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ECOLOGICAL APPROACH TO THE CONSERVATION OF AGRICULTURAL PLANTS ON THE EXAMPLE OF TRITICUM AESTIVUM L.: MORPHOMETRIC, PHYSIOLOGICAL AND BIOCHEMICAL REACTIONS

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Abstract. Having the ability to organise a programme of their individual development depending on the prevailing cultivation conditions, the adaptive response of plants to stress includes changes in regulatory and metabolic structures, the level of interconnections of which combines different types of adaptive reactions. Recently, the development of biotechnological methods has made it possible to determine which characteristics change in response to a particular stress and has shown that genetic control of adaptation mechanisms to various types of adverse effects is a complex system of signal transduction pathways and regulatory factors.

Recently, the development of plant forms with an increased level of osmotolerance to osmotic stresses has been associated with the use of biotechnological methods. An important role in maintaining plant osmotolerance belongs to free proline, which is one of the most multifunctional stress metabolites in plants.

The germination of seeds under simulated water stress was studied. Stress was created by 0.5 M and 0.8 M mannitol solution. In the presence of mannitol, a decrease in the number of seedlings was observed in all tested variants. At the same time, genotypic differences were observed. To study the salt tolerance, the seeds were germinated for 10 days in a 0.5 diluted Murashige-Skoog solution with the addition of 20.0 and 25.0 g/l of seawater salts. On day 10, the content of free proline in the leaves of young plants was analysed. An increase in the level of proline was observed in response to salt stress. Genotypic differences were observed in the degree of the reaction. The nature of the accumulation of free proline under normal and stressful conditions provides evidence in favour of the functioning of the plant gene. Under osmotic stress, the level of free proline increases as a result of synthesis. These genotypic features may indicate an indirect effect on the endogenous genes of the plant. At the initial stages of research, the stress-response relationship can be established by changing the conditions of stress and recovery.

The fundamental role of proline in osmotic regulation and increasing the ability of plants to withstand cell dehydration caused by salinity, drought or extreme temperatures is well understood.

Key words: proline, winter wheat, growth parameters, soil drought, osmotic resistance.

ЕКОЛОГІЧНИЙ ПІДХІД ДО ЗБЕРЕЖЕННЯ СІЛЬСЬКОГОСПОДАРСЬКИХ РОСЛИН НА ПРИКЛАДІ *TRITICUM AESTIVUM* L.: МОРФОМЕТРИЧНІ, ФІЗІОЛОГІЧНІ ТА БІОХІМІЧНІ РЕАКЦІЇ

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Анотація. Маючи здатність організовувати програму свого індивідуального розвитку в залежності від переважних умов культивування, пристосувальна реакція рослин до стресу включає зміни в регуляторних і метаболічних структурах, рівень взаємозв'язків яких поєднує різні види адаптивних реакцій. Останнім часом розвиток біотехнологічних методів дав можливість встановити, експресія яких характеристик змінюється у відповідь на дію того чи іншого стресу та показав, що генетичний контроль механізмів адаптації до різних видів несприятливих впливів представляє складну систему шляхів передачі сигналів та регуляторних факторів.

Отримання форм рослин із підвищенням рівнем осмостійкості до осмотичних стресів за останній час пов'язують із застосуванням біотехнологічних методів. Важлива роль у підтримці осмотолерантості рослин належить вільному проліну, який є одним із найбільш багатофункціональних стресових метаболітів у рослин.

Досліджували проростання зернівок за умов модельованого водного стресу. Стрес створювали 0,5М та 0,8 М розчином маніту. У присутності маніту спостерігали зниження числа проростків у всіх тестованих варіантів. У той же час спостерігали генотипові відмінності. Для дослідження солестійкості зернівки впродовж 10-ти діб пророщували на 0,5 розведеному розчині Мурасіге – Скуга із додаванням 20,0 та 25,0 г/л солей морської води. На 10-ту добу аналізували вміст вільного проліну у листках молодих рослин. Відмічали підвищення рівня проліну у відповідь на дію сольового стресу. За ступенем прояву реакції спостерігали генотипові відмінності. Характер акумуляції вільного проліну за нормальних та стресових умов дає доказ на користь функціонування функціональності роботи гена рослини. За дії осмотичних стресів рівень вільного проліну зростає в результаті синтезу. Відзначені генотипові особливості можуть вказувати на опосередкований вплив на ендогенні гени рослини. На початкових етапах дослідження можуть бути встановлені при зміні умов стрес – відновлення.

Фундаментальна роль проліну в осмотичній регуляції та підвищенні здатності рослин протистояти зневодненню клітин викликаному засоленням, посухою або екстремальними температурами, досить добре вивчена.

Ключові слова: пролін, пшениця озима, ростові параметри, грунтова посуха, осмостійкість.

Introduction. In recent years, the dramatic change/deterioration of the environment has required the scientific community to create and disseminate advanced biological technologies aimed at accelerating the process of sustainable plant breeding. Advanced biological technologies cover a growing number of agriculturally valuable crops [4, 11].

Wheat is one of the main food crops in the world, as manipulations with the crop are aimed at accelerating the breeding process when creating new high-yielding varieties resistant to biotic and abiotic stresses [3, 7, 14].

Modern climate change stimulates the emergence of new anthropogenic factors that combine with unfavourable environmental factors to increase the stress load, resulting in the problem of combined stresses and the need for plants with high adaptive potential. Obtaining plant forms with an increased level of resistance to abiotic stresses using the latest biotechnological methods is becoming increasingly important. However, along with the undeniable achievements, there are problematic issues that require special attention.

In this regard, the task of establishing a reliable marker(s) for monitoring the vital activity of the organism, a parameter that could respond to external influences, comes to the fore. In this way, the difference/contrast in the reaction of sensitive and resistant forms, varieties, and genotypes of plants can be revealed. Molecular markers, which are a special statistical characteristic of a variety, form or genotype, are not subject to environmental influences [5, 11, 13]. In addition, they are quite expensive and require special training from the user or a specialist. In view of this, the search for a systemic indicator that coordinates and interacts with all areas of metabolism is constantly underway. One of the most promising endogenous substances from this point of view is the amino acid L - proline (proline, *pro*) [15, 16, 17].

The peculiarities of this compound are based on its molecular structure, namely the presence of an α-nitrogen atom in the perelidine ring. As a result, proline (pro) is not a substrate for the action of amino acid metabolism enzymes, such as racemases, aminotransferases, and decarboxylases. Instead, pro metabolism is controlled by its own synthesis/degradation/transport systems. The level of free proline is a highly dynamic indicator, sensitive to external factors. Plant species with different degrees of natural resistance can accumulate different amounts of proline even under normal conditions [14]. At the same time, the accumulation of this compound under the influence of various biotic and abiotic stresses is an indisputable fact. That is, the physiological multifunctionality of free proline is simultaneously realised: detoxifying, osmoregulatory, and stabilising [12].

Goal. The aim of our work was to determine and analyse the level of proline in winter wheat at the early stages of grain germination, morphometric parameters without and after exposure to stress in winter wheat plants.

Materials and Methods. The object was seedlings of winter wheat genotypes. The genotypes were obtained at the plots of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine. The free proline content was determined according to the method [14] on day 10 of the experiment. The whole seedling (ground and root parts) was analysed. To provide the required biomass, tissues from 15-20 plants were ground, mixed, and a total weight was taken from the mixture. The viability of seeds was determined on the 10th day in % germination to the total number of grains. The experiment was conducted in triplicate. The results were statistically processed using.

Results and discussion. It is known that, in general, the level of free proline (pro) is self-regulated by the expression/repression of genes for its synthesis/degradation enzymes: Δ1-pyrroline-5-carboxylate synthetase (P5CS) [2, 14]. Under stressful conditions, the level of pro may increase. On the other hand, the level of resistance to osmotic stress is not a stable trait, even for one plant, but changes in ontogeny [15]. In addition, the increased content of pro in certain plant organs may occur as a result of its movement from the zone of its synthesis. Therefore, the study of the free pro content is a complex procedure; it should be linked to the activity of seed germination under stressful conditions, maintenance of viability, speed of recovery from stress, and morphometry.

Osmotic stresses were created by adding mannitol (0.5M, 0.8M) or seawater salts (20.0 g/l – 2mw, 25.0 g/l – 2.5mw). The T0 grain obtained after transformation and the original genotypes were tested. The control was the grain that was (germinated) in water (Table 1).

Table 1: **Germination of wheat grains under stress conditions (from planted)**

Genotipe	7 days					10 days				
	H_2O	2mw	2,5mw	0,5M	0,8M	H_2O	2mw	2,5mw	0,5M	0,8M
UK – 065	98,30 ±1,25	90,0	70,0	95,5	5,0	98,30 ±1,25	90,0	70,0	95,5	50,0
UK – 107 h	93,74 ±2,15	80,0	94,4	90,0	He pic	93,74 ±2,15	80,0	94,4	90,0	97,9

During germination, the linear dimensions of the roots and aerial parts were monitored, and the number of roots was counted. The experiment was stopped on day 16 due to the onset of seedling death.

Morphometry data, which was performed on day 10, are presented in Table 2.

Table 2: **Dimensions of wheat seedlings obtained under stress conditions**

Germination conditions	UK – 065		UK – 107 h	
	Above ground part	Root	Above ground part	Root
H_2O	4,01 ± 0,73	5,28 ± 1,76	3,78 ± 2,50	3,68 ± 2,35
2mw*	0,3	0,5	0,3	0,5
2,5mw*	- **	0,75 ± 0,38	- **	1,05 ± 0,13
0,5M*	- **	3,00 ± 0,25	- **	3,50 ± 0,28
0,8M*	- **	1,1	- ***	- ***

Note: * - grains were counted; ** - the aerial part was not released (under the skin); *** - no germination was observed

The morphometric analysis carried out on day 10 showed: a) low concentrations of salt and mannitol (2.0% of sea water salts, 0.5M mannitol) caused the same reactions from the aerial part and root system in all wheat seedlings of genotype UK – 065; b) the effect of increased salinity concentration caused significant variability in the length of the control root, which could be due to different degrees of stress damage. It is known, that it is the root system that suffers from salinity in the first place. Seedlings of UK-107 h reacted similarly to the increase in the concentration of stress factors. This reaction rather indicates a tendency to adaptation. The viability of the grains is indicated by a higher percentage of germinated seeds, which was observed on the 10th day on the medium with 0.8M mannitol. Other genotypes, namely UK 322 and UK 209 h, were also tested under stress conditions. Similar to the genotype UK – 065, the germination process stopped after 10 days. Further exposure to osmotic stresses caused the death of young plants – the aerial part of the seedling darkened, the root system gradually died. Table 3 shows the germination rates for different wheat genotypes.

Table 3: **Germination of wheat grains (Triticum aestivum L.)**

Genotipe	Germination conditions		
	H_2O	2,0% sea soil	0,5M manitol
UK - 322	80,75 ± 3,50	60,30	73,61
UK- 209 h	89,30 ± 4,17	50,40	51,20

With an increase in stress load (2.5% seawater salts or 0.8M mannitol), germination was further inhibited or stopped altogether. Thus, the seeds obtained after transformation of the genotype UK 322 germinated in the presence of 0.8 M mannitol only in 10% of cases. Germination under salinity conditions was in the range of 0-10%. Although, at first glance, the effect of salinity seemed more aggressive, in our opinion, the genotypes responded to an increase in the osmotic pressure of the medium. First, the in situ response of plants to short-term air drying was determined. Seeds were germinated in water for 7 days (A). Young plants (2.0-2.5 cm in size) were kept on dry filter paper at room temperature for 12 hours (B). After drying, the seedlings were transferred to moistened filter paper for 3 days (C). In our opinion, such rotations of cultural conditions contributed to the disclosure of the adaptive potential of plant organisms and could also reveal the range of normality. At each stage A, B, C, the level of free pro was measured (Table 4).

Table 4: **Free proline content in wheat seedlings under changing cultivation conditions**

Genotipe	Proline mg%/g crude weight		
	Variant A	Variant B	Variant C
Zolotokolosa	23,11	34,11	66,23
Smuglyanka	10,51	41,61	57,18
M - 808	51,60	97,75	100,81
Volodarka	36,12	71,98	86,30

The data in Table 4 undeniably point to two facts. Firstly, in young wheat plants (7-day old), the level of free pro varies widely. Such a range describes the individual characteristics of the organism under normal conditions, namely the germination process itself. There is evidence in the literature that moderate amounts of proline promote cell division and plant growth (A). At the same time, large amounts of the amino acid can have a negative effect, causing a slowdown/stoppage of growth [10, 13]. Secondly, short-term (12 hours) drying, followed by humidification, caused sharp fluctuations in the pro content.

At the same time, the absolute values increased significantly. Such digital values indicate the development of significant water stress. The extent of the damage may be evidenced by the fact that even on the 3rd day after the start of rehydration, the pro level did not yet reach normal values. At the same time, in our opinion, dehydration stress did not cause degradation of plant structural components, since after rehydration the pro level decreased sharply. Such events may indicate the efficiency of the proline metabolism system, and in general, the vital activity of plants of all genotypes. Since the dehydration/rehydration processes caused significant fluctuations in the content of the amino acid, it was considered appropriate to determine the level of free pro under prolonged exposure to simulated water stress, since the prolongation of the stress period should affect the activity of its metabolic enzymes.

The wheat grains were germinated under normal conditions for 7 days and then transferred to a 0.5M mannitol solution. The pro content was determined in the aerial part. Morphometric measurements were performed before the analysis.

Young plants with aerial parts of 1.5-2.0 cm in size and roots not exceeding 2.5 cm were transferred to stressful conditions. On day 7 of the experiment, the following events were observed: a) the size of the aerial part of seedlings of all genotypes remained practically unchanged; b) the number of roots increased and varied widely from 2 to 6. New roots were 0.5-3.0 cm long and thinner. On the 14th day, the following was recorded: a) all control plants were dead. At the same time, a significant chlorophyll fading in the aerial part was noted. The root system, on the contrary, darkened, the root tissue softened.

At the same time, a significant decrease in leaf turgor was observed in viable seedlings.

The size of the aboveground part remained unchanged. There were changes in the root system: parallel processes of rhizogenesis/destruction took place. Older roots were replaced by young roots. Therefore, the average root length on the 14th day was in the range of 0.3-0.7 cm. The experiment was stopped due to the death of the control. The stress-specific response of roots has been reported [6, 16].

In seedling cells, the level of pro varied widely. Since the plants underwent rhizogenesis, this event is an adequate indicator of the organism's resistance. Thus, the accumulation of proline can be considered a consequence of its synthesis (varying in intensity), which is a genotypic property. At the same time, the synthesis did not necessarily occur in the aerial part. Rather, it was carried out in the roots, which were formed de novo [1, 9, 14]. The synthesised proline was transferred to the aerial part with the participation of specialised transporters. In any case, the level of pro was sufficient to maintain vital activity. The data in Table 6 show a significant impact on proline levels.

Conclusions. Thus, in the course of the research, it was found that Free proline in young wheat seedlings was a product of hydrolytic enzymes. The level of proline during germination under stress can be a marker of genotype resistance.

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ВИКОРИСТАННЯ КЛІТИННОЇ СЕЛЕКЦІЇ В ОТРИМАННІ СТРЕС - СТІЙКОГО ТЮТЮНУ (NICOTIANA TABACUM L.) ДО МОДЕЛЬОВАНОГО ЗАСОЛЕННЯ ТА ТЕХНОГЕННОГО ЗАБРУДНЕННЯ

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Анотація. Одним із факторів навколишнього середовища, що приводять до значного зниження врожайності рослин є засолення грунтів. У зв'язку з загальним погіршенням екологічної картини в світі та широким використанням штучного зрошення