Physiological features of the accumulation and distribution of whole protein in the organs of plants of soybean varieties of the Northern ecotype

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Abstract. Soybean is a valuable protein crop. Promotion of its production in the northwestern direction became possible by the creation of varieties of the Northern ecotype. The physiological mechanisms of the adaptive potential of these varieties and, in particular, the features of protein accumulation in grain under contrasting weather conditions are insufficiently studied. Physiological factors that determine the level of whole protein accumulation in vegetative organs and in plant grain were studied on 7 varieties and 2 lines of breeding of the FRC of LGC. Effective assimilation activity of leaves; adaptive changes in the pigment complex, consisting in an increase in the number of carotenoids in adverse conditions; active nitrogen fixation of nodule bacteria lead to increased accumulation of nitrogen in the plant, followed by its remobilization into generative organs. The intensity of nitrogen accumulation and reutilization processes in soybean varieties of the Northern ecotype is especially high in unfavorable conditions of high humidity and lack of heat. In adverse weather conditions, correlations were established between the protein content in leaves and grains and elements of the pigment and symbiotic systems.

1. Introduction

Soybean surpasses most of the world’s cultivated crops in terms of whole protein content. According to some authors, the seeds of this crop can accumulate an average of 38-42% protein with a variation of this indicator from 30 to 50%. The amount of protein in legumes is determined by genotypic features and conditions of cultivation (availability of heat, moisture, nutrients, etc.). Abiotic factors affect the totality of physiological processes of the plant organism, indirectly affecting the accumulation of protein in the grain [1, 2].

The protein complex of legume seeds is formed by the following sources of nitrogen compounds: nitrogen absorbed by the root system and converted by the latter to the level of low-molecular organic compounds (amino acids, amides, peptides); atmospheric molecular nitrogen fixed by nodule bacteria; nitrogen-containing photosynthetic products transported from leaves to seeds; amino acids of proteolized proteins of aging vegetative organs or reutilization. Donor-acceptor relationships of vegetative and generative organs are the leading mechanism in plant adaptation to environmental stress [3, 4].

Soybean varieties of the Northern ecotype are resistant to low temperatures, however, the physiological mechanisms of their adaptive capabilities are not fully disclosed. The study of the formation of qualitative characteristics, including the amount of whole protein in vegetative organs and in grain in
various weather conditions, allows understanding better the adaptive features of new soybean varieties, due to which the production of this crop in the Central regions of the Russian Federation is cost-effective.

The negative correlation between the protein content in grain and the grain yield is an urgent problem in the cultivation of legumes and soybeans. One of the ways to solve this problem is to study the processes of accumulation and distribution of nitrogen in the organs of soybean plants, depending on external conditions.

In connection with the above, the aim of this work is to study the physiological reactions of soybean plants in contrasting weather conditions and their effect on protein accumulation in vegetative organs and in grain.

2. Materials and methods

In 2017-2019, in the field on soybean varieties Zusha, Krasivaya Mecha, Lancet, Mezenka, Svapa, Osmon, Shatilovskaya 17, lines L-216, and L-85 of the breeding of the FRC of LGC. Soybean seeds were treated with strain 634b before sowing. The seedling rate of soybean seeds is 600 thousand germinating seeds/ha. The plot area is 7.5 m², 4-fold replication. The following records and observations were made: sampling for analysis during the budding and full maturation phases [5]; accumulation of protein was determined by the Kjeldahl method using a Velp Scientifica analyzer. The nitrogen content of seeds is calculated using the formula \( \frac{N_{\text{biomass, mg}}}{\text{seed mass, g}} \). The level of nitrogen reutilization was calculated by the balance method as a decrease in its absolute content in vegetative organs during the period of seed filling as a percentage of the maximum content.

The years of research differed by weather conditions (table 1). 2017 was characterized by a temperature 2-3°C below the long-term average one and increased humidity during the germination-budding period, when precipitation exceeded the annual average by 60%, HTC = 1.9. Weather conditions in 2018 and 2019 differed from the normal values of temperature and humidity during the growing season. In 2018-2019, during flowering, the temperature was higher than normal by 3-4°C, precipitation was 30% of the norm. 2018 is generally warm, slightly arid: the sum of effective temperatures for the growing season exceeds the average annual temperature by 352°C, HTC = 1.1. In 2019, there was a lack of heat during fruit formation, and an excess of moisture during bean filling, which negatively affected the formation of fruits. 2019 is quite water-rich, HTC = 1.7.

| Indicators | Months | Average air temperature for the month, °C | \( \Sigma \geq 10^\circ C \) | HTC= | \( \frac{\Sigma \text{precipitation, mm}}{10} \) | \( \sum \text{effect, tt} \) |
|------------|--------|------------------------------------------|----------------------|--------|--------------------------|---------------|
| Average    |        |                                          |                      |        |                          |               |
| long-term  |        |                                          |                      |        |                          |               |
| 2017       | May    | 13.8                                     | 1929.1               | 1.90   |                           |               |
|            | June   | 16.8                                     | 2121.3               | 1.13   |                           |               |
|            | July   | 18.0                                     | 1927.1               | 1.73   |                           |               |
|            | August | 17.0                                     | 16.0                 |        |                          |               |
|            | September | 11.7                                |                      |        |                          |               |
| The rainfall for the month, mm | | | | | | |
| Average    |        |                                          |                      |        |                          |               |
| long-term  |        |                                          |                      |        |                          |               |
| 2017       | May    | 51.0                                     | 366.0                |        |                           |               |
|            | June   | 73.0                                     | 239.9                |        |                           |               |
|            | July   | 81.0                                     | 334.6                |        |                           |               |
|            | August | 63.0                                     |                      |        |                           |               |
|            | September | 67.0                               |                      |        |                           |               |

2.
3. Results

The content of whole protein in the leaves of soybean varieties during the flowering phase ranged from 18.5% in Shatilovskaya 17 in 2019 to 32.1% in Osmon in 2017 (Fig. 1). The maximum concentration of whole protein in leaves for an average of 3 years in the varieties Mezenka (24.1%) and Osmon (24.6%). On average, the plant varieties accumulated whole protein in the stems in 2017 – 17.7%, in 2018 – 7.7%, in 2019 – 7.7%; in the roots – 12.8%, 7.5%, 6.5%, respectively.

**Figure 1.** Whole protein content in leaves during the flowering phase.

In most varieties, the maximum content of whole protein in the grain was noted in the wettest of 3 years in 2017, the values of this trait varied from 39.6% in Mezenka to 43.0% in L-216 (Fig. 2). Krasivaya Mecha is a high-protein variety, in terms of the amount of whole protein in the grain on average for 3 years, it surpassed the other varieties by 1.5-4.0%. The most favorable conditions for the accumulation of nitrogenous substances for this variety were formed in 2019, when the concentration of whole protein in the grain reached 44.0%. The L-85 line, on the other hand, is characterized by low protein content; on average, 38.7% of raw protein was observed in the grain over 3 years.

**Figure 2.** Whole protein content in grain.

In a favorable 2018, the content of whole protein in leaves, nodules and grains on average for varieties is the lowest of 3 years, respectively, 20.8%, 25.8% and 39.7% (table 2). The maximum content of whole protein in leaves (28.6%) as well as in grain was observed in the wettest 2017. This year, 28.8% of whole protein was found in nodules. In 2019, the concentration of whole protein in the
leaves is only 20.1%, but in the nodules this figure is the highest during the research period of 30.6%, and the protein content in the grain is 40.0%.

Table 2. Whole protein content, %.

|                | in leaves (flowering) | in nodules (flowering) | in grain |
|----------------|-----------------------|------------------------|----------|
|                | 2017 | 2018 | 2019 | 2017 | 2018 | 2019 | 2017 | 2018 | 2019 |
|               | ß    | CV, % |       | ß    | CV, % |       | ß    | CV, % |       |
| Reutilization of nitrogen from vegetative organs, % | 68.5 | 47.0 | 53.5 | 40.0 | 75.8 | 25.0 |
| Number of active nodules | 27.6 | 45.0 | 13.7 | 19.0 | 43.6 | 34.0 |
| Active dry weight of nodules, mg/plant | 169.0 | 75.0 | 133.1 | 25.0 | 174.8 | 42.0 |
| Content of Cl a+b, mg/g ACV | 9.1 | 14.0 | 13.4 | 11.0 | 8.7 | 5.0 |
| Nitrogen supply of seeds | 72.0 | 4.0 | 69.0 | 3.0 | 70.0 | 5.0 |
| Amount of nitrogen in the biomass, mg/plant | 849.0 | 10.0 | 669.1 | 20.0 | 492.0 | 26.0 |
| Grain yield, t/ha | 2.3 | 14.0 | 3.3 | 9.0 | 1.8 | 21.0 |
| Collection of whole protein of grain, t/ha | 0.95 | 14.0 | 1.25 | 16.0 | 0.73 | 21.0 |

Redistribution and translocation of nitrogen from vegetative organs to grain or reutilization was maximum in 2019 and amounted to 75.8%, in 2017 – slightly lower than 68.5%, in 2018 this indicator is the minimum for the years of research 53.5% (table 3). The number of active nodules in 2019 is higher than in 2017-2018 by 37-69%, the mass of nodules is 3-24%. The highest content of CL a+b in leaves was observed in 2018. The highest nitrogen content of seeds in 2017 is 72.0. The total nitrogen content of a plant depends on the amount of its biomass and the percentage of nitrogen in it. The maximum amount of nitrogen accumulated in 2017 is 849.0 mg/plant. The whole protein harvest was 0.95 t/ha in 2017, 1.25 t/ha in 2018, and 0.73 t/ha in 2019.

Table 3. Economically valuable indicators of soybean varieties.

| Indicators                        | 2017 | CV, % | 2018 | CV, % | 2019 | CV, % |
|-----------------------------------|------|-------|------|-------|------|-------|
| Reutilization of nitrogen from vegetative organs, % | 68.5 | 47.0 | 53.5 | 40.0 | 75.8 | 25.0 |
| Number of active nodules          | 27.6 | 45.0 | 13.7 | 19.0 | 43.6 | 34.0 |
| Active dry weight of nodules, mg/plant | 169.0 | 75.0 | 133.1 | 25.0 | 174.8 | 42.0 |
| Content of Cl a+b, mg/g ACV       | 9.1 | 14.0 | 13.4 | 11.0 | 8.7 | 5.0 |
| Nitrogen supply of seeds          | 72.0 | 4.0 | 69.0 | 3.0 | 70.0 | 5.0 |
| Amount of nitrogen in the biomass, mg/plant | 849.0 | 10.0 | 669.1 | 20.0 | 492.0 | 26.0 |
| Grain yield, t/ha                 | 2.3 | 14.0 | 3.3 | 9.0 | 1.8 | 21.0 |
| Collection of whole protein of grain, t/ha | 0.95 | 14.0 | 1.25 | 16.0 | 0.73 | 21.0 |

4. Discussion

Weather conditions in 2017 and 2019 (lack of heat and excessive humidity) negatively affected the productivity of soybeans: the grain yield is lower by 30% and 45%, respectively, than in 2018 (table 3). However, the content of whole protein in leaves is highest in 2017, and in nodules – in 2017 and 2019. High nitrogen-fixing activity of nodule bacteria plays an important role in the formation of the protein complex of legumes. In 2017 and 2019, the number and mass of active nodules is higher than in 2018, which contributed to a more intensive accumulation of nitrogen in plants. The correlation between the protein content in leaves and its concentration in nodules in 2019 was found at a high level of r=0.735.

An important stage of nitrogen metabolism is its redistribution between vegetative and generative organs. Thus, the proportion of reutilized nitrogen in soybean grain is 55-85%, depending on weather conditions [9, 10]. In the cooler 2019, the level of reutilization is the highest of the 3 years of research. In other words, under unfavorable conditions, soybean varieties increase the reutilization of nitrogen from the vegetative organs and root system to seeds, which indicates a high level of self-regulation of soybean varieties depending on weather conditions.

The content of pigments is an important factor in endogenous protein formation. In the wettest 2017, a negative correlation was found between the amount of CL a+b and the protein content in
leaves and grain at the level of $r = -0.467$. A positive relationship was noted between the amount of carotenoids and the protein concentration in grain in 2017 ($r = 0.563$) and between the amount of carotenoids and the protein content in leaves in 2019 ($r = 0.493$).

The accumulation of protein in the grain depends on the nitrogen content in the plant per unit weight of the grain and is expressed by the coefficient of nitrogen supply of the grain [11]. In our research, this indicator is the lowest in a favorable 2018 year, as an increase in yield with a limited nitrogen fund in plant biomass leads to a decrease in nitrogen in seeds. A correlation was established between the protein content in leaves and in grain in 2018 at the level of $r = 0.623$.

The amount of nitrogen in a plant depends on the amount of its biomass and the percentage of nitrogen in each of the organs. This indicator in 2018, despite the relatively low protein concentration in the grain, due to the high grain productivity, is at an average level compared to 2017 and 2019 and is equal to 669 mg/plant. The collection of whole protein with grain in 2018 is the maximum and is 1.25 t/ha. In this regard, setting to high productivity with average protein content in seeds can solve the problem of negative correlation between yield and protein content.

The coefficient of variation is a relative measure of variability. The protein content in leaves, nodules and grains, as well as the nitrogen content of seeds, vary slightly by variety at the level of 3 to 12%. The values of reutilization were particularly unstable, which ranged from 25-47% for varieties.

5. Summary
Nitrogen entry into the plant, its distribution to the organs and accumulation in the grain is a complex process, the leading role in which is played by physiological mechanisms. Effective assimilation activity of leaves; adaptive changes in the pigment complex, consisting in an increase in the number of carotenoids in adverse conditions; active nitrogen fixation of nodule bacteria lead to increased accumulation of nitrogen in the plant, followed by its remobilization into generative organs. The intensity of nitrogen accumulation and reutilization processes in soybean varieties of the Northern ecotype is especially high in unfavorable conditions of high humidity and lack of heat. However, in years with an optimal combination of temperature and moisture availability, despite the decrease in nitrogen concentration in plant organs, due to high grain yield, the collection of whole protein is maximum.

The following correlations were established: in the wettest 2017, between the amount of CL $a+b$ and the protein content in the leaves and in the grain $r = -0.467$, between the amount of carotenoids and the protein concentration in the grain $r = 0.563$; in cool and wet 2019, between the protein content in the leaves and its concentration in the nodules $r=0.735$, between the amount of carotenoids and the protein content in the leaves $r = 0.493$; in favorable 2018, between the protein content in the leaves and in the grain level $r = 0.623$.

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