Towards buckwheat breeding on high protein content with use of embryo-endosperm ratio in seeds

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Abstract. Buckwheat breeding on high protein content in seeds was not previously carried out due to the lack of clear selection criteria. Based on the study of the protein content in various organs of buckwheat seed, we proposed a method for selecting a high protein content based on the ratio of the embryo and endosperm in the seed. It was found that the embryo contained 8.3 times more protein than the endosperm (49.9 % and 6.0 %, respectively). At the same time, the contribution of the embryo to the total protein content in the seed is 74 %. The use of correlation analysis shows an independent change in the mass of the embryo and endosperm, which facilitates the use of the “high proportion of the embryo in the seed” trait in the selection process. 9 varieties of buckwheat were studied by embryo mass, endosperm and embryo share in the seed. The most suitable varieties for use in breeding for high protein content are buckwheat Batyr and Bogatyr, which are characterized by low endosperm content and a high proportion of the embryo in the seed. To increase the variation in the content of the embryo and endosperm, it is proposed to use a hybrid material and search for mutations that affect the ratio of the embryo and endosperm in the seed. The basics of the method of growing buckwheat from isolated embryos in soil culture and selecting plants by the mass of viable embryos in the seed are developed.

1. Introduction

Buckwheat (Fagopyrum esculentum Moench) is an important agricultural crop, a source of minerals, high-quality protein, enzyme-resistant starch and biologically active substances [1]. Buckwheat seeds accumulate more protein than rice, wheat, millet, sorghum and corn, conceding only to oats. The protein complex of buckwheat seeds includes 12.5-18.2 % albumins, 43.3-64.5 % globulins, 0.8-2.9 % prolamins, 8.0-22.7 % glutelins and 15 % other proteins [2, 3]. In the scientific literature, there is evidence of a higher content of prolamins (6.24 %) [4], indicating that there are no immunoactive proteins with a molecular weight of 30 Aum that provoke the development of gluten enteropathy. Phagopyritols present in buckwheat seeds protect proteins from degradation over a long period of time [5, 6].

Traditional buckwheat breeding is aimed at increasing the yield and technological qualities of grain, providing the highest yield of grain kernels during processing [7, 8]. However, modern buckwheat breeding, taking into account the development of technologies for deep grain processing, can develop in the direction of creating varieties with a high content of protein, enzyme-resistant starch, unsaturated fatty acids, flavonoids, phagopyritols, phytosterols and other biologically active substances [9, 10]. Based on the high value of buckwheat proteins, special attention should be paid to research that creates the basis for breeding on high protein content in seeds.
The aim of the study was to determine the prospects for breeding buckwheat on a high protein content based on different protein content in the organs of the seed embryo and endosperm, to evaluate different varieties of buckwheat by the ratio of the embryo and endosperm, to develop a selection method for a high proportion of the embryo in the seed.

2. Materials and methods
Determination of endosperm and embryo, as well as the percentage of germ in the milled seed, carried in the seeds of buckwheat varieties in different years are included in the State register of breeding achievements: Bogaty (1938), Demetra (1995), Dozhik (1998), Dikul (1999), Devyatka (2004), Chatyr Tau (2005), Batyr (2008), Bashkir krasnostebelnaya (2009), Temp (2010), Design (2010), Druzhina (2014). The study of the ratio of the embryo and endosperm, as well as the determination of the protein content in the embryo, endosperm and crop was carried out using buckwheat seeds grown in 2010 and 2011. 50 dehulled seeds of each buckwheat variety were analyzed.

The protein content (whole protein) in dehulled fruits, isolated embryos, and endosperms was determined by the Kjeldahl method using an automatic UDK 152 system and a DK 6 programmable heating digester (Velp Scientifica, Italy).

Evaluation of buckwheat genotypes by the content of the embryo and endosperm in the seed was carried out in accordance with method 1, and selection of viable embryos by the high content in the seed was carried out using method 2.

Method 1. The dehulled buckwheat seeds were soaked in Petri dishes for 1 day in tap water. The hatched seeds were divided into endosperm and embryo using a dissecting needle. The organs of the seed were dried during the day to an air-dry state (14% humidity) and the weight was determined. The total mass of the endosperm and embryo was found, and the percentage of the embryo in the seed was calculated.

Method 2. The dehulled buckwheat seeds were weighed and sprouted in Petri dishes between sheets of filter paper soaked in tap water. Embryos with a seedling length of up to 3 mm were isolated from seeds and placed in Petri dishes with wet filter paper, which was previously divided into numbered sectors. The endosperm was dried to an air-dry state (14% humidity). The weight of the dehulled grain, the weight of the dried endosperm, the estimated weight of the embryo and the percentage of the embryo in the seed were determined. The selection of isolated embryos on the basis of “high proportion of the embryo in the seed” was carried out with different intensity.

Statistical processing of data on the weight of the embryo and endosperm, the share of the embryo in the seed was carried out by the method of one-factor analysis of variance. Multiple mean comparisons were performed using the Tukey HSD test (α=0.05). The Pearson parametric correlation analysis method was used to evaluate the relationships between the features. Evaluation of the effectiveness of the method of isolation of viable embryos (method 2 in comparison with method 1) was carried out on the basis of “embryo weight”, “endosperm weight”, “seed weight” using the Student’s test (t-test). The range of variation in the mass of the dehulled seed, embryo, endosperm, and the proportion of the embryo in the seed was estimated using the coefficient of variation.

3. Results
The protein content in the dehulled buckwheat seed and its organs, in the embryo and endosperm differed markedly. In the studied varieties of buckwheat Dikul, Devyatka, Temp, Design, the protein content in the seed (germ and endosperm) varied from 15.8 to 18 % (table 1). At the same time, the embryo contained 8.3 times more protein than the endosperm. If the protein content in the embryo varied from 49 to 52.5 %, then in the endosperm from 5.7 to 6.1 %.
The weight of 1000 isolated endosperms was within the range of 22.3% in the Druzhina variety, and in the endosperm of the highest value of this trait is characteristic of seeds of the Design, Batyr and Bogatyr varieties (27.41; 29.19 and 29.7% respectively).

Variability in the content of the embryo in the seed is a necessary condition for evaluating the initial selection material and conducting breeding for high protein content. The analysis showed that the weight of 1000 dehulled seeds (germ and endosperm) in the studied buckwheat varieties was 23.13-27.95 g, the largest-fruited varieties were Dozhnik, Chatyr Tau and Design with the values of the trait 26.08; 26.93 and 27.95 g, respectively (table 2).

### Table 1. Protein content in various organs of buckwheat seed.

| Variety                     | Protein content, % | Significance of differences on protein content in embryo and endosperm, p | Contribution of embryo in seed protein content, % |
|-----------------------------|-------------------|------------------------------------------------------------------------|-----------------------------------------------|
|                             | dehulled seed     | embryo | endosperm          |                                              |                                               |
| Dikul                       | 16.8              | 52.5   | 6.0                | 0.021835                                     | 72.8                                          |
| Devyatka                    | 15.8              | 48.3   | 5.7                | 0.001869                                     | 72.5                                          |
| Temp                        | 18.0              | 49.7   | 6.1                | 0.001678                                     | 75.9                                          |
| Design                      | 18.0              | 49.0   | 6.1                | 0.007650                                     | 75.7                                          |

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### Table 2. Weight of seed organs in dehulled seeds of buckwheat varieties.

| Variety                                    | Mass of 1000 organs, g | Percentage of embryo in seed |
|--------------------------------------------|------------------------|-----------------------------|
|                                            | embryo | endosperm | ∑  |                             |
| Bashkirskaya krasnostebelnaya               | 5.13  | 18.00     | 23.13| 22.18                      |
| Dikul                                      | 5.45  | 18.36     | 23.81| 22.89                      |
| Demetra                                    | 5.57  | 19.23     | 24.80| 22.46                      |
| Druzhina                                   | 6.00  | 19.59     | 25.59| 23.45                      |
| Chatyr Tau                                 | 6.59  | 20.34     | 26.93| 24.47                      |
| Dozhnik                                    | 6.83  | 19.25     | 26.08| 26.19                      |
| Batyr                                      | 7.12  | 17.27     | 24.39| 29.19                      |
| Bogatyr                                    | 7.38  | 17.47     | 24.85| 29.70                      |
| Design                                     | 7.66  | 20.29     | 27.95| 27.41                      |

* Comparison of means was conducted with Tukey HSD test (α=0.05); identical indexes point out on the absence of significant differences between buckwheat varieties

The range of average mass values of 1000 isolated embryos in the studied buckwheat varieties was 5.13-7.66 g. The largest mass of 1000 embryos was distinguished by the varieties Batyr, Bogatyr, Design (7.12; 7.38; 7.66 g, respectively). The average weight of 1000 isolated endosperms was within the range of 17.27-20.29 g, the highest value of this trait was observed in the Design (20.29 g) and Chatyr Tau (20.34 g) varieties, and the lowest in the Batyr and Bogatyr varieties (17.27 and 17.47 g, respectively). The range of variation in the embryo fraction in buckwheat varieties was 22.18-29.7 %, the highest value of this trait is characteristic of seeds of the Design, Batyr and Bogatyr varieties (27.41; 29.19 and 29.7 %, respectively).

In the studied varieties, the variation coefficient by embryo weight varied from 11.5 to 22.2%, and by endosperm weight from 19.9 to 21.2 % (table 3). The range of variation in the percentage of the embryo in the seed was smaller (10.6-16.9 %). The strongest variation in embryo weight was observed in the Druzhina variety (22.3 %), and in the endosperm weight in the Batyr, Bashkir krasnostebelnaya and Design varieties (19.9 %, 20.6 %, 21.2 %, respectively).

### Table 3. Variability of organ weight in dehulled seeds of buckwheat varieties.

| Variety                                    | Coefficient of variation, % | Percentage of embryo in seed |
|--------------------------------------------|-----------------------------|-----------------------------|
|                                            | embryo | endosperm | seed |                             |
| Bashkirskaya krasnostebelnaya               | 15.2   | 20.6      | 18.7 | 10.6                         |
| Dikul                                      | 17.5   | 17.9      | 16.0 | 14.5                         |
| Demetra                                    | 16.7   | 17.8      | 15.2 | 15.7                         |
Determination of the weight of the seed organs is associated with drying, which is a critical moment for its germination and production of buckwheat plants. Therefore, the original method 2 (Material and methods) was used to select plants based on the “high percentage of the embryo in the seed”, which allows obtaining and germinating viable buckwheat embryos (figure).

![Figure 1. Growing plants of variety Devyatka from isolated mature embryos with high percentage in seed: a) isolated embryos prepared for cultivation in soil, b) buckwheat plants at the beginning of flowering grown from isolated embryos in soil culture.](image)

The study showed that the average mass of 1000 seeds, embryos, and endosperms isolated using methods 1 and 2 did not have statistically significant differences (table 4).

| Method | Weight of 1000 embryos, g | Weight of 1000 endosperms, g | Weight of 1000 dehulled seeds, g |
|--------|---------------------------|-------------------------------|----------------------------------|
| Method 1 | 8.083                      | 20.966                        | 29.049                           |
| Method 2 | 7.789                      | 22.58                         | 30.369                           |
| t-test, p | 0.306171                  | 0.135712                      | 0.274469                         |

Based on the data obtained, we can conclude that the direct (method 1) and calculated methods (method 2) correspond to the estimation of the embryo weight and the possibility of selecting viable buckwheat embryos for use in breeding on high protein content.

4. Discussion
It is known from the literature that the protein content in the flour of various varieties of buckwheat varies from 8.5 to 18.9 % [11]. At the same time, the organs of buckwheat seeds differ markedly in protein content. In the scientific literature, there is evidence that the pericarp of the seed has a protein...
content of 4 % [12]. In our experiment, the average protein content in the dehulled seed was 17.1%, in the embryo – 49.9%, and in the endosperm – 6.0 %. Taking into account the share of the embryo in the seed, the contribution of the embryo to the accumulation of proteins in buckwheat seeds averaged 74 % (table 1). The significant contribution of the embryo to the total protein content in the seed allows using the sign “high content of the embryo in the seed” as a marker in the buckwheat breeding on high protein content.

The study of correlations between the signs “embryo weight”, “endosperm weight”, “total seed weight”, “embryo share in seed” showed that the total weight of buckwheat seed was moderately dependent on the embryo weight (r=0.6; p<0.05) and strongly on the endosperm weight (r=0.96; p<0.05). The percentage of the embryo in the seed was largely determined by its weight (r=0.6; p<0.05). We should note that the weight of the endosperm and the weight of the embryo changed independently (r=0.3; p<0.05), indicating the presence of different regulatory genes. The absence of a close relationship between the weight of the embryo and endosperm was clearly demonstrated by the Batyr and Bogatyr varieties, in which a high mass of 1000 isolated embryos (7.12 and 7.38 g, respectively) was combined with a low mass of 1000 endosperms (17.27 and 17.47 g, respectively).

On the contrary, among the studied set of varieties, the Design variety was characterized by the largest mass of 1000 isolated embryos (7.66 g) in combination with the largest mass of 1000 endosperms (20.29 g).

The range of variation in the weight of the embryo, endosperm, and the percentage of the embryo in the seed in the studied buckwheat varieties was moderate, rarely exceeding the values of the coefficient of variation of 20 %. In this regard, it is better to select for a high proportion of the embryo in the seed separately, taking into account the mass fraction of the embryo and endosperm. Special attention should be paid to the search for mutations that affect the mass fraction of the embryo and endosperm in buckwheat seed. In breeding on high protein content, it is recommended to use hybrid material to increase the variation in the content of the embryo and endosperm.

5. Summary
Buckwheat seeds contain high-quality protein. In this regard, increasing the protein content in buckwheat seeds is an actual direction of breeding. Breeding work is hindered by the lack of clear breeding criteria. Taking into account the significant differences in protein content between the embryo and endosperm, we proposed using the “high proportion of the embryo in the seed” trait as a marker for selecting plants for high protein content. The polymorphism of buckwheat varieties by embryo weight, endosperm and embryo fraction in the seed was studied. Significant differences in the ratio of the embryo and endosperm were revealed between the studied buckwheat varieties. The greatest mass of the embryo and the percentage of the embryo in the seed were different Batyr, Bogatyr, Design varieties. An independent change in the weight of the embryo and endosperm was found, which can be successfully used in breeding for a high proportion of the embryo in the seed and, accordingly, high protein content in the seeds. In this regard, we should pay attention to the search for mutations that affect the ratio of the embryo and endosperm in the buckwheat seed. To increase the variation in the content of the embryo and endosperm, it is proposed to use a hybrid material. The ability of mature isolated buckwheat embryos to germinate on filter paper moistened with tap water, adapt to soil culture, grow into well-developed plants and form seeds is a necessary condition for use in breeding on high protein content. Statistical analysis confirmed the possibility of correct selection of viable buckwheat embryos by mass fraction in the seed for subsequent cultivation in soil culture.

6. References
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