Content of prolin and essential amino acids in spring wheat grain in dry conditions

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Abstract. The article presents the results of the analysis of amino acid composition of grain of spring soft and durum wheat varieties by the amount of proline and essential amino acids under conditions of favorable and dry years in terms of weather factors. An increase in the amount of proline was established under conditions of temperature stress and a lack of moisture in soil, it allows us to consider this fact as an index of resistance to unfavorable environmental factors. The amount of essential amino acids depended on weather and technological factors. The content of the amino acid proline in spring wheat grain should be considered as a signaling function for stressful vegetation conditions. The content of essential amino acids in spring wheat grain increases in favorable years and against the background of moldboard plowing.

1. Introduction

Drought is the main abiotic stressor, significantly repeated in recent years in the main grain-growing regions including Orenburg region. The probability of a severe and very severe drought in the region in May-June is 36%, in July-August it is 44% [1].

Spring wheat is a moderately thermophilic crop and main life cycles normally proceed at temperatures from 13 to 18°C in the tillering phase, 17-19°C in the tillering-earring period and 18-22°C during the earring-ripening period [2]. Changes in the temperature-humidity regime lead to disturbances in the growth and development of plants.

Therefore, according to L.A. Germantsev, V.A.Krupnov [3], in the conditions of chestnut soils of the Trans-Volga region of Saratov region at an average temperature of spring wheat growing season of 16°C, its duration is 95 days, and at 21°C it decreases to 70-75 days. At the same time, an increase in the average air temperature for each 1°C leads to a decrease in yield by an average of 10%.

The negative effect of an increase in temperature can be manifested by an earlier onset of wheat flowering (by one week with an increase in temperature by 1°C) while maintaining the duration of the period from flowering to ripening [4]. Limitation of productivity because of warming is also possible because of an increase in water consumption due to increased transpiration [5].

The parameters of photosynthesis, as well as other indicators, such as leaf temperature, stomatal condition, and biomass accumulation, can be used as indicators of resistance to environmental stress [6]. Plants react to damaging environmental factors both at the level of organism and cells, and at the same time, at different periods of development, different morphological phenotypic characters play a key role in a resistance to drought [7].
Proline is one of these regulators [8]. Proline contributes to the automatic regulation of plant cells to maintain their activity [9] and protects membranes and proteins from harmful effects of high ion concentrations and extreme temperatures [10, 11].

For monocotyledon plants under conditions of water shortage, salinity and osmotic stress, the accumulation of proline is one of the general characteristics [12]. When photosynthesis is limited because of stomatal closure due to the damaging effect of reactive oxygen species, the accumulation of proline is a response. According to Kenny Paul1 et al. [13], the accumulation of proline is a consequence of the joint negative effect of salinity and drought.

The researchers of this issue conclude that proline helps to stabilize membrane and the redox potential of cells, disturbed due to drought and heat stress [14-16].

According to Saffar Abdul (2020) [17], the greatest increase in proline was observed under combined stress (drought + heat stress, - 69.8%) than under the separate influence of these stress factors (by 53.05 and 58.9%, respectively).

The amount of essential amino acids in the human body (valine, leucine, isoleucine, lysine, methionine, threonine, tryptophan, phenylalanine) and conditionally essential amino acids (histidine and arginine) determine the normal course of many physiological functions, and the content of three of them considered critical (lysine, methionine, and tryptophan) limit the use of other amino acids for protein synthesis [18, 19].

Under conditions of combined stress (drought + high temperature), according to some researchers [20, 21], there is an increase in the level of amino acids, in particular, valine, tyrosine, tryptophan. In works of some authors [22, 16] the content of certain essential amino acids (leucine, valine, alanine) in plants accumulates under the influence of drought stress.

2. Problem statement

Plants have developed certain reactions to stress from unfavorable weather factors, one of which is the accumulation of proline in the body during the growing season. The formation of proline in ripe grain is less studied and there is little data on it as the final product of plant development.

Our research addresses this aspect of plant response to drought. In the conditions of Orenburg Cis-Urals, the period of grain ripening is especially arid, therefore, data on the nature of formation of amino acid composition of grain, in particular, proline and essential amino acids, is one of links in the study of plant resistance to drought in this area.

The aim of the research is to analyze grain amino acid composition of spring soft and durum wheat varieties by proline and the content of essential amino acids at different air temperature conditions and moisture reserves in soil during the growing season and sowing according to different methods of basic soil cultivation.

3. Methods and materials

The varieties of spring soft wheat Uchitel and Ulyanovskaya 105 and varieties of spring durum wheat Orenburgskaya 10 and Bezenchukskaya 210 were the objects of study. The varieties belong to the mid-season type.

The varieties were sown against the background of two methods of basic tillage - plowing and nonmoldboard loosening. The agrotechnics in the experiment corresponded to the requirements for the establishment of field studies. The seeding rate is 4.5 million viable seeds per hectare.

The analysis of seeds for the content of amino acids was carried out on the equipment of the Test Center of the Center for Collective Use of the BST RAS in accordance with GOST 55569-2013 using a capillary electrophoresis system "Kapel 105".

The study of correlations was carried out according to Spearman's method [23]. The coefficients of variation and the share of the contribution of the studied characters to the variance were according to B.A. Dospekhov [24].

Weather factors of 2017 are characterized as favorable, especially during the period of vegetative development (HTC of June is 0.59, of July it is 0.66) with dry period of grain ripening (HTC 0.06). In
2018, the beginning of the growing season (May) was optimal in terms of the hydrothermal regime (0.60), and later the conditions were characterized by acute aridity (June HTC 0.19, July HTC 0.22). 2019 is characterized by high aridity of the active growing season (HTC 0.18 in May, 0.17 in June) and high moisture supply (HTC 1.36) during grain ripening period. Growing conditions 2018 and 2019 regarded as unpleasant.

4. Results discussion
After analysis of the amount of proline in grain of spring soft and durum wheat varieties, it was found that it significantly increases in dry years with a lack of moisture in the soil against the background of the extreme temperature regime of air (figure 1).

On average, according to experience, the increase was 0.74% with fluctuations over the years from 0.59% (in 2018) to 0.89% (in 2019), i.e. up to a twofold increase. There is a tendency for a greater increase for proline against the background of soil cultivation by moldboard plowing in comparison with non-moldboard loosening of fall plowing. The aridity of the first half of the growing season of spring wheat (2019) leads to a more significant increase in the amount of this amino acid in comparison with the sharp dryness of the second half of the growing season (2018).

Calculation of the dependence of the proline content in grain of spring wheat on the hydrothermal coefficient of the growing season showed the correlation coefficients for May −0.58, June −0.58, and July +0.80.

![Figure 1](image)

**Figure 1.** The content of proline in grain of spring soft and durum wheat varieties.

In durum wheat variety Bezenchukskaya 210, the proline content in grain in dry years was less than in other durum wheat variety Orenburgskaya 10. Soft wheat varieties did not have significant differences in this indicator.

The results obtained in our experiments show that an increase in the amount of proline under drought conditions is one of the indices of resistance to a lack of moisture against a background of temperature stress. This is also emphasized by [25-28], who found that the proline content is the best indicator for assessing the drought resistance of wheat.

The nutritional value of protein is determined by the amount and ratio of essential amino acids. The amino acid composition depends on the genotype, weather factors, doses and type of fertilizers, and other factors. In an arid zone, grain formation occurs under the influence of high air temperatures and a lack of soil moisture. At the same time, the presence of quantitative and qualitative changes in the amino acid composition of spring wheat grain cannot be ruled out. Calculation of the coefficient of variation
of the content of essential amino acids for different methods of soil cultivation, depending on weather factors, revealed variety-specificity (table 1).

In spring soft wheat Uchitel, a significant variation of this trait is found in lysine during sowing against the background of nonmoldboard loosening and valine in both tillage options. For cultivar Ulyanovskaya 105, high variation was obtained for arginine against the background of tillage and for valine for both backgrounds of autumn plowing. In durum wheat Orenburgskaya 10 having a general low variation in the amino acid content from weather conditions, the amino acid valine and histidine were characterized by a higher coefficient of variation against the background of nonmoldboard loosening of autumn plowing.

**Table 1.** Coefficients of variation of the content of essential amino acids in grain of spring soft and durum wheat varieties according to the options for basic tillage, %.

| Amino acid     | Tillage method          | Uchitel | Ulyanovskaya 105 | Orenburgskaya 10 | Bezenchukskaya 210 | Average |
|----------------|-------------------------|---------|------------------|------------------|-------------------|---------|
| Arginine       | tillage                 | 14.10   | 40.08            | 4.92             | 43.03             | 25.53   |
|                | nonmoldboard loosening  | 14.52   | 2.71             | 9.95             | 2.50              | 7.42    |
| Lysine         | tillage                 | 5.15    | 6.64             | 8.06             | 24.04             | 10.97   |
|                | nonmoldboard loosening  | 19.21   | 11.25            | 3.45             | 20.32             | 13.56   |
| Phenylalanine  | tillage                 | 11.09   | 6.18             | 4.76             | 18.76             | 10.20   |
|                | nonmoldboard loosening  | 7.94    | 16.16            | 10.49            | 10.30             | 11.22   |
| Histidin       | tillage                 | 5.39    | 6.19             | 8.11             | 20.40             | 10.02   |
|                | nonmoldboard loosening  | 10.18   | 16.46            | 12.03            | 9.80              | 12.12   |
| Leucin + Isoleucine | tillage            | 2.68    | 9.83             | 3.06             | 22.81             | 9.59    |
|                | nonmoldboard loosening  | 18.44   | 12.02            | 7.20             | 13.92             | 12.89   |
| Valin          | tillage                 | 24.16   | 20.15            | 12.62            | 26.77             | 20.92   |
|                | nonmoldboard loosening  | 28.87   | 24.05            | 10.19            | 21.12             | 21.06   |
| Threonine      | tillage                 | 4.32    | 9.88             | 1.47             | 22.21             | 9.47    |
|                | nonmoldboard loosening  | 11.67   | 9.27             | 8.81             | 17.89             | 11.91   |
| Average        | according to tillage    | 9.56    | 14.14            | 6.14             | 25.43             | 13.81   |
|                | nonmoldboard loosening  | 15.83   | 13.13            | 8.87             | 13.69             | 12.88   |
| Average        |                         | 12.69   | 13.63            | 7.51             | 19.56             | 13.35   |

For the durum wheat variety Bezenchukskaya 210, the highest average variability of the amino acid content in the grain (19.56%) was obtained with a higher value against the background of plowing (25.43%). At the same time, this variety obtained the highest variability in the content of amino acids (from 20.40% for histidine, to 43.03% for arginine according to the background of plowing and from 20.32% for lysine to 21.12% for valine against the background of nonmoldboard loosening).

Thus, the content of amino acids in the grain of spring wheat is a rather variable indicator with varietal characteristics, determined by weather factors and agricultural techniques.

The study of the contribution of the factors under consideration to the content of amino acids showed (table 2) that the varieties had the least influence, with the exception of the indicator for histidine (contribution of 13.80%). The greatest contribution to the content of amino acids was made by weather factors with an inhibitory effect on the amount of proline, rather significant for valine and a practical absence for histidine.
A rather significant (11.03-23.40%) effect of soil tillage methods on the content of amino acids phenylalanine, histidine and threonine should be noted. In addition, the interaction Variety-Tillage had a high influence (9.90-16.97%) on the dispersion of lysine, leucine + isoleucine and histidine.

Table 2. Contribution of the studied factors to the dispersion of the amino acid content in grain of spring wheat varieties.

| Amino acids      | Contribution (%) to the variance of amino acid content |
|------------------|-------------------------------------------------------|
|                  | Weather conditions | varieties A | tillage method B | Interaction AB |
| Arginine         | 28.4               | 0.22        | 0.74             | 3.34           |
| Lysine           | 45.3               | 1.74        | 4.97             | 9.90           |
| Phenylalanine    | 20.2               | 6.57        | 23.4             | 4.98           |
| Histidine        | 8.3                | 13.8        | 19.0             | 16.97          |
| Leucine +        | 49.5               | 0.86        | 1.83             | 11.69          |
| Isoleucine       |                    |             |                  |                |
| Valine           | 67.9               | 0.32        | 0.09             | 5.65           |
| Threonine        | 28.6               | 3.00        | 11.03            | 5.39           |
| Proline          | 93.15              | 1.59        | 0.06             | 1.03           |

The study of the relationship between amino acid content and the yield of spring wheat showed that the correlation coefficients (according to Spearman [22]) vary from the average positive for lysine (0.55), valine (0.59), leucine and isoleucine (0.51), weakly positive for arginine (0.40), to significantly negative for proline (-0.76) and insignificant for phenylalanine (0.04) and histidine (0.26).

Improvement of growing conditions contributes to an increase in the content of essential amino acids (figure 2).

Differences on average for amino acids in favor of the values of a favorable 2017 reach 0.06-0.10% in absolute terms, which is 10.2-16.9% in relative terms for plowing; on the background of non-moldboard loosening, these differences are within 0.07-0.11% in absolute values and 12.3-19.3% in relative values. There is a tendency towards an increase in the amount of essential amino acids against the background of plowing.

Figure 2. The content of essential amino acids in grain of spring wheat according to options for basic tillage, % for absolutely dry matter.
5. Conclusion
The content of proline in grain of spring wheat should be considered as a signal function for stressful vegetation conditions.

The content of essential amino acids in grain of spring wheat increases in favorable years and after moldboard plowing.

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