2-бөлім

# ӨСІМДІКТЕР ФИЗИОЛОГИЯСЫ ЖӘНЕ БИОХИМИЯСЫ

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Раздел 2

# ФИЗИОЛОГИЯ И БИОХИМИЯ РАСТЕНИЙ

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Section 2

# PLANTS PHYSIOLOGY AND BIOCHEMISTRY

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**Activities of protein components in bean seeds of kazakhstan, russian and other accessions**

Common bean specimens (Phaseolus vulgaris L.) of Kazakhstan, Russian and other foreign collections have been studied for protein content, lectin and proteinase inhibitor activities. All studied specimens have revealed high protein content (23.2% -30.8%) and lectin activity with its relation to particular genotype and origin. In course of the study on the lectin activity, a wide range of variations for this parameter has been found for twelve bean specimens. The maximum difference between the specimens has reached 17.5 mg / mL with highest values of 38.3 and 55.8 mg/mL. Two varieties, "Jubileynaya White" and "Zhuravushka" of Russian selection, one local variety "Assol" and "The Iranian" of Iranian selection have indicated the most expressed lectin activities. Trypsin inhibitor activity has appeared to be approximately twice higher than that of the chymotrypsin, and the highest inhibitor activity of trypsin and chymotrypsin has been detected in the samples with the highest lectin activities. Apart from investigating protein components activity, promising specimens have been identified by testing the highest proteins content, lectin and proteinase inhibitor activities which may serve as powerful sources of bean protein components with reference of their effects on different cell models, to develop biotechnological approaches for extraction and application to agriculture.

**Key words:** Phaseolus vulgaris L., lectins, proteinase inhibitors, plant protection.

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**Қазақстандық, ресейлік және шетел селекциясының кәдімгі үрмебұршақ (*Phaseolus vulgaris* L.) сорт үлгілерінің белоктық компонентінің белсенділігі**

Ақуыз, лектин белсенділігі және протеиназа ингибиторларының мөлшері бойынша қазақстандық, ресейлік және шетел селекциясының кәдімгі үрмебұршақ (Phaseolus vulgaris L.) сорт үлгілерінің салыстырмалы талдауы жүргізілді. Барлық зерттелген сорт үлгілері белоктың және лектиндік белсенділіктің жоғары мөлшерімен ерекшеленді, сонымен бірге бұл параметр сорт үлгілердің генотип ерекшелігіне және шығу тегіне байланысты. Лектиндік белсенділігін зерттеу кезінде осы көрсеткіштің кең диапозонда өзгеретіні анықталды. Сорттар арасындағы максималды айырмашылық 17,5 мг/мл, белгінің максимальды шегі 38,3 и 55,8 мг/мл аралығында болды. Трипсин ингибиторларының белсенділігі химотрипсин белсенділігіне қарағанда екі есе жоғары болды, сонымен қоса трипсин және химотрипсин ингибиторларының жоғары белсенділігі лектиннің мөлшері жоғары сорттарда байқалды. Белокты компоненттердің белсенділігін зерттеу негізінде белок, лектин және протеиназа ингибиторларының мөлшері жоғары перспективті сорт үлгілері алынды, олар үрмебұршақтың өнімділігі жоғары және ауруларға тұрақты формаларын алуда бастапқы бағалы материал бола алады.

**Түйін сөздер:** Phaseolus vulgaris., лектиндер, протеиназ ингибиторлармы, трипсин, өсімдікті қорғау.

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**Активность белкового компонента семян фасоли обыкновенной (*Phaseolus vulgaris L.*) казахстанской, российской и зарубежной селекции**

Проведено сравнительное изучение сортообразцов фасоли обыкновенной (Phaseolus vulgaris L.) казахстанской, российской и зарубежной селекции по содержанию белков, активности лектинов и ингибиторов протеиназ. Все изученные сортообразцы отличались высоким содержанием белка и лектиновой активностью, которое зависело от особенностей генотипа и происхождения. При изучении активности лектинов установлен широкий диапазон варьирования данного показателя. Максимальные различия между образцами достигали 17,5 мг/мл при крайних значениях признака 38,3 и 55,8 мг/мл. Активность ингибиторов трипсина была примерно в два раза выше, чем химотрипсина, причем наибольшая активностью ингибиторов трипсинов и химотрипсинов отмечена для образцов с наибольшей активностью лектинов. На основе изучения активности белковых компонентов выделены перспективные образцы с наибольшим содержанием белка, активностью лектинов и ингибиторов протеиназ, которые могут служить ценным исходным материалом для получения высокопродуктивных и устойчивых к заболеваниям форм фасоли.

**Ключевые слова:** Phaseolus vulgaris L., лектины, ингибиторы протеиназ, трипсин, защита растений.

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# ACTIVITIES OF PROTEIN COMPONENTS IN BEAN SEEDS OF KAZAKHSTAN, RUSSIAN AND OTHER ACCESSIONS

## Introduction

A holistic approach to plant protection issues and increasing level of environmental safety has become one of the most extensively developing areas of modern agriculture. With this respect, development of herbal medications for agriculture and medicine based on various protein components looks quite relevant. The Republic of Kazakhstan has a growing market potential for the effective application of biological products in agriculture, livestock breeding, oil and gas sector, and etc. However, Kazakhstan today has no large-scale production of biological products, and current needs are satisfied by importing such products from abroad. A range of microbiological preparations have successfully been implied for the needs of industry, agriculture, livestock breeding and environmental protection. At the same time, development of phytogenic preparations does not meet modern requirements. The use of herbal medications in combination with modern farming techniques would pave way to better use of not only soil potential but also proper implication of biological potential of plants.

Beans does not belong to traditional crops of Kazakhstan, though since recently the demand in beans has been registered to increase year by year. Along with proteins possessing nutritional value, legumes contain antinutrient compounds that are also belong to proteins, including, in particular, lectins and enzymes such as glucanase, chitinase, proteinase inhibitors and α-amylase which play a significant part in plant disease resistance and abiotic stress factors.

Two last decades have shown a great progress in the study on plant lectin activity against pathogens, specifically nematodes and insects [1, 2].

Proteolytic enzymes carry out a range of physiological functions in living organism, starting from the digestion of dietary protein and seed stored proteins to the series of specific regulatory processes. Important evidence in favour of active role of inhibitors for proteolytic enzymes in plant defense against insects may be observed in the studies revealing the induction of these proteins synthesis in response to the damage of plant tissue intactness. The systemic response to desintegration of a plant tissue is manifested by the onset of the synthesis of specific inhibitors in plants of

different genera [3, 4]. For example, out of three forms of soybean cystatin (*Glycine max L.*), only one (L1) appeared to be constituent, while two others (N2 and R1) were detected in response to wounding or under methyl treatment being absent in intact, unaffected plant [5].

Searches for effective insect proteinase inhibitors are in progress being undertaken in different directions. It is known that many of peptide precursors (propeptides), released at the activation of proteinase zymogens, are able to serve as highly effective inhibitors of mature enzyme molecules. Inhibitors of proteolytic enzymes may play significant part in plant protection, against insects as other pests. Various nematodes invading plants cause usually significant damage of agricultural production in Kazakhstan [6]. Protective role of plant inhibitors could stretch out to a range of pests. As in the case with insects and other pests, plant proteinase inhibitors are able to inhibit enzymatic activity of pathogeneous microorganisms.

Hence, there is a great need to study new sources of lectins and proteinase inhibitors for expansion of researches in the field of agricultural biotechnology and plant protection products. Hence, the aim of present work is to classify bean specimen by the content of protein components and identify new sources of lectins and proteinase inhibitors for the development a new generation of biological products on this basis.

**Materials and methods**

The study was carried out using common bean (*Phaseolus vulgaris* L.) cultivars of Kazakhstan and foreign collections which were available at the the Department of Molecular Biology and Genetics, al-Farabi Kazakh National University. 12 varieties, and namely: «Aktatti», «Assol», «Biychanka», «Zhuravushka», «Pearl», «Iran», «Kamelia», «Red Goya», «Pinto», «Ufa», «Fatima», and «Jubilee White» harvested under crop rotation in the mountain and steppe (plain) zones of Almaty region have been used for investigation. The climate of the Almaty region is sharply continental with average January temperatures of -15 ° C on the plains and – 6…- 8 °C in the foothills, and average July temperatures of + 16 ° C and + 24… + 25 ° C, respectively. The annual precipitations comprise up to 300 mm in the steppe zone, and from 500-700 to 1,000 mm in the mountains. Vegetation season lasts 70-225 days in the foothills and on the plain.

The protein content was determined by a Kjeldahl method [7]. In the seeds rough protein along with total content of soluble proteins in 0.2% NaOH solution have been detected.

To determine lectin activity, the extracts were obtained by homogenization of 2 g plant tissue in 6 ml of 0.9% Na-phosphate buffer. Lectin activity has been determined by common techniques of the reaction of hemagglutination [8].

Evaluation of lectin activity was performed by the hemagglutination reaction with lectins in rabbit or rat blood. Erythrocytes were isolated by washing the blood five times with 0.2M sodium chloride. The extent of lectin activity was assessed either visually or by measuring the lectin titre. The reaction was conducted at room temperature and measured in 2 hours after the onset of titration. Phytohemagglutinating activity of lectins was expressed as the value opposite to minimal protein concentration at which the hemagglutination was observeable [mg / ml] $^{-1.}$

The activity of proteinase inhibitors was determined spectrophotometrically at 280 nm by measuring cleaved products of protein substrate treated by the enzymes (trypsin and chymotrypsin) [9, 10]). Addition of inhibitors binding and thus converting trypsin or chymotrypsin into inactive complexes, is accompanied by reduced extinction. Optical density was measured by using a multichannel «Benchmark Microplate Reader» spectrophotometer (BioRad, USA). All solutions were stored at +4°C for not longer than a fortnight. Substances under investigation were extracted with distilled water at the ratio of 1: 4 000 at +4°C overnight. Then the extract was centrifuged. Trypsin/chymotrypsin activities were detected at $25^0$C in the resulting supernatant.

The activity of trypsin inhibitor (mg / g) was calculated by using the formula:

$$ATI = \frac{CTR \times (\Delta ETP - \Delta EOP) \times VTR \times K_{EXTR} \times K_{DEL}}{V_{INH} \times \Delta E_{TS}},$$

where ATI is the activity of trypsin inhibitor, mg / g of protein; $C_{TR,}$ the trypsin concentration, mg; $V_{TR,}$ the volume of trypsin solution, mL; $V_{ING,}$ the volume of TI solution, mL; $K_{EXTR,}$ the ratio of the extract volume to the weight of plant tissue; $K_{DEL,}$ the dilution index of TI solution; $\Delta E_{TEST,}$ the extinction of test sample; and $\Delta E_{TS,}$ the extinction of trypsin solution. The quantity of trypsin inhibitors able to supress 1 unit of the trypsin activity, was counted as the unit of the trypsin inhibitor activity. The experiment was carried out in three replicas. Chymotrypsin inhibitor activity was calculated using similar approach. The content of the trypsin (or the chymotrypsin) inhibi-

tors was expressed in milligrams of related inactivated enzyme (trypsin or chymotrypsin) per 1 g of the flour.

All the experiments were conducted in triplicates. The outputs obtained have been statistically processed to be presented in the figures as average arithmetic meanings with standard errors.

## Results

Common bean is distinguished as rich vegetable crop supplied by highly digestible protein which is valuable by its abundant amino acid composition. According to a number of the authors, seeds of common bean may preserve in average 20-30% of protein. However, the content and the quality of seed protein in beans was shown to be significantly depending on the genotype [11, 12].

Comparative study on bean specimens of Kazakhstan and foreign breeding indicates their variation in protein content ranging from 23.2% to 30.8% in different accessions. Maximum concentration was observed in such specimens as Iranian (30.8%, foreign selection) and «Zhuravushka» (30.7%, Russian selection). The lowest content was found out in the «Red Goya» (23.2%, foreign selection) and «Fatima» (23.4%, Russian selection). Kazakhstan specimens «Aktatti»and «Assol» have been shown to take intermediate place. (Table 1).

**Table 1** – Protein content in bean seeds (percentage to dry weight)

| # | Specimen | Originating country | Protein content,% | |
|---|---|---|---|---|
| | | | Total,% | Albumin-Globullin fraction,% |
| 1 | «Aktatti» | Kazakhstan | 29.9±0.3 | 76.2±0.9 |
| 2 | «Assol» | Russia | 28.7±0.5 | 76.6±0.8 |
| 3 | «Biychanka» | Russia | 25.6±0.3 | 80.5±1.0 |
| 4 | «Zhemchuzhina» | Russia | 25.6±0.4 | 78.5±0.8 |
| 5 | «Zhuravushka» | Russia | 30.7±0.5 | 73.6±0.8 |
| 6 | «Iranian» | Iran | 30.8±0.5 | 77.2±0.9 |
| 7 | «Camellia» | USA | 26.6±0.7 | 77.8±0.8 |
| 8 | «Pinto» | USA | 26.4±0.3 | 79.8±0.9 |
| 9 | «Red Goya» | USA | 23.2±0.4 | 82.3±0.9 |
| 10 | «Ufa» | Russia | 28.2±0.5 | 78.7±0.9 |
| 11 | «Fatima» | Russia | 23.4±0.4 | 77.3±0.9 |
| 12 | «Jubilee White» | Russia | 28.2±0.6 | 76.7±0.9 |

Bean proteins consist mainly of globullins and albumins, which are considered to be more valuable by their amino acid composition. Lighter solubility in water and neutral salt solutions predicts greater digestibility and, accordingly, higher biological and nutritional value of these legume proteins. By applying the consequent extraction method, it has been shown that the albumin-globullin fraction dominated in the seeds of all the bean varieties and lines studied to make up 76.2-82.3%. Samples with a maximum content of albumin and globullins have been identified among foreign-bred varieties: «Red Goya» (82.3%) and «Biychanka» (80.5%). Minor content has been attributed to «Aktatti» (76.2%), «Zhuravushka», and «Assol» (76.6%) varieties.

It is known that plants producing seeds abundant in energy deposits normally accumulate significant amounts of antinutritional agents, i.g. lectins, proteinase inhibitors and etc. This is applied to different phabaceous crops, and particularly beans. With this regard, the next stage of current study has been to assess the lectin activity of bean specimens. The intensity of the hemagglutination reaction depends on reaction conditions. That is why it is

necessary to count the origin of erythrocytes and procedures of their treatment. In the first series of experiments, visual evaluation of the lectin activity was undertaken by implying the hemagglutination reaction with blood from rabbits and rats. Visual grading of agglutinating effect allows quick, impartial and quite precise determination of the hemagglutination intensity.

Our experiments have established that the hemagglutination intensity might decrease in case of using the rabbit erythrocyte suspension instead of that from rat blood. The hemagglutination reaction with rabbit erythrocytes has slowered 1.5-2 times when compared with rat erythrocytes, and the intensity did not exceed 5 points for all of the sample common beans tested.

At the same time in rat erythrocyte testing system, agglutinating activity was rather high in all specimens to vary, depending on the genotype, from 10 to 13 points. The agglutinating activity has been shown to reach 13 points for the «Jubilee White» and «Zhuravushka» varieties, 12 points for the «Zhemchuzhina» variety, and 10 points for the Ufa and «Biychanka» varieties. Also, hemagglutination reaction in the «Zhuravushka» specimen has been noticed to start 2-3 min earlier, if compared with other specimens revealing relatively high lectin activity.

Further studies on hemagglutinating activity were carried out by measuring the titre of lectins exclusively in rat blood cells. These experiments have revealed that all of the samples investigated possessed quite high lectin titre in range of 38.3 – 55.8 mg / ml $^{-1}$, independent of the bean origin. At the same time, the degree of variation in lectin activity among the specimens of the Russian collection have been noted to be higher than that of the samples from Kazakhstan and other countries. Highest variation in these characters have achieved 17.5 mg / mL for the Russian specimens, 3.0 mg / ml for domestic specimens, and 7.2 mg / ml for other foreign accessions. Three bean varieties, «Jubilee White», «Zhuravushka» and «Assol», have been shown to possess the highest values of the lectin titre reaching 55.8, 52.4 and 50.7 mg / ml $^{-1}$, respectively (Fig. 1).

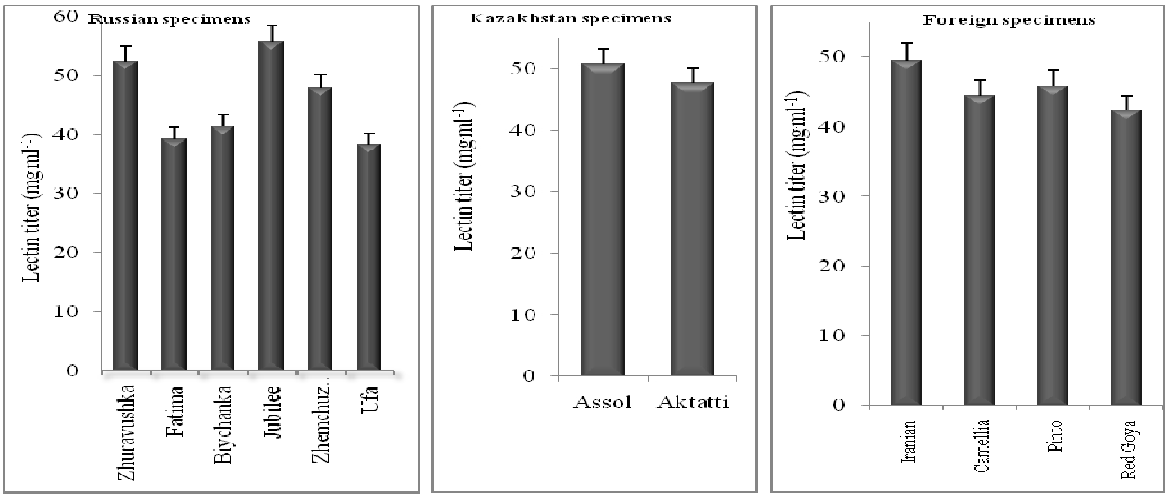
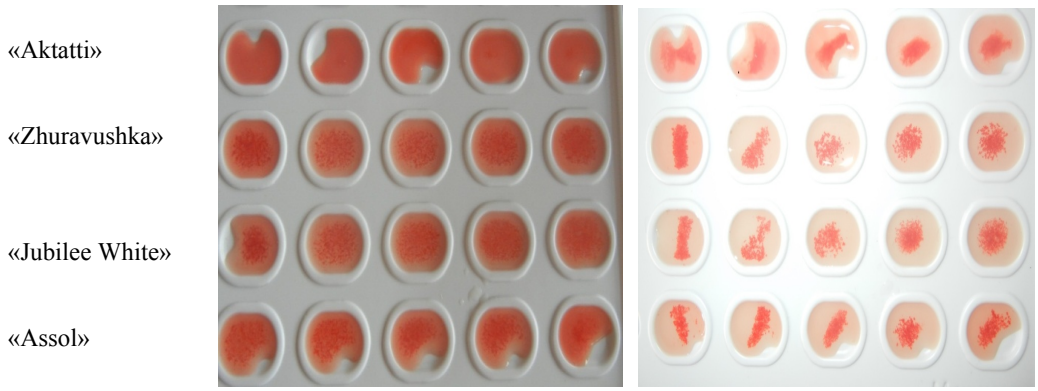



**Figure 1** – Hemagglutinating activity of bean seed lectins

«Fatima» and «Ufa» varieties have shown rather low lectin agglutinating activities of 39.2 mg / ml $^{-1}$ and 38. 3 mg / ml $^{-1}$, respectively. By analyzing these genotypes it has been determined that they are differentiating in lectin activity. Most promising specimens have been revealed to be «Jubilee White» and «Zhuravushka» from Russian specimens, the «Assol» variety from Kazakhstan specimens, and «The Iranian» out of the number of other external specimens. No correlation between the protein content and lectin activity has been identified. The correlation index has been estimated to range from r = -0.3 to r = 0.6.

In the figure we can see the reaction of hemagglutination of four different species.




A – Start B – After 30 minutes

**Figure 2** – The reaction of hemagglutination

In our experiments, the activity of trypsin and chymotrypsin inhibitors in different bean specimens has been determined. The activity of trypsin inhibitors (ATI) has been shown to be approximately 2-2.2 times greater than that of chymotrypsin (ACI). The ATI of Russian varieties has been detected to vary from 2.5 mg / g to nearly 5.5 mg / g, whereas ACI has ranged from 1.9 mg / g to almost 3.3 mg / g (Figure 3).

Lectin proteins are located in different plant organs, including shoots, roots, tubers, bulbs, nodules and generative organs [13]. Its amount and place in plant can be changed in a wide range. Also activity of these proteins depends on different factors (biotic and abiotic). In connection with this information almost all parts of the plants were checked. The highest activity in all species was determined in seeds. «Aktatti» has demonstrated hemagglutination in the first minute, «Zhuravushka» and «Red Goya» in the second one. The reaction is very strong and even 5-times water dilution could not destroy agglutinates. Experiment with the specie called «Camellia» has shown that the reaction started later, but it didn't impact on its power.

In other organs lectin activity is different. In roots of the «Zhuravushka» reaction started after 15 minutes from the beginning of the process. The first three dilutions have demonstrated vivid agglutination, but the last two ones can be characterized as moderate: erythrocytes went to the edges of plate and formed a circle. The same effect has been observed in seeds and leaves of this specie, but the reaction started only after 30 minutes.

«Red Goya» has demonstrated synchronous hemagglutination in leaves, roots and shoots after 20 minutes from the beginning. Reaction was also moderate, erythrocytes formed a circle. In case of «Aktatti» and «Camellia», reaction of hemagglutination started in shoots earlier than in roots and leaves. Hemhemagglutination was moderate. After jolting the sediment was been divided into several pieces.

According to Table 3 we can see the total amount of lectins in seeds which varied from 20.7mg/100 g до 33.7 mg/100 g.

**Table 2** – Lectin content in different organs of beans (mg of raw material/100 g)

| Specimen | Seeds | Shoots | Leaves | Roots |
|---|---|---|---|---|
| «Aktatti» | 33.7 ±1.6 | 22.8±0.8 | 20.8 ±0.8 | 18.2±0.4 |
| «Zhuravushka» | 29.4 ±1.8 | 18.3±1.4 | 16.4 ±1.4 | 10.4±0.9 |
| «Camellia» | 22.3 ±4.2 | 12.2±0.7 | 11.5 ±0.5 | 8.7±0.2 |
| «Red Goya» | 20.0 ±2.5 | 11.8±0.9 | 11.3 ±0.8 | 8.5±0.3 |

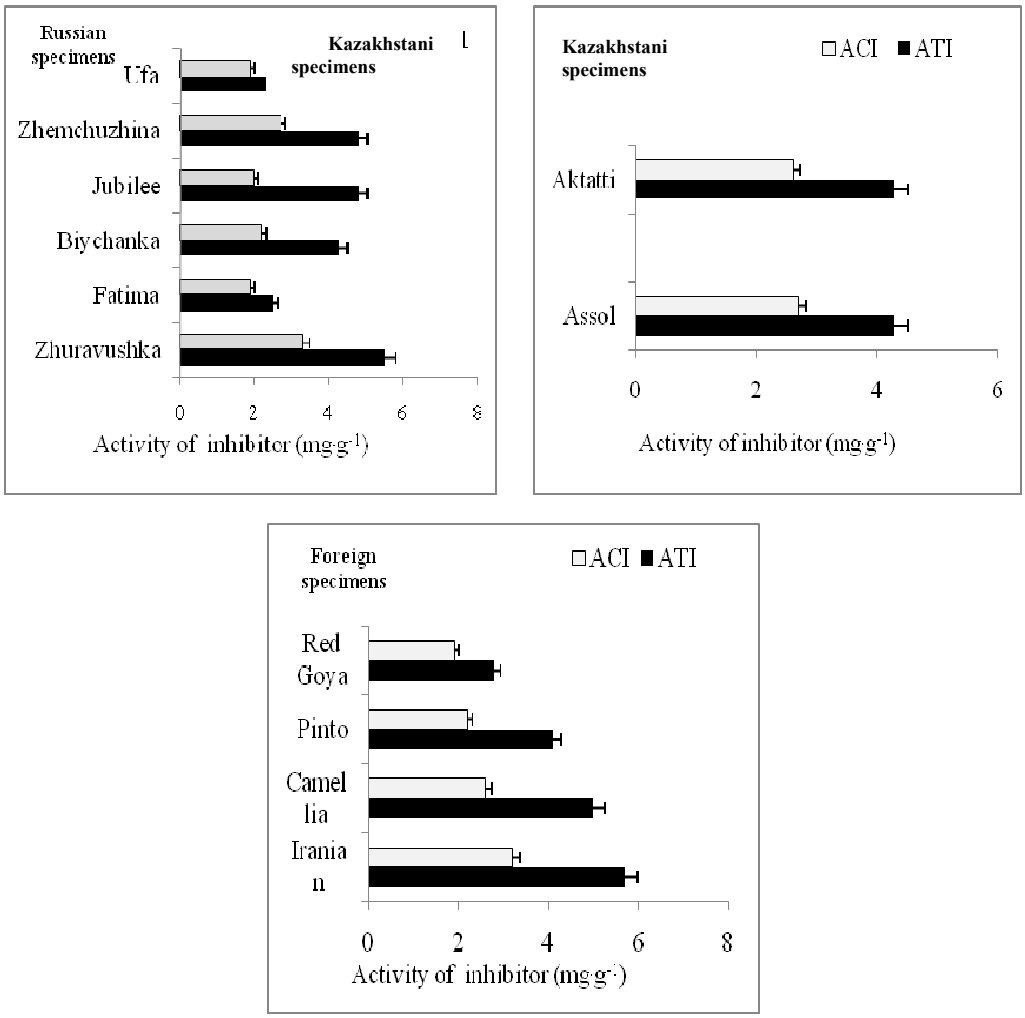



**Figure 3** – The activity of trypsin (ATI) and chymotrypsin (ACI) inhibitors of seed in bean specimens

In leaves and shots the total amount of lectins is almost the same, but it has not been higher that 22,8 mg/100 g. The lowest lectin content has been determined in roots of the all species. Probably it is connected to the undeveloped root system because lectin activity in legumes depends on symbiosis with microorganisms. According to lectin activity all organs have been ranged in the following order: seeds, shoots, leaves, roots. The activity of trypsin and chymotrypsin inhibitors in specimens of Kazakhstani selection has variegated lightly between the varieties. Other foreign varieties have also shown to possess different activities of trypsin and chymotrypsin inhibitors; such activities have been depicted to range from 2.8 mg / g to 5.7 mg / g, and from 1.9 mg / g to 3.2 mg / g, respectively. Among the specimens studied, «The Iranian», «Zhuravushka»and «Assol» have been marked as indicating highest ATI and ACI values: 5.5 mg / g and 3.3 mg / g for «Zhuravushka»; 5.7 mg /g, and 3.2mg / g for «The Iranian», and 4.3 mg / g and 2.7 mg / g for «Assol», respectively. Notably, these specimens have been also remarkable by high protein content. Statistical assay has shown no correlation between protein content and ATI. The correlation index r has ranged between r = 0.01 and r = -0.60. No significant linkage has either been found between lectin activities and proteinase inhibitors. The correlation index has changed from 0.2 to 0.4.

## Discussion

Wide distribution of phabaceous crops in world agriculture is primarily explained by their ability to accumulate and store in seeds and vegetative organs large amounts of excellent, easily digested protein

enriched by essential amino acids. When compared with the cereals, beans conclude in the seed 1.5-2.5 times higher quantity of protein-containing substances. Detailed composition and exact ratio of protein fractions is significant part of qualitative characteristics for bean protein complexes. In this study, comparative analysis of bean varieties and lines by the protein content quantification, identification of protein-rich genotypes has been fulfilled to summerize that protein content and quality have occurred to be dependent on particular genotype and specific growth conditions (Table 2). Similar results were earlier shown for other phabaceous plants [14, 15].

Phabaceous beans are known to include also non-food components, such as lectins, inhibitors, etc. Presence of non-dietary compounds in crops is, in most of the cases, an adaptive trait which had developed in the course of evolution. Proteinaceous antinutritional components, such as lectins and proteinase inhibitors, play an important role in plant defence against insects and plant pathogens. Nowadays, negative impact of antinutrients on the viability of certain pests is being successfully examined with assistance of a broad spectrum of plant lectins [16-19]. Plant proteinase inhibitors are capable of effective inhibiting the activity of serine and cysteine proteinases active in the insect digestive tract, thereby abolishing insects survival and diminishing their biomass and development [20]. Several studies have shown that damage of tomato and potato aerenchyma (air-conducting tissues) by Colorado beetle or its larvae may cause a rapid induction of plant trypsin and chymotrypsin inhibitors [21].

Virtually, all phabaceous plants contain non-food components which amount may increase in response to environmental conditions. Concentration of bean toxic substances has been evidenced to exhibit a wide range of variation to be determined by the genotype, vegetation and harvesting conditions, as particular place of production for the beans. While examining the lectin activity among 12 bean accessions from Kazakhstan, Russian and other foreign collections, it has been established that the highest difference between the samples could reach 17.5 mg / mL at the extreme values of this parameter making up 38.3 and 55.8 mg / ml (Fig. 1), which is consistent with other data [22].

According to scientific sources, common laws of lectin activitiy in plant development have been described. [23]. Its activity increases in mature seeds and also it can be changed in dependence on abiotic and biotic factors. The lowest content of lectins has been determined in roots. Visibly it was because all experiments were conducted on 14-days shoots with weak roots and lectins activity in legumes can not exist without symbiotic microorganisms locating in this part of plants. Studying dependence of lectin localization and its amount in different tissues and steps of development expands our knowledge about the main functions of these chemicals and helps unveil its diversity.

Previously, it has also been repeatedly speculated that high activity of trypsin inhibitors, for instance, in soybeans, stemmed out from evolutionary selection as a method of plant protection from attacks of pathogens and elimination by animals and birds. Low ATI was, as a rule, the character intrinsic for those accessions which turned out to be poorly adapted to growth conditions [24]. In our experiments, varying activity of trypsin and chymotrypsin inhibitors may witness in favour of diverse stability of the varieties and lines under research (Fig. 2). For example, the «Zhuravushka» specimen, resistant to bacterial diseases, has been noticed to possess the highest activity of proteinase inhibitors. Further studies should clarify the relationships of the activity of proteinase inhibitors and intervarietal sustainability in bean collections to the most common pathogens typical for Kazakhstan involved in the formation of protective mechanisms for plants. Such studies are currently being conducted for wheat, barley, lupin and other valuable crops [25, 26-27].

Thus, the study has shown a wide polymorphism of various bean specimens by their content of protein, lectin activity and proteinase inhibitors. Samples with high activity of these protein components by means of bio-screening described in this paper may serve as a valuable stock resource of bean lines to be highly resistant to diseases and also, on their basis, the source of bioproducts advanced by agriculture.

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