

## METABOLIC REACTION OF PROLINE AND ITS DISTRIBUTION IN TOBACCO PLANTS AT THE INITIAL STAGES OF DEVELOPMENT UNDER CONDITIONS OF SALT AND WATER STRESS

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*Scientists are actively searching for and introducing plant species that are resistant to adverse environmental factors. The use of introductions enriches the diversity of species composition. The aim of the study was to investigate the distribution of free proline (Pro) in the vegetative organs (aboveground and underground organs) of the tobacco varieties under investigation. Seedlings were subjected to simulated osmotic stresses for 3 hours by adding mannitol (0,8 M) and seawater salts (25,0% g/l). The content of free proline was measured in Samsun and Dubec varieties, while in the experimental samples we observed a characteristic decrease/stabilisation.*

**Key words:** tobacco, proline, salinity, water stress, sustainability, metabolism.

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**Метаболічна реакція проліну та його розподіл у рослин тютюну на початкових етапах розвитку за умов сольового та водного стресів. Броннікова Л.<sup>1,2</sup>**

*Активна діяльність науковців направлена на пошук та привнесення в культуру різновидів рослин до стійкості несприятливих чинників довкілля. Використання інтродукції збагачує різноманіття видового складу. Метою дослідження було вивчення розподілу вільного проліну (Pro) у вегетативних органах (наземних та підземних органах) досліджуваних варіантів тютюну. Проростки впродовж 3 годин піддавали модельованим осмотичним стресам додаванням маніту (0,8 М), та солей морської води (25,0% г/л). Вимірювали вміст вільного проліну в сортів Самсун та Дюбек, тоді як у дослідних зразках спостерігалось характерне зниження/стабілізація.*

**Ключові слова:** тютюн, пролін, засолення, водний стрес, стійкість, метаболізм.

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### Introduction

In general, the stress tolerance of plant organisms is one of the most large-scale and complex problems. This is due to numerous genotype/environment interactions. At the same time, these relationships are constantly changing under the influence of unpredictable factors.

Among abiotic stresses, salinity and drought are the most common. At the same time, the areas that are being lost are increasing, and the qualitative and quantitative diversity of plant forms is decreasing. In the wild, there is a complete loss of certain genotypes. The global population growth is causing an acute shortage of food. In some regions, the issue of a humanitarian catastrophe is being raised. Therefore, the task of obtaining plant forms resistant to abiotic factors is becoming a priority (Ahmed et al. 2023, Atta et al. 2023, Imran et al. 2021, Wu et al. 2022, Islan et al. 2022, Hasegan et al. 2000).

Obtaining a new plant form (the purpose of the experiment) determines the choice/creation of a transgene when it comes to a form with an increased level of stress resistance, it is necessary to operate with indicators that are guaranteed to be associated with phenotypic reactions to maintain active life under stressful conditions. Stress resistance of plants can be maintained both by cellular reactions and by cooperative (coordinated) work of the organism. When both types of defence are combined, a plant may have a specialised metabolism aimed at accumulating a certain physiologically active compound. This compound must meet certain requirements. Firstly, its amount should not adversely affect the course of biochemical reactions and disrupt key metabolic chains. Secondly, the compound can be transported to separate compartments both within the cell and between organs.

Compatible low-molecular weight protective compounds play a significant role in a number of plant

defence mechanisms against osmotic stresses. First of all, L-proline (*pro*). It is known that in general, the level of free pro is self-regulated by the synthesis/degradation/transport system (Kaur et al. 2017, Yang et al. 2021, Funck 2020). The synthesis gene is  $\Delta 1$ -pyrroline-5-carboxylate synthetase (P5CS); the degradation gene is proline dehydrogenase (PDH); pro is transported by a system of intrinsic and common transporters (Ray, Penna 2013, Dubrovna et al. 2022).

It was found that under stressful conditions, pro accumulates in significant amounts. It is a product of synthesis within the cell. The high content of pro in certain plant organs can occur as a result of synthesis, as well as as a result of its movement from the synthesis zone (Kaur et al. 2017, Alvarez et al. 2021, Islam et al. 2022). It is also an established fact that there is a relationship between the development of a plant organism and the activity of one of the enzymes that regulate the level of free pro, namely proline dehydrogenase (PDH) (Zou et al. 2023, Yang et al. 2021, Dubrovna et al. 2022). Literature data indicate achievements in obtaining plants with an increased level of resistance to osmotic stress using constructs related to proline metabolism (Alvarez et al. 2022, Mykhalska et al. 2021, Dubrovna et al. 2021, Munaweera et al. 2022).

Since the accumulation of proline in an intact plant depends on a number of factors, it is advisable to perform a comparative analysis of the plant and a cell culture that can be obtained from the plant for correct interpretation. The cell culture response can further identify specific and nonspecific adaptation responses.

### Methods and materials

The object of the study was young tobacco seedlings of the Samsun and Dubec varieties. Osmotic stresses were created by adding mannitol or seawater salts to a semi-dilute solution of macronutrients according to Murashige-Skoog. Mannitol, concentration 0.5 M, modelled water stress; sea water salts (sea salt), concentration 20,0 g/l, modeled natural complex salinity.

Seedlings were immersed in experimental solutions with their roots. The duration of osmotic stress was 3 hours. Plants kept in a semi-dilute solution of macro salts served as a control. After a short-term stress, the experimental plants were subjected to recovery in the control solution.

After 3 hours of stress exposure and after 3 hours of recovery, the content of free proline was measured in the plants. The pro content was measured in seedlings (aerial part) by cutting off the roots according to the standard method (Andriushchenko et al. 1981). The data were statistically processed.

### Results and discussion

In general, diagnosing the level of plant viability is important for breeding. It is known that the damaging effect of salinity and water stress increases in proportion to the duration of the factors. Obviously, at the initial stages of stress, there can be no visual difference between the forms. In our case, the appearance of seedlings was similar in all variants. At the same time, given the sensitivity of the systems associated with the regulation of pro levels, changes in this direction would be quite expected. At the 3rd hour of stress exposure, the level of free pro was measured.

Under normal conditions, (n.o.), before the experiment, the level of pro in winter wheat genotypes was insignificant and amounted to mg% /crude matter, in tobacco plants of Samsun and Dubec varieties, respectively,  $9,94 \pm 1,44$  and  $14,53 \pm 2,25$ . In general, changes in the level of pro occur while maintaining the overall metabolism and do not go beyond the normal reaction. They are possible due to the coordination of the activity of synthesis/degradation systems (Kaur et al. 2021, Alvarez et al. 2022, Mykhalska et al. 2021, Yang et al. 2021, Funck et al. 2020, Rai et al. 2013, Dubrovna et al. 2022). The absence of contradictions is maintained by the spatial separation of enzyme functioning compartments.

Changes in amino acid levels after two hours are shown in figure 1.

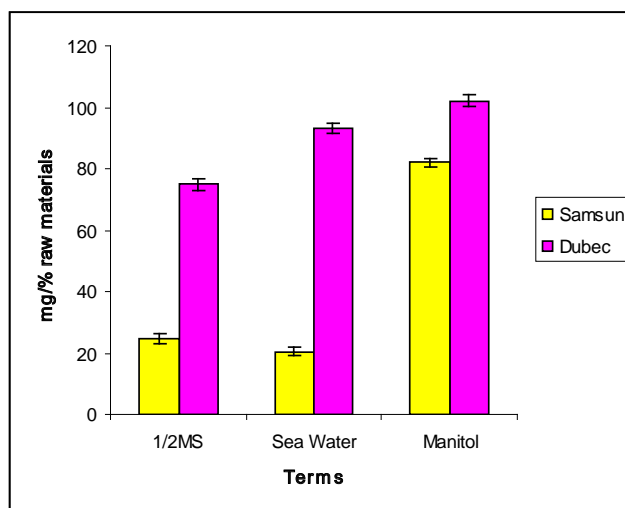


Fig. 1. The content of free proline in the aerial parts of *Nicotiana tabacum* L. plants 3 hours after the onset of osmotic stress

The diagram shows a significant increase in the content of free pro two hours after the start of the experiment. This fact was evident under all external conditions. Under normal conditions, the increase in the level of the amino acid was more significant than

in the control plants. This event can most likely be explained by a decrease in the level of *pro* degradation in these variants.

The two-hour stress caused significant changes in the accumulation of free *pro*. It should be noted that the nature of the changes in the variants was identical, depending on the type of stress factor, namely, a decrease in the *pro* content against salinity (ionic stress) and an increase in this indicator in the presence of mannitol (molecular osmotic).

Under stressful conditions, the level of *pro* is determined by its synthesis system. In the experiment, an aqueous solution of trace elements ensured its flow. In addition, in this experiment, this assumption is supported by the fact that the content of the amino acid in the transformed and control objects under the influence of salinity was similar. Seawater salts slightly reduced the activity of proline synthesis at the second hour of stress compared to the normal parameters, equally in both variants.

The effect of mannitol was manifested in different ways. In the control variants, the absolute value of *pro* content coincided with the indicators of non-stressed plants. In transgenic variants, the level of proline increased. It is possible to assume that in the first case, the stressful effect on metabolism did not become harmful; in the second case, the fact of stimulation is permissible. In our opinion, the movement of proline from the root part is less likely, since moisture loss was minimised by the use of an aqueous solution. Perhaps, this is the indirect effect of the introduced construct, since the direct effect of the *PGD* gene is not realised under stress conditions.

Thus, analysing the nature of free proline accumulation at the initial stages of salt and water stress, it becomes obvious that there is a reorganisation of the functioning of its metabolic systems. This event contributes to the maintenance of the overall metabolism, which is visually manifested in the absence of stress lesions.

In general, the activity of the organism's vital functions should be most pronounced when the environment changes (Ahmed et al. 2022, Atta et al. 2023, Mykhalska et al. 2022, Huizbers, et al. 2017, Maghsoudi et al. 2018). After the stressful effect, the plants were restored in a semi-dilute solution of microelements according to Murashige-Skug. The level of *pro* was analysed 3 hours after the start of rehabilitation. The data are shown in figure 2.

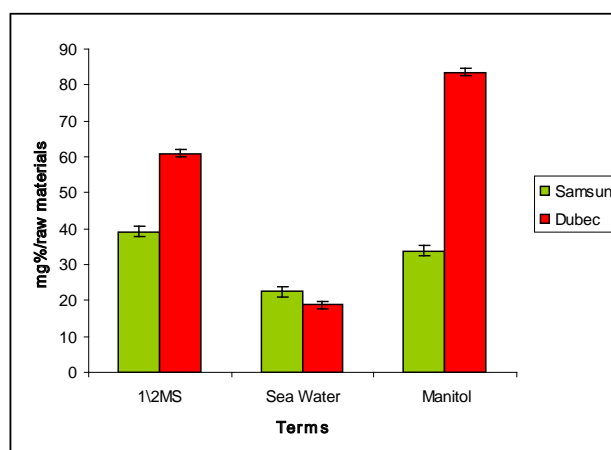


Fig. 2. Free proline content in the aerial parts of tobacco plants 3 hours after the beginning of recovery from osmotic stresses

Under normal conditions, no changes in amino acid accumulation occurred, which confirms the stable course of metabolic reactions, both general and associated with *pro* metabolism, in all variants.

Comparison of figures 1 and 2 showed that the stress → normal transition was manifested in different ways in the control and experimentally obtained variants. Thus, recovery from salinity did not lead to changes in the level of free *pro* in the original form, but caused its increase in resistant plants. The following assumption can be made about the reasons.

In all variants, the absence of stress load restored the activity of the *PGD* gene in parallel with the decrease in the activity of the *pro* synthesis gene. In the control variant In the control variant, activation/decrease events occurred with the same intensity, which was reflected in the stabilisation of *pro* levels. In the resistant variants, the activity of the *PGD* gene was significantly inferior to that of the *P5CS* gene due to the efficiency of the introduced construct. The difference between the forms observed at the 3rd hour of recovery, in our opinion, is a manifestation of individual peculiarities of functioning.

Recovery after mannitol exposure was different. Comparison of Figures 2 and 3 revealed an increase in the level of *pro* in the aerial part of all variants two hours after the stress pressure was removed. The increase in control and resistant forms was 14,7% and ~ 44%, respectively. In the analysis of *pro* during recovery from salinity, it is possible that the accumulation of proline in the aerial parts under the action of molecular osmosis occurred due to its synthesis in

these organs and additional movement from the root part, where the amino acid was synthesised.

Salt and water stress are both types of osmotic stress. At the same time, both are distinguished by their peculiarities in terms of the mechanism of their harmful effects. Water stress (drought) causes significant dehydration of the plant organism and a set of associated pathological transformations of various compartments. Salt stress has a damaging effect due to the ionic structure of salt molecules. The variety of salinity types and the combination of several types of salts can introduce additional components into the mechanism of toxicity.

In both cases, the aggressive force of osmotic stresses is determined by the duration of their impact, during which negative changes increase and may become irreversible. Although resistance to osmotic stresses can be manifested in different ways, plants combine different resistance strategies.

The above suggests that adaptation to osmotic stresses is limited and depends on a number of factors.

First, it is the nature of the genotype that determines the sensitivity/resistance of the organism. Secondly, the variability of adaptation in response to external changes. Third, the availability of resources to maintain vital functions under stressful conditions. Fourth, the ability to recover quickly.

At the same time, we observed different types of adaptations to salinity and water stress. At the same time, we observed different types of recovery.

### Conclusions

The data obtained from the analysis of the nature of free proline accumulation allow us to draw the following conclusions. The effect of salt and water stress on the proline metabolism system is observed at the initial stages of plant development and under the influence of stress factors. The adaptation of control and resistant plants to salinity and water stress is different. The level of free proline under stressful conditions in tobacco plants is maintained due to its increased synthesis.

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