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CHARACTERIZATION OF BEAN VARIETIES ON THE BASIS OF PROTEIN MARKERS

ABSTRACT: The biochemical marker phaseolin and isozymes were used in this work to display the variation of common bean germ plasm. Fifteen bean genotypes of different origin i. e. selections were studied. From 8 analyzed enzymic systems, enzymes MDH, SKDH, ME and IDH were polymorphic, while there were no differences in zymograms for enzymes PGM, PHI, PGD, and ADH. Analysis of phaseolin revealed two types: S and T. The S type of phaseolin was found in most of analyzed genotypes (9). Phaseolin type T was found in varieties of Novi Sad selection: Zlatko, Sremac and Aster, domestic population Žuto-zeleni Stepanovićevo and Jovandeka, Croatian variety Slavonski žuto-zeleni. Those varieties were developed from domestic populations from north-west region of Balkan, Slavonia, and Vojvodina.

KEY WORDS: common bean, germ plasm, phaseolin, isozymes

INTRODUCTION

Bean is an unavoidable food component in diets of people living in many Balkan countries, and elsewhere in the world. It is main source of protein and energy, and is gaining importance in human diet.

Origin of bean (*Phaseolus vulgaris*) is America. It was brought to Europe from Central America during second Columbus voyage. It was brought to Balkan from two directions: from Turkey — south, and From France and Italy — north. Crossing of main trade ways, soil and climatic conditions, and other differences led to great divergence of bean in our surroundings (Vasić, 2004). Domestic populations of tall, climbing beans, of short bean and introduced American varieties with shrubby straight stem, and small, white with round grains prevailed earlier. Today, mostly domesticated populations and modern, bred bean varieties are grown (Vasić et al., 2001).

It is thought that domestication of bean was done in two regions, one is Central America, and second is the area of Andes in South America (Gepts et al., 1986). It is still unclear if there was small centre in Columbia around that region, where transfer of genes from wild relatives to domestic varieties was done (Beebe et al., 1997).

Proofs supporting diversity of two centers of origin come from study of variability of grain size (Evans, 1973), phaseolin (Gepts et al., 1986), morphology (Singh et al., 1991), isozyme (Koenig and Gepts, 1989, Singh et al., 1991a), and DNK markers (Haley et al., 1993).

Storage protein phaseolin and isozymes

From total protein content in bean 50 and 75% are globulins (Alli et al., 1994). There are two types of protein inside this group, the dominating one — phaseolin, and lectin or phytohemagglutinin (Staswik et al., 1986). Phaseolin the main reserve bean protein is soluble in high salt concentration. It contains from 35 to 50% of total nitrogen in seed (Ma and Bliss, 1978, Lioi, 1989). Phaseolin is coded with loci complex from 6 to 9 genes. Alleles coding polypeptides of each phaseolin type are co-dominant. Reserve proteins are reliable markers in studies of domestication and dispersion of bean varieties, and in analysis of phytogene relationship between species inside *Phaseolus* genus. In comparison with *Phaseolus vulgaris* L., bean and string bean, other species of this genus have not been studied enough in the context of molecular characterization.

Bean as a self-pollinated plant species presents an excellent material for isoenzymic fingerprint. Low level of heterozygosity makes it possible for each species to be characterized with one or two isozymic profiles (Weeden, 1984).

The aim of this work was to evaluate 15 bean varieties, using phaseolin seed protein and isozymes analysis, the genetic variability as well as to relate their origin to the Mesoamerican and Andrean gene pools. The results may contribute to improvement of germ plasm bank management and may improve the efficiency of the breeding process.

MATERIAL AND METHODS

Fifteen bean genotypes of different origin i. e. selections were studied in this paper. Eight varieties of Department of vegetables, Research institute of field and vegetable crops (IFVC), Novi Sad: Zlatko, Sremac, Balkan, Belko, Dvadesetica, Levač, Maksa and Aster, domestic population: Greenish-yellow Stepanovićevo and Jovandeka, Bulgarian varieties Prelom and Ludogorje, variety Medijana from Smederevska Palanka, American variety C-20, and Slavonski žuto zeleni from Croatia.

Stem tissues of 5 days old seedling homogenized in 50mMTrisHCl, pH 6.8 in which 1% mercaptoethanol was added, was used for isozymic analysis.

Isozyme systems: malate dehydrogenase (MDH), malic enzyme (ME), phosphohexose isomerase (PHI), phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM), shikimate dehydrogenase (SKDH), isocitrate dehydrogenase (IDH), alcohol dehydrogenase (ADH) were analyzed according to Stuber et al. (1988).

Preparation of samples and 1D-SDS PAGE electrophoresis of phaseolin were done according to Rodino et al. (2001). Four individual seeds were tested from each samples.

RESULTS

From 8 analyzed enzymic systems, enzymes MDH, SKDH, ME and IDH were polymorphic, while there were no differences in zymograms for enzymes PGM, PHI, PGD, and ADH (Fig 1). Genotypes Jovandeka and Aster had faster traveling variant of malic enzyme and malate dehydrogenase, while rest of them had slow traveling allelic variants (Fig. 2a and b). Three different allelic variants were found for enzyme shikimate dehydrogenase (Scheme 1) and two for locus *Idh1* isocitrate dehydrogenase.

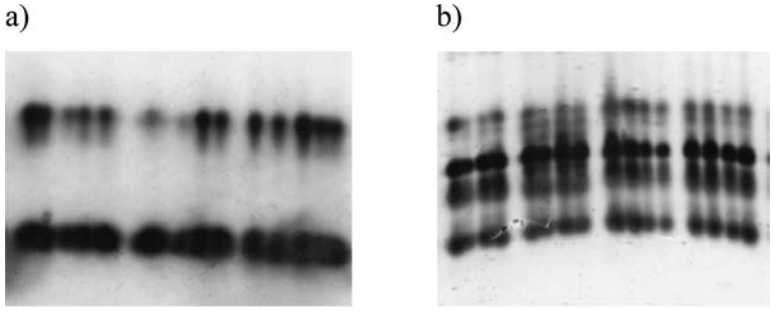


Fig. 1. — Zymogram pattern of PGM (a) and PHI (b) bean genotypes

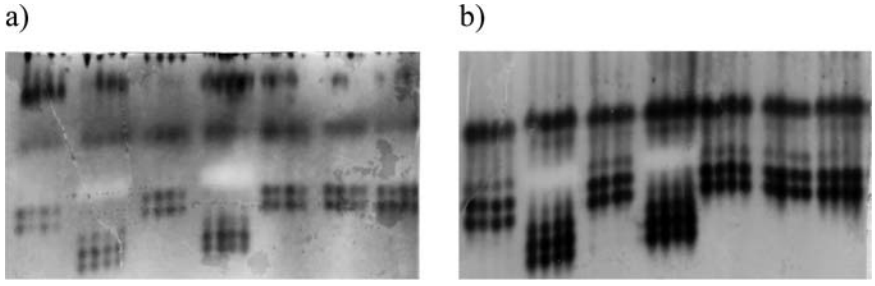
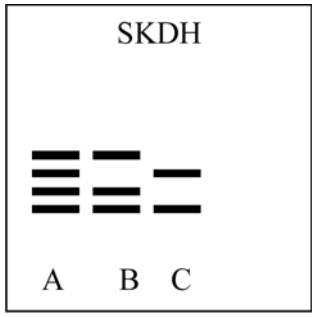


Fig. 2. ME (a) and MDH (b.) zymograms of bean genotypes from the left to the right: C-20, Aster, Ludogorje, Jovandeka, Prelom, Medijana, Greenish-yellow Stepanovićevo



Scheme 1. — Presentation of SKDH zymogram pattern of bean genotypes

Analysis of phaseolin revealed two types: S and T. S type of phaseolin was found in most of analyzed genotypes (9 from 14) (Tab. 1). Phaseolin type T is found in varieties of Novi Sad selection: Zlatko, Sremac and Aster, domestic population greenish-yellow Stepanovićevo and Jovandeka, Croatian variety Slavonski žuto-zeleni (Fig. 3).

Tab. 1. — Bean varieties, origin, type of phaseolin and isozymic variants

Bean variety	Origin	Type of phaseolin	MDH	ME	SKDH	IDH
1. Zlatko	IFVC	T	S	S	B	F
2. Sremac	IFVC	T	S	S	A	F
3. Balkan	IFVC	S	S	S	C	F
4. Belko	IFVC	S	S	S	C	F
5. Dvadesetica	IFVC	S	S	S	B	F
6. Levač	IFVC	S	S	S	C	F
7. Maksa	IFVC	S	S	S	C	F
8. Greenish-yellow Stepanovićevo	domestic population	T	S	S	A	S
9. Medijana	S. Palanka	S	S	S	C	F
10. Prelom	Bulgaria	S	S	S	C	F
11. Jovandeka	domestic population	T	F	F	B	S
12. Ludogorje	Bulgaria	S	S	S	C	F
13. Aster	IFVC	T	F	F	B	S
14. C-20	USA	S	S	S	C	F
15. Slavonski žuto-zeleni	Croatia	T	S	S	B	F

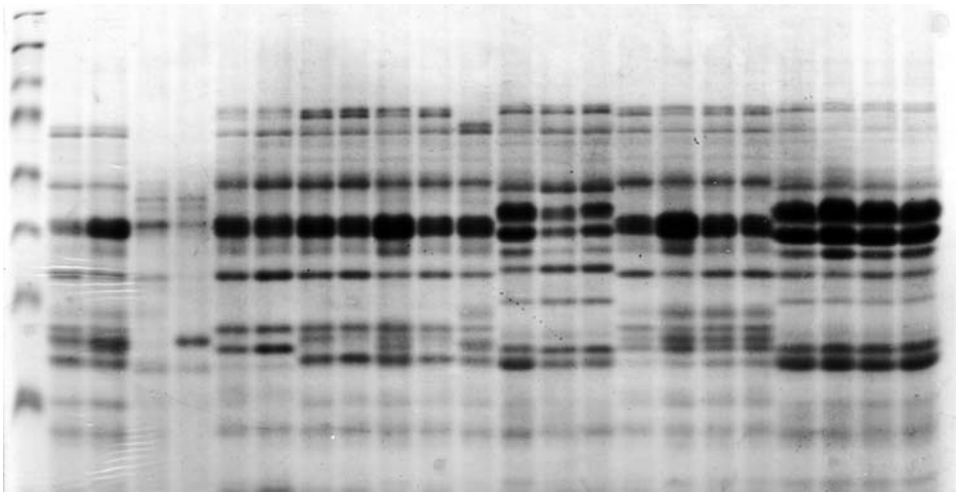


Fig. 3. — Different types of phaseolin obtained by SDS PAGE electrophoresis: 1. protein marker (170-11 kDa), 2,3 control S type phaseolin, 4,5 control C type, 6,7 control S, 8—11 C-20, 12—15 Aster, 16—19 Ludogorje, 20—23 Jovandeka

DISCCUSSION

The variability at the protein level has been well documented for *P. vulgaris* (Weeden 1984, Koening and Gepts, 1989). Isozyme analysis (Koening and Gepts, 1989) and the analysis of phaseolin seed storage protein pointed out to two different groups of *P. vulgaris*. It was found out that there was a relationship between geographic distribution and phaseolin type in wild and cultivated bean varieties. Samples from Central America had primarily S phaseolin type, with a few exceptions having M type. Samples from Andes had primarily T phaseolin type, and some had C, H, A, J, or I type. There are bean varieties with S and C/T phaseolin type, revealing that multiple events of gene recombination happened during domestication process (Brown et al., 1982, Gepts et al., 1986).

The origin of Serbian bean germ plasm is unclear. The biochemical marker phaseolin and isozymes were used in this work to display the variation of common bean germ plasm. The S type of phaseolin was found in most of analyzed genotypes (9 from 14) (Tab. 1), which revealed that in the process of development of new varieties under climatic conditions of our country and the region, germ plasm from Central America was used. According to Genčev et al. (2002) Bulgarian bean varieties with dominating S phaseolin were better adapted, to climatic conditions of high temperature, and irregular rain falls, in comparison to others.

Phaseolin type T was found in varieties of Novi Sad selection: Zlatko, Sremac and Aster, domestic population greenish-yellow Stepanovićevo and Jovandeka, Croatian variety Slavonski žuto-zeleni (Fig. 3). Those varieties were

developed from domestic populations from north-west region of Balkan, Slavonia, and Vojvodina.

Zeven et al. (1999) showed that T phaseolin type predominated in Holland gene bank. It was found in 132 genotypes from analyzed 157, which revealed Andes origin.

By combination of data for phaseolin and seed size one can conclude that at least three independent domestications took place. In Central America domestication led to varieties with small seed and S phaseolin type; in Columbia small seed and B phaseolin type, and in region of South Andes large seed and T phaseolin type. Low frequency of B phaseolin type pointed out that it was a minor center (Gepts et al., 1986). Origin of C, H and T phaseolin type has not been cleared yet. They have not been found in Central America. Brown et al. (1981) suggested that C phaseolin type could be created by translocation or uneven crossing over in hybrids between two lines having T and S type. This event could take place after introduction of varieties with S phaseolin type into Andes region. Results obtained in this work suggested that both gene pools were used in process of introduction and breeding of common bean in Serbia.

Data on isozymic variability in combination with data on phaseolin type give a fine picture on genetic diversity of bean varieties (Santalla et al., 2002). Analysis of specific region of genes for phaseolin, identification of variation in exon and intron, offers more precise data on genetic diversity (Kami et al., 1995).

CONCLUSION

It was confirmed by experiment that significant polymorphism of enzymic system was not expected since commercial bean varieties were studied. Different allelic variants were found for enzymes: MDH, ME, SKDH and IDH. Most of studied genotypes had S type of phaseolin, and T type was found in just a few. Germ plasm from Central and South America was used in the process of creation new varieties under climatic conditions of our country and the region. Analysis and characterization of varieties of Department of vegetables, Research institute of field and vegetable crops, Novi Sad at the level of protein was done for the first time. Obtained results present a solid starting base for further investigation of gene bank and application of molecular markers.

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КАРАКТЕРИЗАЦИЈА СОРТИ ПАСУЉА НА ОСНОВУ ПРОТЕИНСКИХ МАРКЕРА

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Резиме

У раду је проучено 15 сорти пасуља различитог порекла и селекција, из банке гена Завода за повртарство Научног института за ратарство и повртарство, Нови Сад. Анализирано је 8 ензимских система и резервни протеин фазеолин. Различите алелне варијанте нађене су за ензиме: MDH, ME, SKDH и IDH. Већина анализираних генотипова (9) има S тип фазеолина. Сорте новосадске селекције: Златко, Сремац и Астер, домаће популације Жуто зелени Степановићево и Јовандека, хрватска сорта Славонски жуто-зелени имају T тип фазеолина. Новосадске сорте су настале избором из домаћих популација из северозападног подручја Балкана, Славоније и Војводине.

На основу добијених резултата закључено је да се у процесу стварања нових сорти у климатским условима наше земље и региона користила гермплазме из Средње и из Јужне Америке. По први пут су извршене анализе и карактеризације сорти Завода за повртарство Научног института за ратарство и повртарство, Нови Сад, на протеинском нивоу. Резултати полиморфизма фазеолина и изоензима представљају добру полазну основу за даља истраживања банке гена пасуља и примену молекуларних маркера.