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Bronnikova L.
Postgraduate student,
Department of Plant Physiology and Introduction
Oles Honchar Dnipro National University
Junior Research Fellow
Institute of Plant Physiology and Genetics,
National Academy of Sciences of Ukraine
Zlenko_lora@ukr.net
orsid.org/0000-0002-8103-0548

Khomenko L.
Candidate of Agricultural Sciences
Senior Research Fellow
Department of Plant Genetic Improvement
Institute of Plant Physiology and Genetics,
National Academy of Sciences of Ukraine
Lidole@ukr.net
orsid.org/0000-0002-3776-6208

Dikun M.
Category 1 Engineer
Grain Quality Laboratory
Institute of Plant Physiology and Genetics,
National Academy of Sciences of Ukraine
Mariiadykun@gmail.com
orsid.org/0000-0003-4187-0865

WINTER WHEAT (*TRITICUM AESTIVUM* L.) CELL CULTURES AS A MODEL SYSTEM FOR STUDYING

Random changes, especially stress, cause plants to undergo significant metabolic and structural modifications. Most of these are adaptive responses that make the organism more suited to new conditions. Salinisation is the most harmful abiotic stress. In the case of severe stress, the effectiveness of the genotype's vital functions becomes apparent over a long period of time. Cell culture allows us to avoid problems: physiological, biochemical, and other changes that occurred due to the stressor. The aim of this study was to analyse the content of free proline and protein in different cell cultures of wheat varieties subjected to prolonged osmotic stress. However, the accumulation of this osmolyte alone cannot serve as an indicator of successful adaptation. Under conditions of severe stress, which significantly limits the availability of external trophic and energy resources, proline can act as a reserve source of nitrogen and carbon. Therefore, the Pro pool in cells subjected to prolonged stress can change significantly. When studying the dynamics of changes in wheat plants, we found that the level of proline in callus cells in the Volodarka variety decreased as stress pressure increased, and the synchronous decrease in amino acid content during prolonged salt stress in the Zolotokolos and Smuglyanka varieties was 18.3 ± 3.4% and 21.4 ± 4.6%, respectively. In wheat, reserve proteins (gliadin and glutenin) belong to this category of proteins. In wheat grains, they account for 75-85% of the total protein composition; the gliadin/glutenin ratio depends on the specific variety or genotype of the plant. In addition, these proteins are rich in arginine and glutamine. The remaining part of the endosperm proteins consists of albumins and globulins, many of which have enzymatic activity. We observed a significant depletion of the protein pool, independent of the type of salinity. Since glutenin is also enriched with proline, the increase in the content of this amino acid in cells during salinisation could be *the result of their hydrolysis. Indirect evidence of this may be the decrease in osmolyte content on the 25th day. The progressive decline in glutenin reserves led to a decrease in proline.*

Key words: salinisation, proline, protein, winter wheat, cell selection.

Introduction. For successful life activity, plants, as attached organisms, must continuously adapt to dynamic (often critical) environmental factors. Changes (deterioration) in living conditions are no longer local problems; they have become global. They also affect Ukraine [2, 15]. The forecasts are not encouraging. Natural abiotic stresses are significantly modified/intensified by the introduction of by-products of industrial activity. Local changes in the environment are becoming ecological disasters. There is a growing need for forms that are characterised by a high level of complex resistance to abiotic stresses. In this regard, there is a need to improve biological technologies aimed at obtaining resistant (adaptive) genotypes [4, 5].

A special place in the development of this problem is occupied by It is and will remain the main component of the diet of most of the world's population. Significant human and material resources are spent on obtaining wheat varieties with improved stress resistance, yield and grain quality. It is obvious that macrostructural changes, as well as general integral indicators (growth, productivity), depend on the activity of cellular physiological and biochemical reactions [2, 15]. Wheat is also a valuable food product for humans, can be used as animal feed, is processed in the production of starch and ethanol, and is of interest as a potential source of technical biofuel. Areas of genetic improvement of wheat grain quality cover all of the above-mentioned industries.

In addition to scientific development, other pressing issues are also included, namely: resistance to biotic and abiotic stresses [3, 14].

In vitro culture provides unique opportunities for studying cellular-level reactions. The approaches used in this process will allow for the selection of cells with altered metabolism. Such cell variants can become the starting material for the search and isolation of genes associated with specific sections of metabolic chains. They will be the subject of further manipulations aimed at obtaining regenerants [15, 16]. It is extremely important to conduct a comparative study of the cell line and the regenerant plant, which will reveal the expression of the new characteristic in the whole plant. This will allow the polymorphism of new forms to be identified, primarily by adaptive traits [17, 18].

Optimisation of the standard protocol is ensured by continuous dynamic monitoring of cell metabolism. When studying the dynamics of changes in free proline and protein in wheat varieties, we found that under stressful conditions, some of these substances are first transferred to the tissues that are directly affected by the stressor. In our opinion, such a redistribution of the protective compound may contribute to the prolongation of the viability of genotypes. Cell culture excludes this physiological reaction. In this case, the level of proline and protein is the result of the interaction of the synthesis, transport and catabolism systems.

The aim of this study was to analyse the content of free proline and protein in different cell cultures of wheat varieties subjected to prolonged osmotic stress. Methods and materials. For the study, wheat varieties selected by the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine, Volodarka, Smuglyanka, Zolotokolosa, which are included in the National Register of Variety Standards [19], were selected. Callus was induced from sterile plant sprouts, which was grown over 4 passages to obtain the required amount of biomass on Hamburg B5 medium [20]. In the experiment, the callus was exposed to sulphate-chloride (sea water salt) and sulphate salting at a concentration of 20.0 g/l. According to the literature [14-16], this concentration creates severe salt stress. The resulting callus was divided vertically and transferred to a fresh medium in equal parts. The variants were kept under salinity conditions during a standard passage (30 days). During this time, under normal conditions, cell cultures go through all stages of development.

The proline content was determined using a standard method [7]. Protein was determined using the Lowry method [8]. The data were obtained in three replicates, and statistical processing was performed using standard methods of variational statistics with the use of Microsoft Excel. The difference between the data was considered significant at $p \le 0.05$.

Results and discussion. The in vitro system ensures standardisation of cultivation conditions. Under severe stress, both physiological and genetic responses of the studied organism are revealed. This makes it possible to distinguish between general and specific characteristics of the organism. A convenient criterion is the amino acid proline (Pro) [1, 13].

Diagram 1 shows the levels of free Pro in cell cultures of wheat of different genotypes, measured on the 15th day of the experiment. By this time, under normal conditions, the culture is in a stage of stationary growth, when genotypic characteristics are maximally revealed. Under unfavourable conditions, physiological adaptation reactions may occur during this time, rather than primary stress-induced responses.

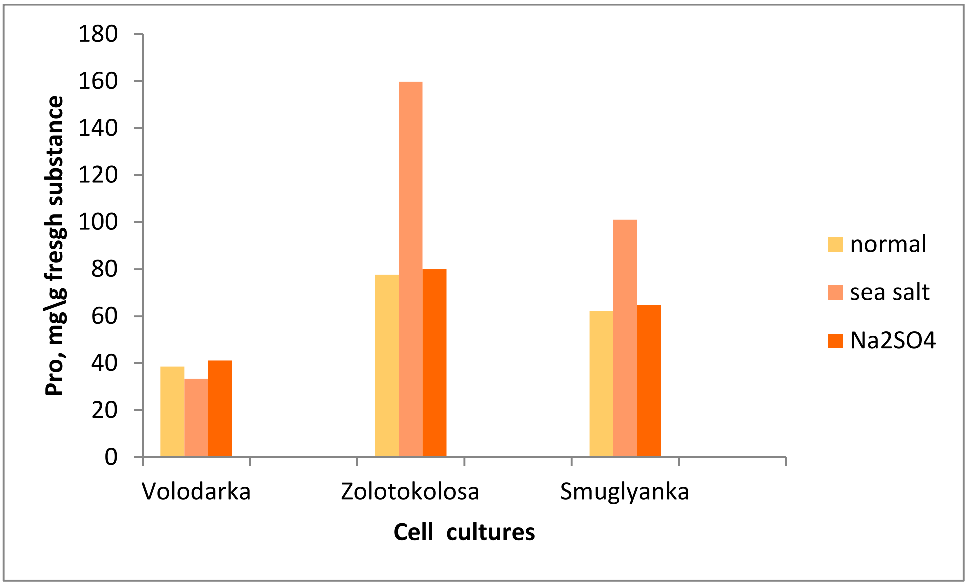

Fig. 1. **Free proline content in cell cultures of Volodarka, Zolotokolos and Smuglyanka wheat varieties cultivated under normal conditions and under salt stress.**

The diagram shows that the level of amino acids in cells decreased as stress pressure increased. At the same time, the type of salinity did not affect the decrease. In both cases, the decrease was ~ 20 %. A similar effect was observed in other genotypes. The synchronous decrease in amino acid content during prolonged salt stress in the Zolotokolos and Smuglyanka varieties was 18.3 ± 3.4 % and 21.4 ± 4.6 %, respectively. Salinisation is one of the most aggressive stress factors, as its damaging effect is the result of the combination of osmotic, ionic and oxidative stresses. The accumulation of free Pro in cells is aimed at compensating for pathological changes [6, 10, 11]. However, the accumulation of this osmolite alone cannot serve as an indicator of successful adaptation. Under conditions of severe stress, which significantly limits the availability of external trophic and energy resources, proline can act as a reserve source of nitrogen and carbon. Therefore, the Pro pool in cells exposed to prolonged stress can change significantly.

Figure 2 shows the free Pro content in wheat culture cells (genotype Volodarka) at the initial and final stages of testing on a salt background.

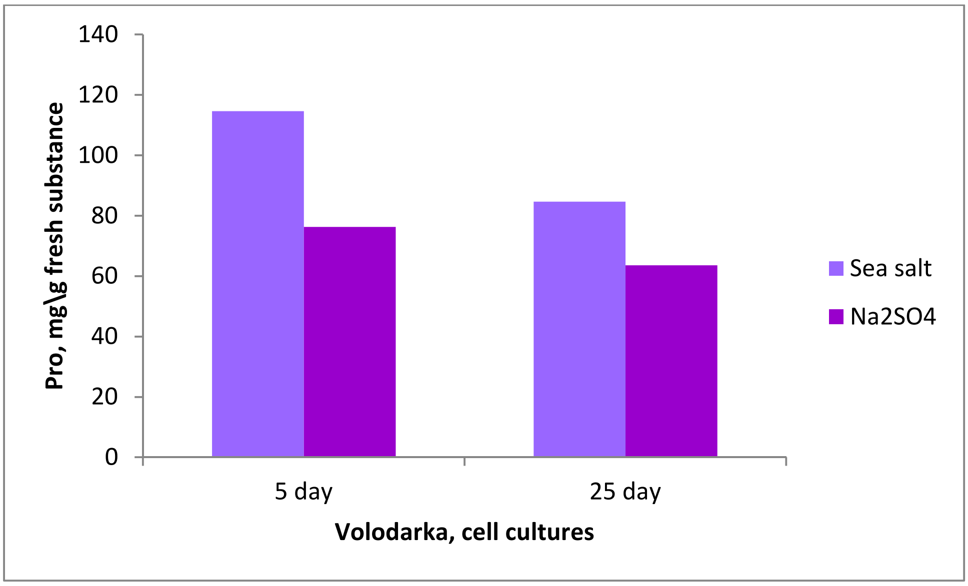

Fig. 2. Free proline content in callus cells of Volodarka wheat variety cultivated under salt stress

Other authors who have studied this parameter also point to the priority of dynamic indicators [9, 12]. Thus, in callus cultures of barley of a number of salt-tolerant genotypes, the amino acid content in cells increased, stabilised, or decreased with increasing cultivation duration. The author believes that this is how the proline-forming ability of genotypes is realised. In our opinion, a high level of cellular Pro does not guarantee the viability of the organism, since its accumulation can occur not only as a result of increased synthesis, but also as a result of the degradation of proline-enriched proteins [1, 16].

In wheat, reserve proteins (gliadin and glutenin) belong to this category of proteins. In wheat grains, they account for 75-85% of the total protein composition; the gliadin/glutenin ratio depends on the specific variety or genotype of the plant. In addition, these proteins are rich in arginine and glutamine. The remaining part of the endosperm proteins consists of albumins and globulins, many of which have enzymatic activity. However, viable cell culture differs qualitatively from grain. Under normal conditions, callus biomass increases as a result of active dynamic metabolism. Under severe stress (salinisation), metabolism decreases, and prolonged exposure may result in cell death due to the destruction of cell structures [13, 17, 18].

Since the experiment showed that callus cultivation on a salt medium was accompanied by an increase in Pro content, electrophoresis of gliadin and glutenin fractions was performed as possible sources of amino acids. The analysis revealed a number of phenomena. First, the extract lacked the gliadin fraction, which, at first glance, could indicate their hydrolysis.

Gliadin, especially ω-gliadin, consists almost entirely of repeating glutamine and proline residues [18]. However, from our point of view, this was a reflection of the properties (reactions) of the cell culture as a research object, since it was observed regardless of genotype and cultivation conditions. The influence of the in vitro system, which affects the nature of intracellular metabolism, cannot be ruled out either. In any case, according to our assumption, this event was not the cause of the increase in proline levels. Electrophoretic analysis of glutenins was also performed. Figure 3 shows the culture indicators on the 25th day.

A significant depletion of the protein pool was observed, regardless of the type of salinity. Since glutenins are also enriched with proline, the increase in the content of this amino acid in cells during salinity could be a consequence of their hydrolysis. Indirect evidence of this may be the decrease in osmolyte content on day 25.

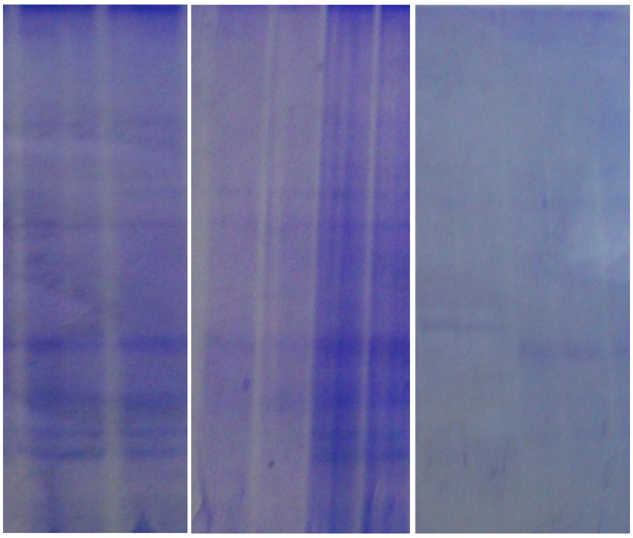

Fig. 3. **Electrophoresis of wheat callus glutenin fractions; 25th day; 1,2 – Volodarka genotype; 3,4 – Zolotokolos genotype; 5-10 – Smuglyanka genotype; 1 – 6 normal conditions; 7,8 – sea water salts; 9,10 – sodium sulphate**

The progressive decline in glutenin reserves led to a decrease in proline. The response of a cell culture to prolonged exposure to any stress can be manifested both in the form of stress-induced pathologies and in reactions associated with maintaining the viability of the system. In our case, in wheat culture, salinisation caused synchronous events of proline content increase and a decrease in the total glutenin pool. This phenomenon is also characteristic of intact plants; such targeted metabolism ensures the stress resistance of young seedlings. In wheat cell culture subjected to prolonged exposure to salt, the consumption of reserve proteins ensures the protection/preservation of physiologically necessary cell compartments. Thus, in our opinion, the observed reactions were a manifestation of the culture's adaptation to stressful conditions. This was evidenced by the resumption of callus growth when transferred to normal conditions in the next passage. However, the relative increase in callus biomass (a classic indicator of cell culture growth and development) in this case was significantly lower than that recorded for cells that did not experience stress. This may support the assumption that further continuation of the stress exposure could have led to a lethal outcome.

Conclusions. Testing of cell cultures of the wheat varieties we studied, obtained from varieties under conditions of prolonged salinisation, showed dynamic development of proline and protein in the adaptive responses of the cell population.

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Броннікова Л. І.
аспірантка, кафедра фізіології рослин та інтродукції
Дніпровський національний університет імені Олеся Гончара,
молодший науковий співробітник
Інститут фізіології рослин та генетики,
Національна академія наук України
Zlenko_lora@ukr.net
orsid.org/0000-0002-8103-0548

Хоменко Л. О.
кандидат сільськогосподарських наук, старший науковий співробітник
відділ генетичного вдосконалення рослин
Інститут фізіології рослин та генетики,
Національна академія наук України
Lidole@ukr.net
orsid.org/0000-0002-3776-6208

Дікун М. О.
інженер 1 категорії
лабораторія якості зерна
Інститут фізіології рослин та генетики,
Національна академія наук України
Mariiadykun@gmail.com
orsid.org/0000-0003-4187-0865

КЛІТИННІ КУЛЬТУРИ ОЗИМОЇ ПШЕНИЦІ (TRITICUM AESTIVUM L.) ЯК МОДЕЛЬНА СИСТЕМА ДЛЯ ВИВЧЕННЯ ДИНАМІЧНОГО МЕТАБОЛІЗМУ ІНДИКАТОРІВ

Випадкові зміни, особливо стреси рослини демонструють істотні метаболічні та структурні модифікації. Переважна їх частина є адаптивними реакціями, які роблять організм більш пристосованим до нових умов. Засолення є найбільш шкодочинними абіотичними стресами. У випадку дії жорсткого стресу ефективність життєдіяльності генотипу стає очевидною через тривалий проміжок часу. Клітинна культура дозволяє уникнути проблем: фізіологічні, біохімічні, та інші зміни, які відбулись на дію стресора. Метою даного дослідження був аналіз вмісту вільного проліну та білку у різних клітинних культур сортів пшениці, які піддавали тривалим осмотичним стресам. Однак, накопичення даного осмоліта, саме по собі, ще не може служити показником успішної адаптації. В умовах жорсткого стресу, істотно обмежує доступність зовнішніх трофічних і енергетичних ресурсів, пролін може виступати резервним джерелом азоту і вуглецю. Тому пул Pro в клітинах, що піддаються дії тривалого стресу, може істотно змінюватися.

При дослідженні динаміки змін в рослинах пшениці ми встановили, що рівень проліну в клітинах калюсу у сорту Володарка знизився в процесі посилення стресового тиску, а синхронне спадання змісту амінокислоти при тривалому сольовому стресі у культури сортів Золотоколоса та Смуглянка склало, відповідно 18,3 ± 3,4% і 21,4 ± 4,6%.

У пшениці, до категорії таких протеїнів відносяться запасні білки (гліадин і глютенін). У зерні пшениці вони становлять 75-85% загального протеїнового складу; при цьому співвідношення гліадин/глютенін залежить від конкретного сорту чи генотипу рослини. Крім того, ці білки багаті аргініном і глутаміном. Частина, що залишилася білків ендосперму доводиться на альбуміни і глобуліни, багато з яких мають ферментної активністю.

Нами спостерігалося істотне виснаження білкового пулу, незалежне від типу засолення. Так як глютенін також збагачені проліном, то зростання вмісту цієї амінокислоти в клітинах при засоленні могло бути наслідком їх гідролізу. Непрямим свідченням може бути зниження змісту осмоліту на 25-у добу. Прогресуюча спад резерву глютенінів приводила до зменшення пролина.

Ключові слова: *засолення, пролін, протеїн, пшениця озима, клітинна селекція.*

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