ADAPTATION REACTIONS OF COMMON WHEAT
(TRITICUM AESTIVUM L.) AND EMMER
(T. DICOCCUM SCHRANK EX SCHÜBL.)
SEEDLINGS UNDER OSMOTIC STRESS AND TREATMENT
WITH METAL NANOPARTICLES

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Aim. To study the adaptive reactions of common wheat (Triticum aestivum L.) and emmer (T. dicoccum Schrank ex Schübl.) seedlings using the parameters of oxidative homeostasis under polyethylene glycol (PEG)-induced osmotic stress and metal (Fe, Cu, Mn, Zn) mixture nanoparticles treatment. Methods. Biochemical assays: spectrophotometric measurements of the activity of antioxidant enzymes (SOD, CAT), the content of proline and products of lipid peroxidation; mathematical statistics. Results. The state of oxidative homeostasis of common wheat (cv. Favorytka, cv. Trypilska) and emmer (cv. Holikovska) seedlings under PEG-induced osmotic stress conditions was investigated and analyzed. The development of lipid peroxidation processes under stress conditions was observed only in the cv. Trypilska seedlings. It was established that proline content in common wheat seedlings of both cultivars under stress had significant (10 : 1) predominance in roots, while in roots and shoots of emmer seedlings proline content increased equally. SOD activity in the leaves of the studied cultivars under stress conditions was not changed. At the same time, an increase of SOD activity by 30 % under osmotic stress was shown in the roots of common wheat of cv. Favorytka, while it decreased by 25 % in the roots of cv. Trypilska. It was established that CAT activity in the roots of both cultivars of common wheat decreased by 25 and 38 %, respectively, whereas in emmer of cv. Holikovska this parameter increased by 35 % under osmotic stress. Presowing treatment using a colloidal solution of a mixture of biogenic metal nanoparticles contributed to the induction of SOD activity in the roots of cv. Favorytka, CAT activity in the leaves of cv. Trypilska, and CAT activity in the roots and leaves of cv. Holikovska compared to non-treated plants. It should be noted that the treatment of plants with metal nanoparticles promoted the restoration of CAT activity in the roots under osmotic stress conditions to the level of the control plants in both wheat varieties. Conclusions. It was established that osmotic stress provoked the development of oxidative processes and inhibition of the activities of antioxidant enzymes, in particular, SOD and CAT, in the seedling roots of common wheat cultivars. It was shown that emmer wheat seedlings of cv. Holikovska can maintain redox homeostasis and avoid oxidative damage under osmotic stress conditions. It was found that the seedlings of common wheat and emmer demonstrated different strategies of osmotic regulation under osmotic stress, which is confirmed, in particular, at the level of proline accumulation. It was shown that the application of colloidal solutions of metal nanoparticles induced an antioxidant protection system and reduced the oxidative processes, which are inevitable effects of drought. The obtained results indicate that common wheat cultivars are more susceptible to drought compared to emmer wheat of cv. Holikovska.

Keywords: common wheat, emmer, superoxide dismutase, catalase, proline, lipid peroxidation, adaptation.

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INTRODUCTION

The search for the means of adapting wheat to drought due to global climate changes to ensure food safety is a complicated task for scientists, requiring additional investigations [1–3]. International researchers work at solving this problem, elaborating new approaches to enhance cereal productivity using modern methods of agricultural [4] and breeding technologies [5–8]. The most relevant requirement, which should be met by the perspective cultivars, is adaptivity, i.e. the capability of resisting environmental factors, decreasing productivity and yield. Non-compliance with adaptivity requirements leads to a rise in price for agricultural products [9]. Expert community of Wheat Yield Consortium (WYC) are of the opinion that slower tempo of the increase in productivity is related to exhausted possibilities of enhancing it due to the factors, which ensured the thrust in wheat productivity rise as a result of “green revolution” [10]. At the same time, being one of the leading food crops, wheat may have suffered the most from so called “genetic erosion” [11]. At present only two cultivars – common wheat Triticum aestivum L. and, to a lesser degree, durum wheat T. durum Desf. cover practically the whole area of the crop [12]. The reduction in the genetic make-up, and thus in the diversity of genes, conditioning the resistance to biotic and abiotic factors makes fields susceptible, and the volume and quality of the yield – unstable [7, 13].

Recent decades have witnessed the renewed interest to non-traditional species of wheat, first and foremost, spelt (Triticum spelta L.) [14, 15] and emmer (T. dicoccum Schrank ex Schübl.) [13, 16]. A common feature of these species is their grain being tightly enclosed with husks, which makes it hard to thresh – hull content [12]. Hull presence limits the cultivation of these species, as it complicates their processing with the purpose of obtaining pure grain, which requires special equipment and additional energy losses. However, husks are reliable protection for the grain kernel and young seedling during the period from sowing to sprouting [17]. The resistance of young and mature plants to pests and diseases is also ensured by the resistance genes [18], which allows avoiding chemical means of protecting plants and meets the requirements of organic agriculture [12].

Drought and increased temperatures have negative effect on the growth and development of plants, water regime, induce shorter ontogenesis stages, disrupt photosynthesis processes (light absorption, fixation of CO₂) and respiration which leads to the loss of cereal productivity [19, 20]. A cascade of chain reactions is developed on the cellular level, which conditions the disruption of redox homeostasis and, as a result, the increase in the production of reactive oxygen species (ROS) [21]. The induction of different protection mechanisms occurs in plants under these conditions: elimination of ROS, synthesis of antioxidants, accumulation of osmotic active substances, activation of signaling cascades [22, 23] which lead to the formation of different strategies of adaptation to drought.

Therefore, global warming and droughts trigger general concern for the production of grain and other crop products. The solution to this problem is impossible without accumulating new knowledge about metabolome self-regulation mechanisms under drought conditions [24], in cereal crops, in particular. The study of plant adaptation to drought is described in numerous articles in global scientific literature, but so far, none of the investigations led to desired enhancing of plant tolerance to osmotic stress, which is evidently related to insufficient knowledge of physiological and biochemical foundations of forming adaptive reactions under these conditions.

One of the mechanisms of compensating negative impact of drought is the application of mineral nutrition elements [25]. In this respect, a promising method is the application of nanofertilizers in the form of non-ionic colloid metal nanoparticles, but the introduction of such methods requires thorough study of their impact on biological objects. It is believed that nanoparticles of essential metals are involved in different metabolitic processes, including photosynthesis, respiration and assimilation of nitrates, which is conditioned by their cofactor function [26]. In particular, Cu, Fe, Mn and Zn nanoparticles are likely to modulate synthesis and functions of antioxidant enzymes [27]. Thus, one of the mechanisms of stress-protective activity of metal nanoparticles may be the regulation of ROS level and functioning of cellular antioxidant system.

The aim of this work was to study adaptive reactions of common wheat and emmer under polyethylene glycol (PEG)-induced osmotic stress and treatment with the colloid solution of metal (Fe, Cu, Mn, Zn) mixture nanoparticles.

MATERIALS AND METHODS

The cultivars of common wheat Triticum aestivum L. (cv. Favorytka as a highly resistant cultivar, Trypilska – drought susceptible) and emmer (T. dicoccum Schrank ex Schübl. (Holikovska – drought-resistant) were used.
to study the specificities of defensive reactions of different genotypes under osmotic stress. The roots and leaves of 7-day-old seedlings were used in the study. The plants were grown on Hoagland’s solution with the osmotic potential of –0.3 MPa. The negative osmotic potential in the medium was created using PEG 6000 (Carl Roth, Germany) according to Michel and Kaufmann [28]. PEG was not added to control plants. To investigate the possibility of eliminating the negative impact of osmotic stress on seedlings, the seeds of the investigated cultivars were subject to presowing treatment via processing with the colloid solution of metal nanoparticles mixture (Fe, Cu, Mn, Zn) in the concentration of 2 mg/l for 12 h. The colloid solutions of metal nanoparticles were elaborated by the Chair of technology of construction materials and materials science, NULES of Ukraine, and obtained via dispersion of granules of the relevant metals with electric impulses of 100–2,000 A in water [29].

The development of oxidative processes was estimated by the content of thiobarbituric acid (TBA) reactive substances [30]. Plant tissues (200 mg) were homogenized with 3 ml 0.1 M buffer of Tris-HCl (pH 7.6). The homogenate was added 1 ml of 0.67 % TBA solution and 2 ml of 20 % trichloroacetic acid. The reaction mixture was boiled on water bath for 30 min and centrifuged at 1,500 g for 10 min. The absorption was defined at λ 533 nm using spectrophotometer Shimadzu UV-1500 (Japan). The content of TBA-reactive substances was estimated using the coefficient of molar extinction for malondialdehyde (ε = 1.55·10⁵ M⁻¹·cm⁻¹) and expressed in μmol per g of fresh weight.

The content of proline was determined according to the method of Bates et al. (1973) [31]. For this purpose, the plant material (0.15 g) was homogenized in 2 ml of 3 % solution of sulfosalicylic acid and centrifuged at 7,000 g for 10 min. The reaction mixture, containing 1 ml of supernatant, 1 ml of acid ninhydrin solution (1.25 g ninhydrin in 60 % acetic acid and 40 % 6M H₃PO₄) and 1 ml of iced acetic acid, was incubated on boiling water bath for 60 min. After cooling, the tubes were added 3 ml of benzene, mixed and kept at room temperature for 30 min until the separation of two phases. The absorbance of solutions (benzene fraction) was measured at λ 520 nm to the pure benzene. The estimation of proline content in the investigated samples was conducted by the calibration curve and expressed in μg per g of fresh weight.

To determine the activity of antioxidant enzymes, plant tissues were homogenized in liquid nitrogen with the addition of 50 mM potassium-phosphate buffer (pH 7.8). The obtained homogenate was centrifuged at 12,000 g for 15 min at 4 °C. The activity of superoxide dismutase (SOD, EC 1.15.1.1) was measured according to Gianopoulos and Ries (1977) [32]. For this purpose, 50 ml of the extract were added to the reaction mixture, containing 1 ml of 0.0015 % riboflavin, 1 ml of 5.82 % methionine, and 1 ml of 0.154 % of nitroblue tetrazolium. The absorption of the solution was estimated at λ 560 nm after the incubation of the reaction mixture in light for 15 min. SOD activity was estimated as the amount of the enzyme, inhibiting the rate of formazan formation by 50 %, and expressed in conditional units per mg of protein.

Catalase activity (CAT, EC 1.11.1.6) was determined according to Aebi (1984) [33]. For this purpose, the reaction mixture, containing 2.9 ml 50 mM potassium-phosphate buffer (pH 7.0), 90 μl of the extract and 10 μl H₂O₂, was prepared. Absorption was measured at λ 240 nm for 60 sec. The estimation of CAT activity was conducted using extinction coefficient for H₂O₂ (ε = 39.4 mM⁻¹·cm⁻¹), the enzyme activity was expressed in μmol H₂O₂ per mg of protein.

The studies were conducted in three biological and three analytical repeats. The statistical analysis of the data obtained was conducted in Microsoft Excel and Statistica 8.0. The comparison of samples involved the use of arithmetic mean (M) and standard error of mean (SEM). The reliability of the difference between the compared groups was estimated using Duncan’s criterion. The difference p < 0.05 was considered statistically reliable for all the indices.

RESULTS

The investigation of the content of TBA-reactive substances in the roots and leaves of common wheat and emmer demonstrated that the development of lipid peroxidation under osmotic stress was observed only in the seedlings of cv. Trypilska (Fig. 1). For instance, the content of TBA-reactive substances in the roots of seedlings increased by 60 % compared to the control variant, and in the leaves – by 27 %.

Presowing treatment of biogenic metal nanoparticles with the colloid solution had protective effect on common wheat seedlings, cv. Trypilska, inhibiting the development of oxidative processes under osmotic stress compared to the variant without any treatment with nanoparticles. No reliable changes by this parameter were determined in other species under the effect of metal nanoparticles.
The results obtained demonstrated the changes in proline metabolism in the investigated species under the stress. It was established that proline content in different parts of common wheat seedlings is different, with considerable prevalence (10 : 1) in the roots (Fig. 2). Less intense accumulation of proline was observed in the roots of emmer seedlings, cv. Holikovska, compared to other cultivars. At the same time, no reliable significant changes in proline content were determined in the leaves of common wheat seedlings, contrary to emmer, where this index was 7 times higher.

After the presowing treatment with the mixture of metal nanoparticles, the decrease in proline content was observed in the roots and leaves of emmer under osmotic stress by 66 and 36 % respectively, compared to the variant, not treated with nanoparticles. The established changes may demonstrate the involvement of alternative protective mechanisms, in particular, the systems of antioxidant protection.

The investigation of the activity of the main antioxidant enzymes, including SOD and CAT, established the multi-vector defensive reactions of the investigated species under prolonged osmotic stress. An increase of SOD activity by 30 % under osmotic stress was shown in roots of common wheat of cv. Favorytka, while it decreased by 25 % in the roots of cv. Trypilska (Fig. 3). At the same time, the level of this enzyme activity in the roots of emmer of cv. Holikovska corresponded to control values. SOD activity in the leaves of the studied cultivars under stress conditions was not changed.
Common wheat cv. Trypilska and Favorytka were characterized with the decrease in CAT activity in the roots by 25 and 38 % respectively (Fig. 4), while in emmer of cv. Holikovska this index increased by 35 % under osmotic stress. The changes in the leaves by this parameter were observed only in cv. Favorytka, where CAT activity was reliably decreased by 19 % under cultivating on PEG-containing medium.

The treatment with the colloid solution of biogenic metal nanoparticles mixture promoted the increase in SOD activity in the roots of cv. Favorytka almost twice, while in the roots of cv. Holikovska an increase in CAT activity by 34 % was observed compared to the control variant. An increase in CAT activity by 30 and 16 %, respectively, was observed in cv. Trypilska and Holikovska compared to the control. It is noteworthy that presowing treatment with metal nanoparticles mixture promoted the restoration of CAT activity under osmotic stress up to the level of control variant values in the roots of both common wheat cultivars.

DISCUSSION

A wide range of scientists have come to the consensus on the issue that drought resistance should be viewed in the complex with changes in processes of growth and development of plants [34]. On early stages of ontogenesis, plants are especially sensitive to drought, thus, the clarification of the specificities of adaptive reactions under PEG-induced osmotic stress may bring us closer to the realization of adaptation strategies in cultivars of common wheat and emmer. The results of our investigation demonstrated that the cultivation of wheat seedlings under osmotic stress did not result in
the development of oxidative damage in cultivars with high tolerance to PEG-induced drought (Holikovska and Favorytka) which may demonstrate the restoration of redox balance and control over the processes of ROS formation in plant cells.

Drought-induced proline accumulation is known to be one of adaptive reactions of many cereal crops [35]. The results of our investigations demonstrate that osmotic regulation and maintenance of water balance of cells under stress takes place with some participation of proline, which is also confirmed with considerable accumulation of this aminoacid in the roots of the investigated cultivars. The root system has direct contact with the medium of negative osmotic potential, and thus should ensure the conditions to preserve the water content, sufficient for metabolic processes, and make water outflow into the medium impossible. In this respect, the accumulation of osmotically active proline in the roots is an expected reaction of plants under osmotic stress [36].

According to the scientific literature, proline accumulation in the leaves may result from changes in plant metabolism under drought, aimed at the decomposition and decrease in protein synthesis or transformation of some aminoacids into proline [37]. The role of proline in plant leaves under stress is not limited with osmotic regulation only. Proline is known to act as chaperon, ensuring the protection of protein molecules, has antioxidant properties and participates in the regulation of nitrogen content [38]. At the same time, the mechanism of maintaining osmotic balance depends on the genotype. In particular, the capability of accumulating proline in response to drought may serve as a criterion to enhance drought-resistance of wheat in the breeding programs (for instance, the selection of genotypes with higher content of proline under drought conditions compared to normal conditions) [39].

Our results are in good agreement with, for instance, the investigation of Marcińska et al. [40] where a 2–5-fold increase in proline content was registered in the leaves of common wheat seedlings (T. aestivum) depending on the PEG content in the cultivation medium. The authors determined that the accumulation of this aminoacid occurred more intensely in cultivars with higher drought-resistance compared to drought-sensitive ones. It is known that exogenous treatment with proline may decrease the negative effect of stress, in particular, pollution with heavy metals, and promote avoiding excessive moisture loss by plants [41].

Osmotic stress is usually accompanied with the increase in ROS generation and activation of the antioxidant system of plants. The rate of changes in the content and activity of its components and the capability to maintain redox-balance at the level, which allows avoiding critical oxidative damage of cells, is required to ensure the survival of plants under unfavorable environmental factors. For instance, the study of Zhang and Kirkham [42] demonstrated that under drought similar SOD and CAT reaction is observed in 10-day-old wheat seedlings with different genome ploidy. At the same time, the authors indicate lower efficiency of antioxidant systems of common wheat compared to emmer, depending on the degree of lipid peroxidation. The study of Sairam, R. K. et al. [43] demonstrate higher SOD activity and lower content \( \text{H}_2\text{O}_2 \) and malondialdehyde in emmer under drought on different stages of ontogenesis which also demonstrates more profound mechanism of antioxidant protection of plants of this species. It is noteworthy that usually stress is induced in seedlings/mature plants, previously grown under optimal moisturization conditions. The osmotic stress system, used in our study, allows estimating the condition of plants, which grew under osmotic stress since germination.

Little is still known about the impact of metal nanoparticles on different levels of antioxidant protection of plants. Our results demonstrate the protective effect from the treatment with metal nanoparticles mixture, observed in the roots of the investigated species, where a decrease in the content of TBA-reactive substances took place on the background of the increase in SOD and CAT activity. It is believed that the treatment with metal nanoparticles may mediate the activation of the system of antioxidant protection, promoting rapid adaptation of plants under stress conditions [26, 44]. Metal nanoparticles may be the source of ROS and promote the intensification of reactions within the antioxidant system [45, 46]. Therefore, they may facilitate pre-adaptation of plants to the effect of unfavorable environmental factors [47] which is in agreement with our results. Moreover, some metal oxide nanoparticles manifest enzyme-like activity, in particular, these are nanoparticles of Fe, Cu and Mn oxides [45]. Taking into consideration the fact that SOD cofactors are Cu, Fe, Mn, Zn, and CAT contains heme Fe [48], nanoparticles of the relevant metals could be involved in the composition of antioxidant enzymes. Some investigations demonstrate that the treatment with nanoparticles of Cu oxide promotes the increase in SOD activity in duckwheat plants (Lemna minor L.) [49], rice (Oryza
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It was established that osmotic stress provoked the development of oxidative processes and inhibition of the activities of antioxidant enzymes, SOD and CAT, in the common wheat seedling roots. It was shown that emmer wheat seedlings of cv. Holikovska can maintain redox homeostasis and avoid oxidative damage under osmotic stress conditions. It was found that the seedlings of common wheat and emmer demonstrated different strategies of osmotic regulation under osmotic stress, which is confirmed, in particular, at the level of proline accumulation. It was shown that the application of colloidal solutions of metal nanoparticles induced an antioxidant protection system and reduced the oxidative processes, which are inevitable effects of drought. The obtained results indicate that common wheat cultivars are more susceptible to drought compared to emmer wheat of cv. Holikovska.

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модельованого осмотичного стресу. Розвиток перексисионного окислення ліпідів спостерігали лише у проростках м’якої пшениці сорту Тріпільська. Показано, що у проростках пшениці м’якої обох сортів вміст проліну за умов стресу значно (10 : 1) переважав у коренях, тоді як у надземній та підземній частинах проростків двозернянки вміст проліну збільшувався одинаково. Змін активності СОД у листках досліджуваних сортів за дії стресора не виявлено. Разом з тим, у пшениці м’якої сорту Фаворитка спостерігали підвищення активності СОД в коренях на 30 % за умов осмотичного стресу, тоді як у коренях сорту Тріпільська активність СОД знижувалась на 25 %. У коренях обох сортів пшениці м’якої спостерігали зниження активності КАТ на 25 та 38 %, відповідно, тоді як у пшениці двозернянки сорту Голиковська цей показник зростав на 35 % за осмотичного стресу. Передпосівна обробка колоїдним розчином суміші наночастинок біогенных металів сприяла активації СОД у коренях сорту Фаворитка, КАТ у листках сорту Голиковська та в листках і коренях сорту Голиковська. Варто зауважити, що обробка сумішшю наночастинок металів опосередковувала відновлення активності КАТ у коренях обох сортів пшениці м’якої за умов осмотичного стресу до рівня значень контрольного варіанту. **Висновки.** Встановлено, що осмотичний стрес пропоковує розвиток окиснильних процесів та притягнення активності антиоксидантних ензимів СОД та КАТ у коренях проростків пшениці м’якої. Показано, що проростки пшениці двозернянки сорту Голиковська можуть підтримувати редук-гоеоестаз та уникати окиснювальних пошкоджень за умов осмотичного стресу. З’ясовано, що проростки пшениці м’якої та двозернянки демонструють різні стратегії осмотичної регуляції за умов осмотичного стресу, що пояснюється, зокрема, на рівні акумуляції проліну. Виявлено, що обробка колоїдним розчином суміші наночастинок металів індукує систему антиоксидантного захисту та дозволяє зменшити прояв окиснильних процесів, що є невід’ємними наслідками дії посухи. Отримані результати свідчать, що сорти пшениці м’якої є більш чутливими до дії посухи порівняно з пшеницею двозернянкою сорту Голиковська.

**Ключові слова:** пшениця м’яка, пшениця двозернянка, супероксиддисмутаза, каталаза, пролін, перексис окиснення ліпідів, адаптація.

**Адаптаційні реакції проростків пшениці м’якої (Triticum aestivum L.) та двозернянки (T. dicoccum Schrank ex Schübl.) при дії осмотичного стреса і обробки наночастинками металів**

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**Цель.** Исследование особенностей формирования адаптационных реакций проростков пшеницы мягкой (Triticum aestivum L.) и двузернянки (T. dicoccum Schrank ex Schübl.) в условиях полиэтиленплилкильти ПЗГ – моделированного осмотического стресса и обработки сместью наночастиц металлов (Fe, Cu, Mn, Zn) по параметрам окисительного гомеостаза. **Методы.** Биохимические: спектрофотометрическое измерение активности антиоксидантных энзимов (СОД, КАТ), содержания свободного пролина и продуктов перекисного окисления липидов; математическая статистика. **Результаты.** Исследовано и проанализировано состояние окисительного гомеостаза проростков сортов пшеницы мягкой (Фаворитка, Тропильская) и двузернянки (Голиковская) в условиях ПЗГ-моделированного осмотического стресса. Развитие перексисного окисления липидов наблюдалось только в проростках мягкой пшеницы сорта Тропильская. Показано, что в проростках пшеницы мягкой, находящихся в условиях стресса, содержание пролина значительно преобладало (10 : 1) в корнях, а в надземной и подземной частях проростков двузернянки содержание пролина увеличивалось одинаково. Вместе с тем, у пшеницы мягкой сорта Фаворитка наблюдали увеличение активности СОД в корнях на 30 % в условиях осмотического стресса, тогда как в корнях сорта Тропильская активность СОД снижалась на 25 %. В корнях обоих сортов пшеницы мягкой наблюдали также уменьшение активности КАТ на 25 и 38 %, соответственно, при этом у пшеницы двузернянки сорта Голиковская этот показатель увеличивался на 35 %. Предпосевная обработка коллоидным раствором смеси наночастиц биогенных металлов способствовала активации СОД в корнях сорта Фаворитка, КАТ в листьях сорта Тропильская, а также в листьях и корнях сорта Голиковская. Стоит отметить, что обработка смесью наночастиц металллов опосредствовала восстановление активности КАТ в корнях обоих сортов пшеницы мягкой в условиях осмотического стресса до уровня значений контрольного варианта. **Выводы.** Установлено, что осмотический стресс провоцирует развитие окислительных процессов и угнетение активности антиоксидантных энзимов СОД и КАТ в корнях проростков пшеницы мягкой. Показано, что проростки пшеницы двузернянки сорта Голиковская могут поддерживать редук-гоеоестаз и избегать окисительных повреждений в условиях осмотического стресса. Установлено, что проростки пшеницы мягкой и двузернянки демонст-
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