

<https://doi.org/10.15407/dopovidi2020.02.087>

UDC 581.143.6

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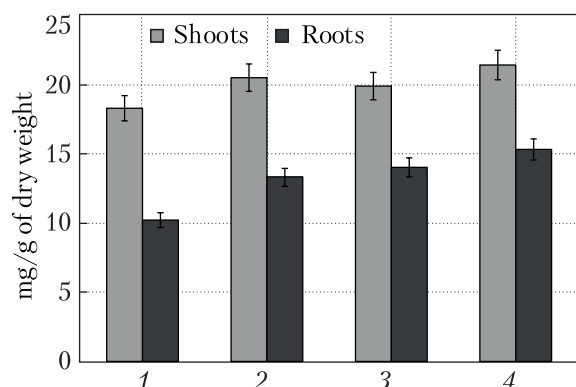
## **Stress temperature and soil drought effects on amino acid composition in winter wheat**

*Presented by the Corresponding Member of the NAS of Ukraine P.M. Tsarenko*

*The effect of short-term high (40 °C, 2 h) and positive low (4 °C, 2 h) temperatures, as well as simulated moderate soil drought, on the total free amino acid and proline contents is studied under controlled conditions on organs of 14-day-old *Triticum aestivum* L. plants, the cultivar Podolyanka. The contents of free amino acids and proline are found to be higher in shoots of control plants and are, respectively, 18.29 mg/g and 0.85 mg/g of dry weight. Under simulated moderate soil drought conditions, the free amino acids and proline contents increase in shoots by 17 % and 71 %, respectively, while in the roots by 50 % and 61 %. During a short-term hyperthermia, the increases in free amino acid and proline content in shoots by 12 and 47 %, respectively, and in roots by 30 % and 23 %, respectively, are also observed. The response to a short-term hyperthermia was less pronounced. The involvement of free amino acids and proline in developing the resistance to abiotic stressors in winter wheat is discussed.*

**Keywords:** *Triticum aestivum*, temperature stress, soil drought, proline, free amino acids.

The study of the climate change impact on the cultivated plants productivity is one of the leading trends in modern biological science. One of the main reasons for the heightened interest in this problem is a marked change in the intensity and frequency of many climatic phenomena in recent years, significant variations in daily temperatures, rainfall, etc. [1]. Temperature affects almost all aspects of plant growth and metabolism. The response to temperature fluctuations depends on the duration and intensity of the stressor action and the phase of plant development. To overcome the stress effects, plants use certain adaptive strategies, which include morphological, physiological, and biochemical responses and determine the growth success and productivity [2]. Another adverse factor, whose effect is enhanced, is the lack of moisture. On the territory of Ukraine in the last quarter of a century, there is a steady tendency of the annual rainfall decrease [3]. Disturbance of the plant water regime under the drought conditions leads to the development of oxidative stress, in protection against which the antioxidant system is involved. To prevent dehydration, plants engage structural and functional rearrangements, the state of the respiratory system is regulated, and stress phytohormones and osmolytics that act as stabilizers for macromolecules and cellular structures are accumulated [4]. Under the action of stres-



**Fig. 1.** The effect of abiotic stress on the content of free amino acids in the shoots and roots of *Triticum aestivum* L. cv. Podolyanka. 1 – control, 2 – hypothermia (+4 °C, 2 h), 3 – hyperthermia (+40 °C, 2 h), 4 – moderate soil drought

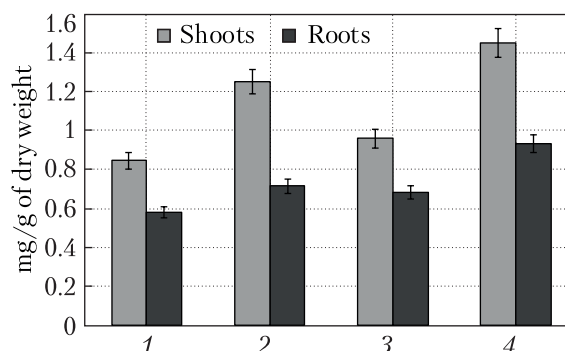
sors, plants accumulate low molecular, highly soluble organic compounds, among which the amino acids, including proline, predominate. Proline accumulation was found to be associated with the development of stress resistance. It prevents the protein aggregation and performs signaling and antioxidant functions [5].

Due to its nutritional and forage properties, wheat has a leading position among cereals in Ukraine and in the world. It is the subject of selection studies aimed at selecting and creating new stress-resistant genotypes. Therefore, the aim of our work was to study the effect of temperature stress and moderate soil drought on the total amino acid and proline content in winter wheat plants and to clarify the involvement of these compounds in the formation of responses.

**Materials and methods.** The seedlings of the high-yielding, frost- and drought-resistant variety *Triticum aestivum* L. Podolyanka from the collection of the Institute of Plant Physiology and Genetics of the NAS of Ukraine were studied. Dry calibrated grains were sterilized in 80% ethyl alcohol solution, washed with distilled water and soaked for 3 h. The seeds were then germinated in a thermostat in cuvettes on filter paper moistened with distilled water at +24 °C for 21 h. Spired seeds were planted in 2-liter containers. Washed, deaned, roasted and dried river sand was used as a substrate. The plants were grown under controlled conditions at 20/17 °C (day/night), light intensity 690  $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ , photoperiod 16/8 h (day/night), relative air humidity  $65 \pm 5\%$ . The substrate humidity was maintained at 60% of the total moisture capacity. Watering was carried out daily with a Knop solution at a rate of 50 ml per container. For the simulation of hyper- and hypothermia, 14-day-old plants were exposed to short-term (2 h) temperatures of +40 and +4 °C under the specified humidity and light regime. Soil drought was achieved by stopping irrigation of 14-day-old plants for 4 consecutive days until the substrate moisture content was halved and leaf fading. Samples previously dried at 100 °C to a completely dry mass were used to isolate the amino acids. The tissue sample was incubated in 3% sulfosalicylic acid and centrifuged at 4000 g, 30 min. The proline content and the total one of amino acids in the obtained supernatant were determined using ion exchange liquid column chromatography on an automatic T339 amino acid analyzer (Czech Republic). The registration of amino acids in the eluate was performed according to the method of detection with ninhydrin. The significance of the difference was estimated according to Student's criterion using a 5% significance level ( $P \leq 0.05$  at  $n = 16$ ). The diagrams show the arithmetic mean and their standard errors.

**Results and discussion.** Our study has demonstrated that the content of free amino acids in the shoots was higher than in the roots of the control plants of winter wheat Podolyanka

**Fig. 2.** Effect of abiotic stress on the content of proline in the shoots and roots of *Triticum aestivum* L. cv Podolyanka. 1 – control, 2 – hypothermia (+4 °C, 2 h), 3 – hyperthermia (+40 °C, 2 h), 4 – moderate soil drought



and amounted to 18.29 and 10.2 mg/g of dry weight, respectively. In the simulated moderate soil drought, the content of free amino acids in the shoots increased by 17 %, while in the roots by 50 % and amounted to 21.38 and 15.3 mg/g of dry weight, respectively. In the case of short-term hypothermia, there was observed an increase in the content of free amino acids in the shoots by 12 %, in the roots by 30 %, which amounted to 20.5 and 13.3 mg/g of dry weight, respectively. The response to the high temperature was less pronounced. The content of free amino acids in the shoots increased by 8 %, while in the roots by 37 % (Fig. 1).

Thus, under stressful conditions, the accumulation of free amino acids was more active in the root system of winter wheat plants. Changes observed following a moderate soil drought and short-term cold stress were more intense than after hyperthermia that corresponds to the frost and drought characteristics of the genotype under study. Increase in the content of free amino acids in winter wheat plants under stressful conditions most probably indicates that these compounds are involved in the formation of the response to changes in temperature and drought. Free amino acids have multivarious functional roles in the plant stress tolerance by acting as compatible osmolytes, being involved in the pH regulation and in the detoxication of reactive oxygen species (ROS), and acting as a nitrogen and carbon reserve, mainly for the synthesis of enzymes and precursors of various secondary metabolites such as flavonoids and lignins, as well as being an available stock of amino acids, which are useful during recovery from stress. Osmoprotective effects of free amino acids at low temperatures in *Brachypodium distachyon* (L.) P. Beauv. plants have been reported in other studies [6]. Arginine, histidine, proline, cysteine, tryptophan, lysine, methionine, and threonine have also been shown to exhibit the antioxidant activity [4].

We established that, under the simulated moderate soil drought conditions, the proline content in the shoots of Podolyanka winter wheat plants increased by 71 %, and in the roots by 61 %, and amounted to 1.45 and 0.935 mg/g of dry weight, respectively. Proline content also increased with short-term hypothermia. Thus, in the shoots, the amount of proline increased by 47 % and in the roots by 23 %, which corresponded to 1.25 and 0.715 mg/g of dry weight. The response to a high temperature was less pronounced. The proline content in the shoots increased by 13 %, while in the roots by 18 % (Fig. 2).

Proline is one of the polyfunctional plant protective compound. Despite its role as an osmoregulator and osmoprotectant, proline also plays a significant role as a signaling molecule and acts as a part of the signaling network in plants during the development, especially under stress conditions. Due to its unique structure, it inactivates hydrogen peroxide and other reactive oxygen species (ROS), causing them to “quench” or break the cascade of free radical reactions [7].

The accumulation of endogenous proline in leaves of transgenic “*Swingle*” *citrumelo* plants was found to induce the activity of genes responsible for the synthesis of antioxidant enzymes [8]. Proline, together with aspartic and glutamic amino acids, acts as a trigger for the osmoregulation, enabling the plant to retain active growth of the root system under conditions of a gradual decrease in soil moisture [9]. At a low positive temperature, *Avena nuda* L. were reported to actively accumulate proline [10], and a strawberry variety, which accumulated more proline and retained the activity of antioxidant enzymes turned out to be more resistant to hyperthermia [11]. Sunflower regenerating plants with double-stranded RNA suppressor of proline dehydrogenase were characterized by a high proline content, high resistance to osmotic stress, and preservation of the pool of photosynthetic pigments under conditions of soil drought [12]. Priming with exogenous proline accelerated the germination of *Vigna radiate* (L.) R. Wilczek. seeds at a low temperature, and, in the seedlings, the content of malondialdehyde decreased [13]. Under the exogenous proline impact, the heat resistance of *Cicer arietinum* L. raised due to the protective effect of amino acids on carbon and antioxidant metabolism [14]. In our previous studies, the simulated moderate soil drought and short-term hyperthermia were established to have a negative effect on the growth and morphological characteristics of the shoots of Podolyanka winter wheat plants, whereas the root system was sufficiently resistant to abiotic stressors. Thus, after the simulated moderate soil drought, the length of the shoots decreased by 19 %, whereas the length of the roots decreased by 11 %. Changes in growth rates and the pattern of dry weight accumulation revealed a greater endurance of the root system [15]. Under stress conditions, proline accumulated more actively in the root system of winter wheat plants. Variations in the accumulation of proline, as well as free amino acids, observed after a moderate soil drought and short-term cold stress were more significant than those occurring after hyperthermia that correlates with changes in morphometric characteristics and corresponds to the signs of frost and drought resistance of the studied genotype. The results indicate that proline is involved in the formation of a response to changes in temperature and drought. The recent investigations on the roles of amino acids in the metabolism, physiology, and different developmental processes of higher plants showed their potential for the induction of stress tolerance. Though a lot of work has been done in order to understand this tolerance mechanism, there are still some gaps in the resistance and tolerance mechanisms. Furthermore, studies have been carried out mainly on some specific amino acids. They revealed that amino acid metabolism could be a vital component for the plant abiotic stress tolerance.

*The paper is based on results obtained in the work under project No. III-82-17.463 “Plant hormonal regulation of the growth and development of cereal plants under influence of negative climatic factors” (2019–2023) funded by the National Academy of Sciences of Ukraine.*

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Received 13.11.2019

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#### ВПЛИВ ТЕМПЕРАТУРНОГО СТРЕСУ ТА ҐРУНТОВОЇ ПОСУХИ НА АМІНОКИСЛОТНИЙ СКЛАД ОЗИМОЇ ПШЕНИЦІ

У контрольованих умовах досліджено вплив короткотривалої високої (40 °С, 2 год) і позитивної низької (4 °С, 2 год) температур та помірної ґрунтової посухи на загальний вміст вільних амінокислот і проліну в органах 14-добових рослин *Triticum aestivum* L. сорту Подолянка. Встановлено, що вміст вільних амінокислот і проліну був вище у пагонах контрольних рослин і становив відповідно 18,29 та 0,85 мг/г маси сухої речовини. За умов модельованої помірної ґрунтової посухи вміст вільних амінокислот і проліну підвищився у пагонах відповідно на 17 та 71 %, тоді як у коренях — на 50 та 61 %. У разі короткотривалої гіпотермії також зафіксовано зростання вмісту вільних амінокислот і проліну у пагонах відповідно на 12 та 47 %, а в коренях — на 30 та 23 %. Реакція на короткотривалу гіпертермію була менш виразною. Обговорюється участь вільних амінокислот і проліну у набутті стійкості озимої пшениці до абіотичних стресорів.

**Ключові слова:** *Triticum aestivum*, температурний стрес, ґрунтова посуха, пролін, вільні амінокислоти.

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#### ВЛИЯНИЕ ТЕМПЕРАТУРНОГО СТРЕССА И ПОЧВЕННОЙ ЗАСУХИ НА АМИНОКИСЛОТНЫЙ СОСТАВ ОЗИМОЙ ПШЕНИЦЫ

В контролируемых условиях исследовано влияние кратковременной высокой (40 °С, 2 ч) и положительной низкой (4 °С, 2 ч) температур и умеренной почвенной засухи на общее содержание свободных аминокислот и пролина в органах 14-суточных растений *Triticum aestivum* L. сорта Подолянка. Установлено, что содержание свободных аминокислот и пролина было выше в побегах контрольных растений и составляло соответственно 18,29 и 0,85 мг/г массы сухого вещества. В условиях умеренной почвенной засухи содержание свободных аминокислот и пролина повысилось в побегах соответственно на 17 и 71 %, тогда как в корнях — на 50 и 61 %. При кратковременной гипотермии также зафиксирован рост содержания свободных аминокислот и пролина в побегах соответственно на 12 и 47 %, а в корнях — на 30 и 23 %. Реакция на кратковременную гипертермию была менее выраженной. Обсуждается участие свободных аминокислот и пролина в приобретении устойчивости озимой пшеницы к абиотическим стрессорам.

**Ключевые слова:** *Triticum aestivum*, температурный стресс, почвенная засуха, пролин, свободные аминокислоты.