Prospects of phaseolin application as a molecular marker in common beans (Phaseolus vulgaris L.) breeding

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Abstract. During the study, the phaseolin types in 24 bean varieties from the Novosibirsk SAU collection were determined: 20 varieties are of T-type, 2 varieties - S-type. Two varieties showed a type of phaseolin previously undetermined by polymerase chain reaction (PCR) method. It was conditionally designated as N-type by us. Statistical analysis of the relationship of phaseolin type with economic and valuable traits showed a reliable relationship with the mass of 1000 seeds and the number of seeds from the plant. A number of other traits showed evidence of differences uncertainty between phaseolin types. Further research with a large number of varieties is therefore required. In the early stages of the breeding process, however, the phaseolin type can be used in the selection of forms with valuable breeding traits.

1. Introduction

In modern food production, there is a popularity increase for vegetarian food, which is considered more healthy as it is rich in vitamins and various micronutrients. However, people who for various reasons are forced to resort to plant food consumption face the problem of the lack of diversity of full-fledged plant protein sources. Currently, soybeans are the main alternative to meat, whereas other legumes are used to a much lesser extent [1]. However, it can be cultivated not in all regions of Russia. Currently there is an active search for leguminous crops, which could get wide spread in sowing and at the same time not yield up to soybeans in nutritional value. One of such crops is common beans (Phaseolus vulgaris L.). It can compete with soy as it does not yield up to it in terms of protein and lysine content. In addition, it has excellent taste and culinary qualities with less likelihood of developing allergic reactions [2]. Thanks to this, it is able to significantly enrich people's diet and improve its quality.

Globally, there is a tendency to increase the acreage of haricot beans [3]. In Russia, this culture has the potential of widespread distribution. However, currently its acreage does not exceed 4 thousand hectares. Low prevalence is associated with insufficient selection elaboration on a number of commercially important features and features providing broad ecological plasticity [4]. Modern bean variety must have high productivity and a short growing period (70-90 days). For modern agricultural production, the role of mechanization is great. Therefore, plants with a height of 55-70 cm with a bush growth type, as well as a high lower bean attachment are required. In turn, in addition to high yield and suitability for mechanized harvesting, varieties must be resistant to a number of the most harmful phytopathogens, as well as possess the required cold resistance [5]. The solution to this problem lies in the widespread use of molecular markers in bean selection, which will speed up the process of obtaining new varieties.
Due to its greatest study, one of the promising molecular markers of common beans is phaseolin. This is the main reserve protein of bean seeds, the content of which reaches 50% of the total protein amount [6,7]. The main advantage of phaseolin as a molecular marker is the significant polymorphism of the types of a given protein. It is mainly represented by T- and S- types that characterize the various primary centers of Phaseolus vulgaris origin [8]. A number of authors indicate the presence of phaseolin types relationship with some quantitative traits, including content of protein, lysine, mass of 100 seeds [9]. As the predominant reserve protein of bean seeds, phaseolin seriously impacts their nutritional value. Based on the observations of a number of authors, methionine content also depends on the phaseolin type. For example, varieties with S-type are more rich in methionine [10]. All the abovementioned traits are important for the breeding study of beans and the creation of varieties that would be able to obtain widespread prevalence in the crops of legumes on the territory of Russia.

The purpose of our study was to evaluate haricot bean samples from the collection of Novosibirsk SAU by phaseolin type, as well as identify the connection with some important quantitative traits characterizing the possibility of applying phaseolin as a molecular marker.

2. Materials and Methods
The materials of the study were seeds, sprouts and vegetating plants of common beans. A total of 24 haricot bean varieties were studied (Table 1).

Genotyping was carried out in the laboratory of enzyme analysis and DNA technologies of the Novosibirsk State Agrarian University. To determine the type of phaseolin, the method of polymerase chain reaction (PCR) was used according to the method published by J. Kami and co-authors [11]. DNA was isolated from three-day sprouts by a commercial set according to the manufacturer's instructions. The following primers were used for the reaction: F: 5’- AGCATATTCTAGAGGCCCTCC-3’; R: 5’-GCTCAGTTCTCAATCTGTTCC-3’. These primers refer to SCAR markers (sequence characterized amplified region). The temperature profile of the reaction consisted of the following steps: one cycle of 5 min 95°C for DNA polymerase activation, 40 cycles (40 sec - DNA chains denaturation at 94°C, 40 sec - primers annealing at 53°C, 40 sec - DNA complementary chains synthesis at 72°C) and final synthesis for 10 min at 72°C. Electrophoresis in 10% polyacrylamide gel (PAAG) were used to separate amplification products. Gels dyeing was carried out with bromide ethidium for 30 minutes.

Determination of protein mass fraction was carried out by biuretic method in Jennings modification [12]. For the reaction, 1.5 g of flour from bean seeds without a shell and germ were taken. 100ml of biuret agent were added to the flour and stirred for an hour in a shaker. After stirring, the intensity of reagent staining was measured by the colorimetric method on the KFK-2MP device. For this purpose, cells with an optical pathway of 5 mm were used. Measurement was carried out in three repetitions for each variety.

The study of bean plants and quantitative traits assessment was carried out on the experimental field of “Sad Michurintsev” study production farm for three years (2017-2019). The soil of the experimental site is characterized by the average humus content of 4.5%, a weak acid medium reaction (pH = 6.28), low supply of nitrate nitrogen (6 - 10 mg/kg), increased labile phosphorus (9.8 - 12.8 mg/100 g) and medium labile potassium (6.2 - 6.4 mg/100 g). The main tillage is ploughing to a depth of 27-30 cm. Sowing of haricot beans was carried out in the third decade of May manually. The quantitative traits of the studied varieties were evaluated according to the VIR method [13]. Statistical data processing was carried out in Excel using generally accepted methods [14].

3. Results
3 types of phaseolin were found in the studied bean varieties in accordance with the pattern distribution on electrophoretograms (Fig.1)
In PCR analysis of bean samples it was found that 20 varieties had a T-type of phaseolin (Table 1), which, according to the literary data, was characterized by the presence of three fragments with lengths of 249 p.n., 264 b.p., and 285 b.p. on electrophoreograms. Specimens with S-type had two fragments 249 b.p. and 270 b.p. long (Nerussa, Zusha chernaya).

In turn, two varieties (Shokoladnitsa, Brunot) showed a pattern with the presence of four lengths fragments: 249 b.p., 264 b.p., 270 b.p., 285 b.p. A data search in the literature did not provide information about a similar configuration of amplicon. It was not possible to reliably identify this phaseolin type at this stage of research, we conventionally denoted it as N-type.

**Table 1. Distribution of haricot bean varieties by phaseolin types.**

| Phaseolin type | S | T | N |
|----------------|---|---|---|
| **Bean varieties** | Zusha chernaya; Nerussa | Veenoorl; Creola; Muhranula; Oran; Stringless; Katia; Bomba; Canario; Krasno-pestraya; Klyuchiki belye | Romanovo; Mechta hozyaiki; Zusha pestraya; Bijchanka pestraya; Lukerja; Inga; Rubin; Zusha belaya; Pestraya; Zolotistaya | Brunot; Shokoladnitsa |

We analyzed the relationship between S- and T-type of phaseolin with a number of quantitative traits that are most important in the selection of valuable genotypes. In the course of the study, the following traits were evaluated: total protein content, seed mass from a plant, number of seeds per plant, mass of 1000 seeds and yield. The average data on the studied traits for the 3 years of study are presented in Table 2.

**Table 2. Data on quantitative traits of common beans.**

| Variety (phaseolin type) | Mass fraction of protein, % | Mass of seeds from a plant, g | Number of seeds from a plant, pcs. | Mass of 1000 seeds, g | Yield, g/m² |
|--------------------------|-----------------------------|------------------------------|-----------------------------------|----------------------|-------------|
| Veenoorl (T-type)         | 19,10%                      | 10,03                        | 25,20                             | 463,70               | 153,10      |
| Creola (T-type)           | 18,30%                      | 14,60                        | 33,40                             | 299,90               | 194,30      |
| Muhranula (T-type)        | 19,30%                      | 9,12                         | 18,50                             | 543,30               | 194,60      |
| Oran (T-type)             | 19,00%                      | 10,96                        | 52,00                             | 256,40               | 174,50      |
Analyzing the relations hip of the “seed mass from a plant” indicator with the studied protein type, it was found that this trait has little variety depending on the type of phaseolin (Cv= 4.96%). The Mann-Whitney criterion also indicates that there are no significant differences between types based on the seed mass from the plant (U_{emp.}= 16; U_{crit.}= 4). The obtained data indicate the impossibility of using phaseolin in the selection of bean varieties based on this trait due to the lack of statistically significant relationship between them. This appears to be due to the significant intervarietal volatility of this trait, which is confirmed by a variation coefficient of 43.05%.

Further, we studied the relationship of phaseolin type to protein content. This indicator is one of the most important in determining the nutritional value of a bean variety. Previously, the presence of the relationship of this trait with the phaseolin type was indicated. However, the data presented in Table 2 indicate low variability of this trait depending on the phaseolin type. This is confirmed by a variation coefficient of 6.30%. In turn, the Mann-Whitney U- criterion with the value of 16.5 (U_{crit.}= 18) allows us to conclude the uncertainty of differences between phaseolin types based on protein content.
content trait. This is due to a small number of varieties with S- and N- types, which does not allow for a more reliable assessment.

As for the number of seeds from the plant, significant variability between types (Cv= 59.76%) was established. The same conclusion was confirmed by the U- criterion with the value of Uemp.= 0 (Ucrit.= 4). The study found that S-type significantly exceeds T-type in the number of seeds from the plant. This allows us to talk about the possibility of applying the phaseolin type as a molecular marker on this basis of this trait.

In turn, when studying the relationship of the mass of 1000 seeds with the phaseolin type, the highest mass of 1000 seeds was shown by varieties with T-type (x̅ = 415.55; s_x̅ = 8.24), whereas varieties with S- type differ in a low mass of 1000 seeds (x = 236.63; s_x = 5.62). The validity of differences between types are proven by the Mann-Whitney criterion equal Uemp.= 1 (Ucrit.= 4).

Also, the value of the variation coefficient confirms the trait variability by type (Cv= 40.58%).

As for the possibility of using phaseolin type as a yield marker, the data obtained was ambiguous. The Mann-Whitney criterion (Uemp.= 7; Ucrit.= 4) indicates that there are no significant differences between types for this indicator. This is due to the complex nature of this trait. The results obtained suggest the need for further multi-year studies with a large number of varieties that would equally represent all major types of phaseolin.

In addition, it should be noted that there is an inverse correlation of -0.64 (Fig.2) between the mass of 1000 seeds and the number of seeds from the plant. This suggests that phaseolin of S-type provides an increase in the number of seeds from the plant, yet to the detriment of their mass, whereas T-type plants have smaller seed number while having large seed sizes.

Figure 2. Correlation analysis of the relationship between the characteristics mass of 1000 seeds and the number of seeds per plant.

4. Conclusion
1. During the study, the phaseolin types in 24 varieties of haricot beans from the collection of Novosibirsk SAU were determined. It was established that 2 varieties were of S-type, 20 of T-type and 2 of N- type, which was not previously determined by PCR.

2. Significant differences between varieties with S- and T-type of phaseolin on the number of seeds from the plant (Uemp.= 0; Ucrit.= 4) and by mass of 1000 seeds (Uemp.= 1; Ucrit.= 4) were found. Varieties with T-type phaseolin showed high mass of 1000 seeds, whereas varieties with S-type had a larger number but smaller sizes. It was also found that these traits have an inverse dependence (r = -0.64).

The data obtained suggest that the phaseolin type can be used as a molecular marker in haricot bean breeding, since there are statistically significant results about its relationship with some quantitative traits (mass of 1000 seeds, number of seeds from the plant). However, in order to introduce this
marker into the breeding process practice, it is required to perform further research using more varieties and forms.

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