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Morphogenetic and biochemical analysis of domestic and external common bean seeds

Abstract. Malnutrition in low-income countries is one of the massive central issues to be solved. It means the scholars should propose effective way of solving this problem by using modern methods of breeding and harvesting. Grain legumes are regarded as principal source of vegetable protein among plants. Grain legumes are abundant by vitamins, minerals, flavonoids, and fiber, and are also convenient for cultivation in plain and alpine zones. Under arid conditions grain legumes are helpful in the exploration of the areas unsuitable for breeding to obtain steady annual harvests. This paper summarizes information on successful introduction and breeding of certain bean varieties obtained from local and foreign collections. It includes the data on biochemical comparison of different bean varieties, amino acids composition of their seeds, structural analysis and statistic data on the correlation of breeding conditions in various areas and the bean yield.

Key words: grain legumes, beans varieties, introduction, biochemical compounds, productivity, adaptability, breeding.

Abbreviations: BAS, biologically active substance; HPLC, high performance liquid chromatography; cvs, cultivars, or varieties

Introduction

The main source of vegetable protein for human diet and farm animals is grain legumes. Beans play a special role as the seeds and unripe fruits (called pods) are used for the food mainly in cooked or canned forms [1].

Bean seeds and pods are known for their high palatability standards [2, 3]. Beans contain proteins (17-32%, which is higher than the amount of protein in meat (20-22%), and fish (18-19%), carbohydrates (mainly starch, 55%), fats (1.8%), fiber, minerals (zinc, copper, potassium, iodine, iron, sulfur, magnesium) and vitamins (A, C, B₁, B₂, B₆, E, and PP) [4-7].

Due to chemical composition, beans reveal strongly positive effect on immune and nervous sys-

tems, increase body resistance to viral and microbial invasions, promote wound healing, regulate metabolism, improve hematopoietic functions, remove gallbladder and kidneys stones, reduce liver's inflammation, make an impact on the activities of alimentary and urinary tracts. Besides, beans are recommended to defeat bronchial diseases, rheumatism, and intestinal infections, due to abundant antioxidants, iron, vitamin B₆, and starch. The composition of white beans includes magnesium, fiber and folic acid. Their shortage may cause megaloblastic anemia. Beans are often cultivated jointly with corn, potatoes and melons. As the crop and nitrogen-fixing species beans can be rotated to substitute cereals and root crops.

According to beans polymorphism, they have an exceptionally wide range of cultivation. Some varieties and forms indicate a lot of differences in

vegetable and reproductive growth, as environmental characteristics. Studying such characteristics is considered to be necessary for further development of science-based farming techniques, which are supposed to be specific for diverse forms, varieties and areas. For this purpose the global gene pool of beans should be extensively explored. It is particularly important to get information on polymorphism of existing bean varieties and lines [1].

The grain legumes also play important role in improving soil fertility. Due to the symbiosis with nitrogen-fixing bacteria, they are able to seize free nitrogen from the air to accumulate it in roots and crop residues in range of 50-100 kg or even more per hectare [8]. Apart from the food, beans may be used as the source of citric acid, green manure, animal feed and heirloom plants [9].

Despite numerous beans benefits as their increased demand in the market, this vegetable crop is still not considered as conventional for our country.

Present-day bean production in Kazakhstan is complied mainly with imported seeds, raw and canned beans from CIS and foreign countries. Meanwhile, southeast regions of this country are extremely favourable for the cropping of high-protein bean varieties.

Modern beans breeding is not supported by relevant physiological and biochemical investigations which would allow to evidence in favor of the impact of changing plant morphogenetic traits on biochemical characters, physiological functions, seed productivity, grain quality and plant adaptiveness to be inherited by the following generations.

The aim of current study is to generate high-protein lines of common bean, put forward their qualitative characteristic based on bean breeding and extensive bean introduction.

Materials and methods

Current study of research subject is based on «The Guidelines for Studying Collection of the Grain Legumes» and «The Classifier of Phaseolus vulgaris L. (Common bean)» [10, 11].

Seeds (11 specimens in the steppe zone, at «The Zhanga Talap Agrobiocenter», 29 specimens in the mountain (footfill) region, at the Institute of Botany and Phytointroduction, 9 specimens in the mountain region, «The Almarasan Canyon») were planted on plots (2 x 10 square meters). Double-row planting with wide row spacings (40 cm x 60 cm) was applied. Harvesting was carried out manually. In addition to the main collection of the mountain region, 6 samples of vegetable and heirloom varieties of French breeding were tested for the first time.

Cv. «Aktatti» known to be widespread in Almaty Region was used as a standard. Collection specimens were planted twice.

Phytochemical screening of beans samples, detection and identification of polyphenolic compounds of local and other samples was performed by Folin-Ciocalteu [12].

Quantitative detection of flavanoids was determined by the complex-forming reaction in presence of 1% alcohol solution of the aluminum chloride.

Total content of flavonoids was estimated based on calculating the quantity of rutin (vitamin P), whereas total content of phenolic compounds based on the amount of gallic acid (3,4,5-trihydroxybenzoic acid). The calculation was conducted by using the following formula:

$$X = A_x \times W \times W_2 / E1\% \times 1 \text{ cm} \times m \times V_a,$$

where

A_x , optical density of sample solution;

W and W_2 , dilutions, ml;

$E1\% \times 1 \text{ cm}$ – specific absorption rate of a standard rutin solution;

m , mass of seed sample, g;

V_a , aliquot volume, ml.

Results and their discussion

To create the university collection of high-protein bush beans varieties, lines and accessions were picked out for further planting.

Common bean, *Phaseolus L.* has numerous varieties which are subdivided into two large groups: American beans and Asian beans. Whereas the first group is known for possessing large seeds, and less frequently small seeds, the second group is referred to have predominantly miniature, small seeds. Four American bean species are considered to be significant for our conditions: common bean (kidney bean, *Ph. vulgaris* Savi L.), lima bean (*Ph. lunatus* L.), runner bean (*Ph. coccineus* Willd.) and tepari (*Ph. acutifolius* Gray).

One of the Asian species displaying great importance for cropping in Kazakhstan is *Ph. aureus* Piperu, or golden bean (mung). It is cropped in Central Asia and Transcaucasian states for gain and green fertilizers.

In current research we have used American varieties including cvs «Red Goya», «Pinto», «Camellia», «Lima», then «Turkish beans», and Asian varieties including purple pole beans, cv. «Violet Maleo», cvs «Iranian», «Nut», «Dermason». There have been also some varieties from Polish («Bomba», «Igolinsky»), Russian («Biichanka», «Zhuravushka», «Fatima»), and locally bred cvs («Aktat-

ti), «Nazym» and «Talgat» as other lines and local specimens).

Apart from this, 5 new French varieties were introduced and 6 French varieties were examined in «Almarasan Canyon» and on plots of the Institute of Botany and Phytointroduction. It is shown that cvs «Argus», «Mystery», «Dream of Venice» and «Triumph of the Farcy Town» have displayed high productivity. It is planned to have this seed material propagated under the steppe zone at the «Zhanga Talap Agrobiocenter».

To record field experiments and land specimens we have used essential software registered as «Planting Manager» [13].

Duration of the vegetation period is a significant factor for determining the opportunity to cultivate or induce the beans in the region. Obviously, it is also one of the most important issues for the breeding. Based on research of the first vegetation season and its duration ranging from 80 to 140 days, bean samples were subdivided into early-ripening, average-ripening and late-ripening beans (see Table 1).

Table 1 – The duration of vegetation season of various common beans samples

Group of ripeness	Number of varieties or specimens		Duration days	Variation index, %%
	Number	Percentage		
Early-ripening	14	37.8	80-85	2.5-5.9
Average-ripening	22	59.5	from 86 to 95	5.7-6.5
Late-ripening	1	2.7	96 and more	-

Groups of samples with different duration of vegetation could be characterized by specific variability of this character.

If early-ripening samples are less variable (2.5 – 5.9%), mid-ripening samples indicate growing variability reaching 5.7 – 6.5%. Because of minor number of late-ripening samples, this index for that case was neglected.

Out of 44 bean samples of different geographical region, more than a half was shown to belong to the average-ripening group, whereas the rest would be represented by early-ripening forms with the exception of a single late-ripening variety. Duration of the vegetation period is dependent on precipitations and temperature. At low temperature and precipitations this period tends to extend. Extremely high or low temperatures in the steppe or mountain zones affect productivity of growing varieties.

Parental samples of common bean and its relatives were chosen based on the specimens representing various geographic origins (Kazakh, American, Chinese, Czech, Polish, Russian and Turkish varieties and lines).

Studying the duration of time for transition from germination to the seedlings has shown that this period depends mainly on climatic factors, such as average daily air temperature and the amount of precipitations.

Transition time from germination to the seedlings was established to depend on growing conditions in the steppe and mountain zones, and specific varietal traits.

A range of the specimens under this study has been shown to possess different period of germination-seedlings transfer varying from 25 to 70 days.

Early-ripening beans have passed that period in 25-40 days (33 days in average), average-ripening beans – 30-60 days (45 days), late-ripening – 33-70 days (52 days). As indicated below in Table 2, the period «germination – seedlings» for different types of common bean is variant.

The period covering seed development and achieving technical maturity has been established to vary from 90 to 105 days, thus lasting 10-15 days longer than habitual annual season of vegetation under local conditions.

Genetically determined composition of the seed protein fractions allows to planning further breeding experiments towards high protein accumulation by using simultaneous analysis of amino acid composition in certain genotypes. Intervarietal polymorphism in common bean has shown that protein and amino acid contents occur to differ among varieties and lines. It has revealed that there would be some opportunities to acquire increased quantities of individual compounds and improve the quality of amino acid composition in the course of breeding.

Table 2 – The duration of the period for «germination – seedlings» in different groups of beans

Group of ripeness	Duration, days	Coefficient of variability, %
Early-ripening	25-40	8.9-10.6
Average-ripening	30-60	12.3-16.8
Late-ripening	33-70	-

To determine amino acid composition six local and foreign accessions have been subject to high-

performance liquid chromatography, or HPLC (see Table 3).

Table 3 – Amino acids composition of commonbean seeds (mg/100g)

Amino acids	Aktatti	Djungarskaya	Zhuravushka	IFGBR- 48	Camellia	Karakoz
Alanine	1405	1144	1025	1248	928	1352
Glycine	644	320	258	526	286	608
Leucine	420	378	425	425	404	480
Isoleucine	390	265	304	384	295	425
Valine	304	205	210	328	220	356
Glutamate	3980	2213	2082	3245	2147	3828
Threonine	462	214	192	448	218	483
Proline	1273	762	705	1064	694	1256
Methionine	335	118	130	290	142	350
Serine	628	416	378	702	415	780
Aspartate	2806	1045	1210	1948	1148	2344
Cysteine	55	18	25	42	24	62
Hydroxyproline	6	3	2	5	2	7
Phenylalanine	692	282	303	556	312	680
Tyrosine	729	304	342	608	356	735
Histidine	588	298	315	526	285	554
Ornithine	5	4	3	4	3	6
Arginine	738	587	538	640	515	715
Lysine	410	368	325	315	286	370
Tryptophan	278	120	112	226	98	268

As seen from the Table 3, the major fractions have occurred to be glutamatic (3980-2082 mg/ 100 g) and aspartic acids (2806-1045 mg/ 100 g), alanine (1405-928 mg/ 100 g) and proline (1273-694 mg/ 100 g). Noteworthy, domestic lines have turned out to surpass external analogues by more than 91% by the glutamate content, more than 2.4 times by the aspartate content, more than 51% by alanine and 83% by proline.

Besides these abundantly presented glucogenic amino acids, it has been noticed that the ketogenic amino acids (leucine, lysine and tryptophan) would

be left in much lower concentrations, what allows to recommending related varieties for manufacturing diabetic products due to a minor risk of forming the ketone bodies.

The content of essential amino acids has been registered to be in range of 27.5-29.8%. Lysine, threonine and serine are biological substances enhancing the growth of human and animals. Interestingly, local lines «Aktatti» and «Karakoz» were shown to contain the greatest amount of sulfur-containing amino acids, methionine and cysteine, which level would be maintained in seeds of these

lines in amounts varying from 335 to 350 mg / 100 g and from 55 to 62 mg / 100 g, respectively. In addition, the line «Aktatti» has appeared to be enriched by lysine (410 mg / 100 g).

Therefore, it is confirmed that the protein content is dependent of the climatic changes, breed-

ing technologies, soil characteristics and genotypic classification of the variety or line.

Studied accessions, varieties and lines could be classified by an Osborne classification presenting bulk of proteins as globulins (phaseolin, 60-90%) and albumins (10-20%) (Table 4).

Table 4 – Composition of essential amino acids (mg/100g) in bean seeds

Essential amino acids	Aktatti	Djungarskaya	Zhuravushka	IFGBR- 48	Camellia	Karakoz
Leucine	420	378	425	425	404	480
Isoleucine	390	265	304	384	295	425
Valine	304	205	210	328	220	356
Threonine	462	214	192	448	218	483
Methionine	335	118	130	290	142	350
Phenylalanine	692	282	303	556	312	680
Histidine	588	298	315	526	285	554
Lysine	410	368	325	315	286	370
Tryptophan	278	120	112	226	98	268
Total amount	3879	2248	2316	3498	2260	3966

Presently pharmacologists are seeking for specific sources of flavonoids, isoflavones and phenolic compounds. In order to add common bean to formal lists of biologically active substances (BAS), some reliable methods of quantification for such compounds are required. For this reason we have analyzed phytochemical composition of some local and foreign varieties and lines of common bean including «Aktatti», «Djungarskaya», «Camellia», «Nazym», «Red Goya» (seeds), «Talgat» (seeds and empty pods) and some others. BAS detection has been carried out by using qualitative reactions and chromatography tools.

In course of the study it has been shown that bean specimens with different geographical origin (Kazakhstan, USA and Russia) would be varying also in the content of flavonoids.

So, line «Talgat» (Kazakhstan) and cv. «Red Goya» (USA) have appeared to lead by total amount of flavonoids (1.49 and 1.22 mg/g of dry weight, respectively), while cv. «Red Goya» (USA) and cv. «Ufinskaya» (Russia) would occur ahead of resting varieties and lines by total quantities of phenolic compounds (2.46 и 3.06 mg/g of dry weight, respectively).

It has been also indicated that cv. «Zhuravushka» (Russia) could be implied as a negative stan-

dard for the flavonoids content, because this BAS fraction has not been virtually detected, though this variety contained phenolic compounds (1.93 mg/g of dry weight), as it is shown in Table 5.

Domestic line «Talgat» has been determined to be one of the most prospective forms by substantial prevalence of the overall flavonoids fraction.

It is clear from the data presented above that parental combinations for the local gene pool of common bean may be chosen in consent with phytochemical analysis of the collection in hand. Newly generated domestic lines have been shown to possess a high phytochemical potential.

Data on stability and variability of morphogenic and structural features of common bean have allowed identifying some prospective forms for further breeding under conditions of Kazakhstan.

These studies are supposed to be a part of complex analysis of adaptive capacity and genotypic stability in altering environmental conditions. Such analysis would promote identifying high-productive and adaptive genetic stocks. To make a structural analysis of plant productivity, we have estimated root and pod length, pod width, number of pods per plant, seed number per pod, seed productivity, and seed mass per pod. We have also tested stem height, length and width of apical leaf and some other parameters.

Table 5 – Phospholipids and flavonoids in other beans varieties

Sample	Aktatti	Djungarskaya	Camellia	Nazym	Red Goya	Talgat		Ufimskaya	Karakoz	Zhuravushka
Part of plant						Seeds	Empty pods			
№	1	2	3	4	5	6	7	8	9	10
Optical density at wave length, $\lambda = 415$ nm (phospholipids)										
Probe, μ l	500	500	500	500	500	500	500	500	500	500
1	0.007	0.029	0.020	0.013	0.086	0.127	0.103	0.008	0.019	0.000
2	0.008	0.032	0.022	0.015	0.094	0.118	0.111	0.006	0.020	0.000
3	0.007	0.031	0.022	0.016	0.119	0.121	0.110	0.006	0.020	0.000
Phospholipids in probe, μ g:										
1	0.85	3.54	2.44	1.59	10.49	15.49	12.56	0.98	2.32	0.00
2	0.98	3.90	2.68	1.83	11.46	14.39	13.54	0.73	2.44	0.00
3	0.85	3.78	2.68	1.95	14.51	14.76	13.41	0.73	2.44	0.00
Dry mass, mg/g:										
1	0.09	0.35	0.24	0.16	1.05	1.55	1.26	0.10	0.23	0.00
2	0.10	0.39	0.27	0.18	1.15	1.44	1.35	0.07	0.24	0.00
3	0.09	0.38	0.27	0.20	1.45	1.48	1.34	0.07	0.24	0.00
Average	0.09	0.37	0.26	0.18	1.22	1.49	1.32	0.08	0.24	0.00
Deviation \pm	0.01	0.02	0.02	0.02	0.20	0.06	0.05	0.02	0.01	0.00
Optical density at wavelength, $\lambda = 765$ nm (phospholipids)										
Probe, μ l	500	500	500	500	500	500	500	500	500	500
1	0.239	0.540	0.702	0.504	0.769	0.716	0.645	0.958	0.631	0.611
2	0.246	0.541	0.698	0.506	0.782	0.704	0.646	0.967	0.669	0.606
3	0.263	0.540	0.716	0.522	0.781	0.723	0.661	0.978	0.642	0.617
Flavonoids in probe, μ g:										
1	7.56	17.09	22.22	15.95	24.34	22.66	20.41	30.32	19.97	19.34
2	7.78	17.12	22.09	16.01	24.75	22.28	20.44	30.60	21.17	19.18
3	8.32	17.09	22.66	16.52	24.72	22.88	20.92	30.95	20.32	19.53
Dry mass, mg/g:										
1	0.76	1.71	2.22	1.60	2.43	2.27	2.04	3.03	2.00	1.93
2	0.78	1.71	2.21	1.60	2.48	2.23	2.04	3.06	2.12	1.92
3	0.83	1.71	2.27	1.65	2.47	2.29	2.09	3.10	2.03	1.95
Average	0.79	1.71	2.23	1.62	2.46	2.26	2.06	3.06	2.05	1.93
Deviation \pm	0.04	0.00	0.03	0.03	0.03	0.03	0.03	0.04	0.06	0.02

Related data is available from the diagrams depicted in figures 1-3.

It is evident that the local line «Aktatti», along with the other foreign varieties, such as cv. «Igolinska» (Poland) and «Red Goya» (USA) possessing average root length of 73.13 cm and 68.53 cm re-

spectively, has a well-developed root-system reaching 67.3 cm length. Cvs «Camellia» (USA) and «Fatima» (Russia) have been shown to develop the smallest root networks.

Consequently, line «Aktatti» together with cvs «Igolinska» and «Red Goya» have been revealed

to outstrip other varieties by this parameter to be further used for phytoremediation activities under steppe conditions.

With the exception of American cvs «Pinto» and «Red Goya», development of the root system is in good agreement with pod length.

It is suggested that the US varieties are distant from the European (Russian and Polish) varieties

by their origin, and Kazakhstan lines are supposed to be closer to the European accessions, as they manifest similar correlation of root and pod lengths. Hence, this parameter of productivity, namely the correlation of root length and pod length could be taken into account in frame of the analysis for genetic relationship between common bean accessions from different geographic regions (Table 6).

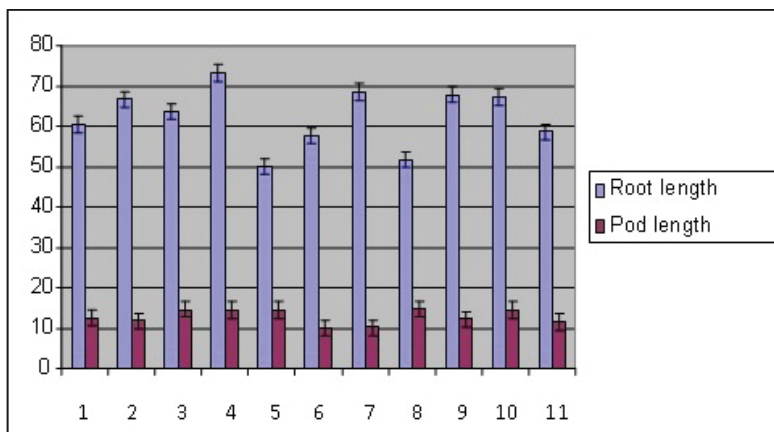


Figure 1 – Root and pod lengths of common bean samples: 1 «Biichanka»; 2 «Bomba»; 3 «Zhuravushka»; 4 «Igolinska»; 5 «Camellia»; 6 «Pinto»; 7 «Red Goya»; 8 «Fatima»; 9 «Aktatti»; 10 «Nazym»; 11 «Talgat»

Table 6 – Leaf size for chosen beans varieties

Beans sample	Country	Leaf length, cm	Leaf width, cm
Aktatti	Kazakhstan	10.4 ± 0.8	8.7 ± 0.4
Biichanka	Russia	11.2 ± 1.1	7.3 ± 0.8
Bomba	Poland	11.3 ± 0.9	9.2 ± 1.2
Zhuravushka	Russia	10.8 ± 0.3	7.6 ± 0.3
Camellia	USA	8.4 ± 0.7	5.7 ± 0.6
Igolinska	Poland	11.2 ± 1.2	9.4 ± 0.9
Pinto	USA	9.5 ± 0.5	8.1 ± 0.4

The widest pod in the steppe conditions belongs to cvs «Bomba» (Poland) and «Fatima» (Russia) with average width of 1.22 cm and 1.18 cm, respectively, and also local line «Nazym» (with average pod width 1.13 cm, Fig. 2).

On the basis of the seed mass accumulation in pods cvs «Zhuravushka» (Russia) and «Camellia» (USA) have been determined to pass ahead of other samples with average mass per pod 4.27 g and 3.57g, respectively.

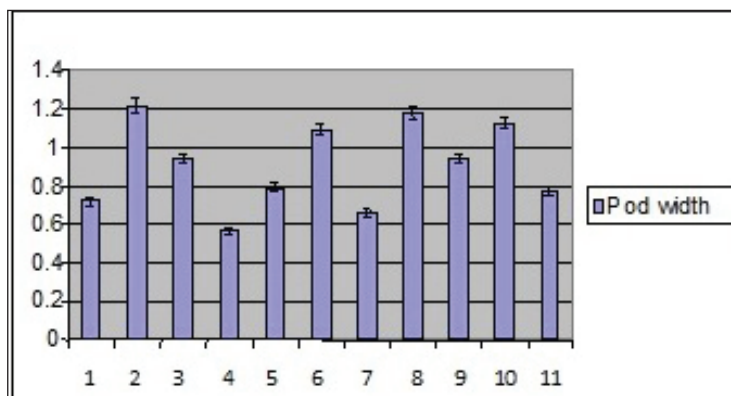


Figure 2 – Pod width of beans varieties and lines: 1 «Biichanka»; 2 «Bomba»; 3 «Zhuravushka»; 4 «Igolinska»; 5 «Camellia»; 6 «Pinto»; 7 «Red Goya»; 8 «Fatima»; 9 «Aktatti»; 10 «Nazym»; 11 «Talgat»

Besides, «Aktatti» (Kazakhstan), «Camellia» (USA) and Russian cvs «Biichanka» and «Zhuravushka» have demonstrated high productivity.

The mass of seeds for these specimens has reached 3.53, 3.54, 3.52, and 4.3 g, respectively (Fig. 3). Obviously, these local and foreign cvs and lines may be recommended for planting in the steppe zone for remark-

able harvests. It is anticipated that bean productivity of these samples would steadily rise under drip irrigation (as in cases of samples from N.I. Vavilov Institute [14] and vegetable bean varieties propagated in the Caspian Region and further mentioned in the proceedings of The Nikita State Botanical Garden of the Ukrainian Agricultural Academy [15].

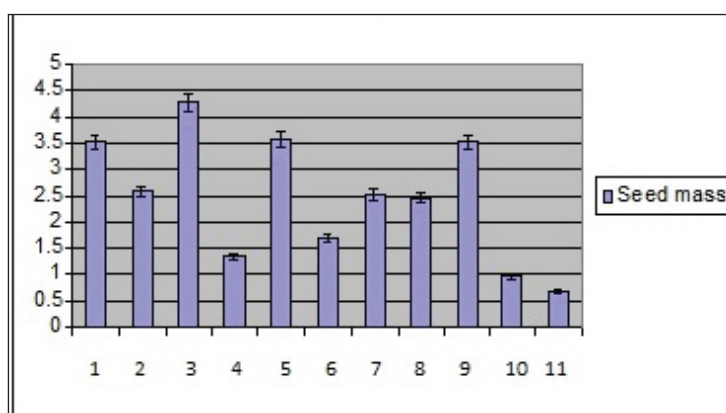


Figure 3 – Mass of seeds per pod of beans varieties and lines: 1 «Biichanka», 2 «Bomba», 3 «Zhuravushka», 4 «Igolinska», 5 «Camellia», 6 «Pinto», 7 «Red Goya», 8 «Fatima», 9 «Aktatti», 10 «Nazym», 11 «Talgat»

Based on this study, major samples have been shown to occur highly productive, early-ripening and adaptive to the steppe and arid zones even under irregular watering. Yield of cv. «Igolinska» has been shown to be reduced due to fungal disease.

Structural analysis of varieties and lines grown in the steppe zone has determined the most prospec-

tive and adaptive cvs («Zhuravushka», «Camellia», «Aktatti», «Biichanka» and others).

It has been shown that local line «Aktatti», along with some foreign varieties, would possess a powerful root system. This group of samples would be suggested for phytoremediation aims in the steppe zones.

Newly generated common bean lines should ideally combine high seed productivity, stability and highly efficient sets of biochemical and phytochemical characters. However, it is possible to determine a single character of a prospective sample, since varieties and lines might possess optimal combinations of essential amino acids or phenolic compounds leaving behind such a crucial trait as plant productivity.

Oppositely, highly productive varieties and lines could be quite poor in amino acid or polyphenol compositions. This versatile nature of common bean and its specific development allows us to use this vegetable crop for different purposes encompassing food and feedstuff production, pharmacology, phytoremediation, biotechnology and landscape design.

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