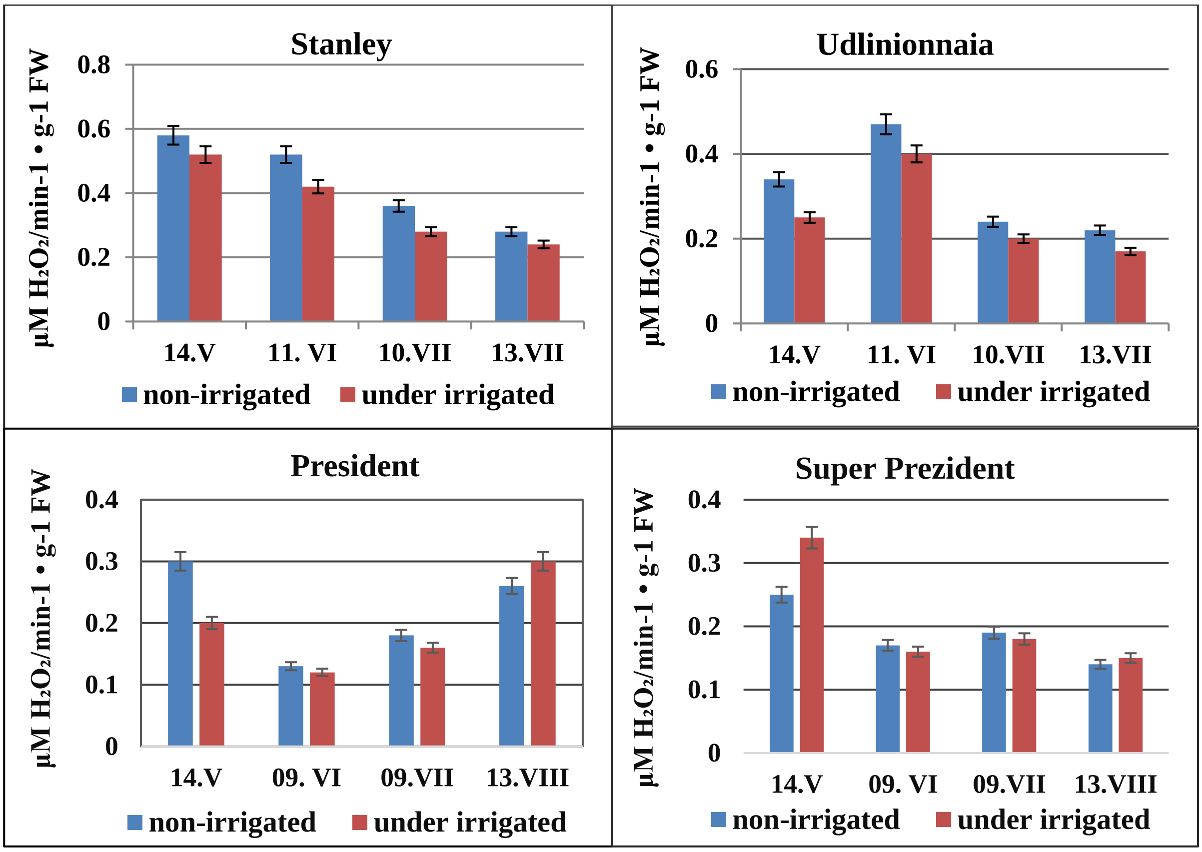

Figure 2. Catalase activity in plum leaves, depending on growth conditions and genotype

Based on the statistical processing of the data (ANOVA test), it was established that catalase activity in plum leaves is primarily determined by variety, the percentage contribution being 40.52%, followed by humidity and the variety-humidity interaction with shares of 25.26% and 1.3% respectively. We note that all sources of variation analyzed have a significant impact for $p \leq 0.001$ (Table 2).

Table 2. Analysis of variance of catalase (ANOVA test)

Source of variation	Degrees of Freedom	Sum of Squares	F-ratio	PI,%
Variety (A)	1	0,0752083	54,16***	40.52
Humidity (B)	1	0,046875	33,76***	25.26
Interaction AB	1	0,00240833	1,73*	1.3
Total	47	0,185592		

*, ** ,*** - significant differences for $p \leq 0,05; 0,01; 0,001$.

On average, during the vegetation period, the highest activity of the catalase enzyme in leaves was recorded in the variant with trees grown under non-irrigated conditions, by 16.59% in the Stanley variety, Udlinionnaia - 19.74%, President-10.58%, and by 10.10% in the Super Prezident variety, respectively, in relation to trees grown under optimal growing conditions (with irrigation). So, both peroxidase and catalase intensify their activity under growing conditions with insufficient moisture, having an important role in the adaptation of trees to variations in environmental factors.

Thus, we can conclude that the dynamics of the activity of the enzymes peroxidase and catalase in leaves during the vegetation period reflects the complexity of the processes of growth and development of plum trees, being significantly influenced by the biological peculiarities of the variety. The intensification of the activity of these enzymes emphasizes their essential role in metabolic processes, as well as their direct involvement in the mechanisms of adaptation and resistance of trees to unfavorable environmental factors. The results obtained indicate a gradual increase in the activity of the peroxidase enzyme, in parallel with the progressive decrease in the activity of catalase. Thus, the data obtained confirm the differentiated and essential role of these enzymes in the metabolism of plum plants. Based on the above, monitoring these biochemical indices is important. Through their specific functions in metabolism and in the mechanisms of adaptation to environmental conditions, these enzymes play a crucial role in ensuring the vigor of trees, optimizing productivity and maintaining fruit quality.

CONCLUSION

The plum varieties studied showed a response to insufficient soil moisture during the growing season, triggering a significant increase in the activity of key antioxidant enzymes (catalase and peroxidase), which represents a plant defense reaction to neutralize oxidative stress caused by drought.

Enzymatic activity during the vegetation period did not present constant values, fluctuating depending on the intensity of metabolic processes in different phenophases of tree growth and development, growth conditions and biological particularities of the varieties.

The research conducted reveals that trees of all 4 plum varieties, grown under non-irrigated conditions, have the highest activity of catalase and peroxidase enzymes in the leaves, compared to those grown under irrigated conditions (soil humidity – 70%).

Under conditions of water stress (drought), throughout the growing season, increased peroxidase activity was recorded, serving as a marker of resistance to fluctuating environmental factors.

The research revealed a higher activity of the peroxidase enzyme in the leaves of the President variety. In the case of catalase enzyme activity, this law is valid for the Stanley variety.

The results obtained can serve as a benchmark for the subsequent development of procedures for modulating the adaptation, growth and development response of fruit species, in the context of climate change.

ACKNOWLEDGMENTS

This research was supported by the subprogram 011101 „Genetic and biotechnological approaches to agroecosystem management under climate change”, funded by the Ministry of Education and Research of the Republic of Moldova and the doctoral project „Cyto-physiological adaptation of plum trees to environmental factors and the impact on productivity”.

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