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Acta Horticulturae
Number 830

Proceedings of the Fourth Balkan Symposium on Vegetables and Potatoes

Volume 1

Editors

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**PROCEEDINGS OF THE
IVth BALKAN SYMPOSIUM
ON
VEGETABLES AND POTATOES**

Volume 1

Convener

L. Krasteva

Plovdiv, Bulgaria

September 9-12, 2008



ISHS Section Root and Tuber Crops
ISHS Section Vegetables

Acta Horticulturae 830
June 2009

Genetic Diversity of the Bulgarian *Phaseolus vulgaris* L. Germplasm Collection through Phaseolin Marker

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Keywords: *Phaseolus* × *vulgaris*, genetic diversity, core collection, phaseolin type, seed coat colour

Abstract

Evaluation of the Bulgarian *Phaseolus* × *vulgaris* L. germplasm collection has been undertaken in order to constitute a representative 'core collection' on the basis of discriminating characters. Investigation on present genetic diversity in local common bean accessions and breeding forms has been carried out through 1D-SDS-PAGE and a densitometry of bands from proteinograms. Four phaseolin types: 'T', 'Ca', 'C' and 'S' have been distinguished indicating a low level of genetic diversity in comparison with the level occurring in centres of origin and primary diversity of this neotropical species. As a highly conservative marker, the phaseolin type is helpful to characterize the primary gene pool of the accessions grown and maintained in a region of secondary diversity, as Bulgaria became more than three centuries ago. In Bulgaria, representatives of the two Andean and Mesoamerican common bean gene pools have been identified, with a predominance of the Andean over the Mesoamerican one. Most local accessions share the 'T' phaseolin type, while the 'Ca' type predominates among the breeding lines and cultivars.

INTRODUCTION

Phaseolin is the most important protein, representing up to 49% of the nitrogen compounds of the seeds (Ma and Bliss, 1978) and belonging to the vicilin family of the seed storage proteins (Blagrove et al., 1981; Gottschalk and Müller, 1983; Brown et al., 1981; Osborn, 1988). Gepts and Bliss (1986); Gepts (1988a, b); Koenig and Gepts (1989a, b); and Singh (1991) subdivided the species into two gene pools using 1D-SDS-PAGE: Andean pool in which 'T' phaseolin type prevails among others, and Mesoamerican pool in which 'S' phaseolin type dominates (Gepts, 1990; Gepts and Bliss, 1986; Gepts et al., 1986). In the neotropical regions where the *P. vulgaris* primary gene pool is distributed, 'S' type is present in both cultivated and wild accessions while 'M', 'I' and 'J' phaseolin types are present only in wild forms (Gepts et al., 1986; Koenig et al., 1990). 'M' type dominates in the Mesoamerican gene pool, but in addition 'S' and 'B' types appear also in this gene pool (Gepts and Bliss 1988; Lioi 1989). 'T' phaseolin dominates in the Andean gene pool (Gepts et al., 1986; Gepts and Bliss, 1986), but 'H', 'C' and 'CH' types also appear in wild and cultivated forms from the Andean gene pool (Koenig et al., 1990). The presence of 'Ca', 'Na', 'Ko', genetically related to 'T' phaseolin (Tolme et al., 1995), as well as variants, such as 'Sb' and 'Sd', close to 'S' type (Gepts and Deboutck, 1991) increase the variability occurring in Andean and Mesoamerican gene pool, respectively. At present about 30 different phaseolin types in cultivated *P. vulgaris* forms and twice more in wild forms are

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known.

The common bean genotypes from the New World is organized into two main germplasm groups represented by Middle American (Central and North America) and Andean South American (south of the Andes) gene pools. The two groups have been subdivided into races, based mainly on morphological, agronomical, and biochemical evaluation (Singh, 1991). Studies with DNA markers have confirmed the existence of these two germplasm groups corresponding to two domestication centres (Gepts, 1993; Miklas and Kelly, 1992; Skroch et al., 1992a, b; Sonnante et al., 1994; Tohme et al., 1996). In Europe, the variability found among the common bean germplasm is partly the result of adaptation to different growing environments of germplasm introductions from both the Andean and the Mesoamerican domestication centres. The contribution of the two New World gene pools, Mesoamerican and Andean, to the evolution of the European germplasm of *P. vulgaris* is not well established.

The aim of the present study was to investigate the *P. vulgaris* genetic diversity in the Bulgarian collection through the identification of phaseolin types, with a view to assist gene bank management and plant breeding.

MATERIALS AND METHODS

In our study, 409 common bean genotypes were investigated. Among them 235 local accessions from the gene bank of the Institute of Plant Genetic Resources in Sadovo, collected from 21 expeditions since 1977, were included. The accessions were sampled from 69 locations in order to cover the whole countries territory and to be representative of the geographical and ecological areas of Bulgaria. Another set of genotypes consisted of 127 breeding lines and cultivars resulting from breeding programmes and available in the working collections of the Mariza Vegetable Crop Research Institute, Plovdiv and of the Dobroudza Agricultural Institute, General Toshevo. The remaining 47 recently introduced accessions came from different gene banks outside the country; such accessions are integrated as parental genotypes in hybridization programmes with local accessions and breeding lines and cultivars from the Mariza and Dobroudza Institutes working collections. In order to compare the electrophoretic profiles of the Bulgarian accessions, controls for phaseolin types 'S', 'T', 'Ca' and 'C', were obtained from CIAT (Centro Internacional de Agricultura Tropical, Cali, Colombia), recognized as the worldwide repository bank of *Phaseolus* genus. The control accessions were sown in the experimental fields of the region of Plovdiv (South Bulgaria) and General Toshevo (North Bulgaria) to observe their protein patterns when they were grown in different geographical locations.

Basic Method - 1D-SDS/PAGE

Identification of phaseolin type was performed in 10 single ripe seeds from randomly selected plants of each accession by one-dimensional SDS/PAGE (Koenig et al., 1970). Two molecular weight markers from protein bands, purchased from Merck, MMW-1 - Standard mixture IV (Art. No15791) and MMW-2 - Standard mixture VIII (Art. No11536) were used for phaseolin zone determination on the proteinograms (Fig. 1a, f). Protein types were identified thanks to referential accessions kindly offered by Dr. D. Debouck from CIAT: ICA Pijao - for 'S' type phaseolin, CDRK - for 'T' type, G2362 - for 'Ca' type, and G21194 - for 'C' type.

Additional Densitometric Method

For the phaseolin identification of variants with close electrophoretic phenotypes, a separation to 1D-SDS/PAGE proteinograms was performed. Ultra Violet Products Gel Documentation System, Version 7.00 Science Park, Milton Road, Cambridge CB4 4FH, England and Gel Work-1D-Advanced, Version 3.01 (<http://www.statsoft.com>) were applied consecutively. The intensity of each fraction of the phaseolin zone on the proteinograms obtained from different accessions was measured and compared. This was useful to distinguish the phaseolin types 'T' and 'Ca'.

Mathematical Procedure

In order to find a criterion for comparison of the phaseolin types normal approximation of binomial distribution was used. Computing procedure following Brownly (1977) was applied to compare proportions of groups identified by phaseolin types in local accessions and breeding genotypes. The criterion verified in this procedure is bilateral. The predominating part of accessions from gene pool and from phaseolin type was considered preferentially in the groups of comparison. The $U_{1,p}$ obtained from the computing of the experimental data was determined and compared to the tabular value of the standard normal distribution marked with $N(0,1)$ and was evaluated at three different thresholds of statistical significance p corresponding to three different probabilities for the verified hypotheses: when the probability of the verified hypothesis corresponds to different thresholds of statistical significance: $p < 90\%$ */, $p < 95\%$ */**, and $p > 95\%$ /****/, the tabular values of the standard distribution $N(0,1)$ are, respectively, 1.64, 1.96 and 2.57.

RESULTS AND DISCUSSION

Screening 409 common bean local accessions, breeding lines and cultivars from the Bulgarian collection allowed us to identify four phaseolin types: 'S', 'C', 'Ca' and 'T'. 'S' corresponds to the Mesoamerican gene pool while 'C', 'Ca' and 'T' correspond to the Andean gene pool. The proteinograms of the total seed storage proteins include the fractions corresponding to the phaseolin types as shown in Figure 1. No difference in phaseolin type was observed from single ripe seeds belonging to each Bulgarian accession except few cases within the breeding lines and cultivars. The different phaseolin types identified in plants from the group of the breeding lines and cultivars corresponded to the same types of phaseolin observed in the parents. The phaseolin fractions on Figure 1 cover all types assessed in the national collection. Phaseolin fractions on the proteinograms are characterized by molecular weights between 45000 D and 51000 D (Brown et al., 1981; Osborn, 1988). The first electrophoretic pattern corresponds to 'S' phaseolin type and represents the phaseolin pattern of 9JE281 local accession, coming from Sovoljano village (in the region of Kyustendil town) (Fig. 1b). 'C' type represents the proteinogram of E5780 accession, coming from Kurdzhali (Fig. 1c). 'T' type represents the proteinogram of cultivar 'Ruets', coming from Ruets village (near by Turgovishte - Noth-Est Bulgaria) (Fig. 1d). 'Ca' type represents the proteinogram of accession E6087, coming from the town of Antonovo (near by Turgovishte) (Fig. 1e). More details on the geographical distribution of these localities are given in Tomlekova et al. (1999).

There was a great similarity in electrophoretic phaseolin phenotypes between accessions from the national collection and the corresponding reference accessions (Fig. 2). All phaseolin types identified in our collection corresponded to the well known types identified in the cultivated forms of the primary gene pool.

No polymorphism in seed electrophoretic profiles was observed in the control accessions used as reference to identify the phaseolin types grown in different geographic locations: control cultivars obtained from CIAT and control accessions collected from Plovdiv and General Toshevo from South and North Bulgaria expressed the same electrophoretic profiles. Identity of the phaseolin zones on the electrophoregrams of reference accessions from different geographic locations demonstrated the conservatory propriety of the selected marker, i.e., phaseolin. The latter is well suited to reflect genetic diversity studies in the *P. vulgaris* national collection of Bulgaria, considered as a centre of secondary domestication.

P. vulgaris accessions were classified in two basic groups, corresponding to the two previously established gene pools – Andean and Mesoamerican pools (A and M groups) – on the basis of phaseolin type characterization of seed storage proteinograms. Clustering accessions according to phaseolin types and gene pools are given in Table 1 and Figure 2. Among the local accessions from Bulgaria, 'T' phaseolin prevails over the other identified types while in breeding lines and cultivars from the Bulgarian collection 'Ca' phaseolin dominates over the remaining ones. The introductions from the Mesoamerican gene pool characterized with 'S' phaseolin type outnumber the introductions from the Andean one

with the identified 'T', 'C', 'Ca' phaseolins. From the 409 studied accessions, 230 entries are determined by a phaseolin type associated with the Andean gene pool (A). This group includes accessions with 'C', 'Ca' and 'T' phaseolin types (Fig. 1c, d, e). The remaining 179 entries possess only one 'S' phaseolin type and are identified as representatives of the Mesoamerican gene pool (M) (Fig. 1b). In Table 2, the two groups of Bulgarian common beans (local accessions: breeding lines and cultivars) are compared according to phaseolin types: only Andean gene pool data are given (A), as Mesoamerican (M) data are analogous. The percentage of the phaseolin types belonging to the Andean gene pool is differently distributed in each one of the two groups: local accessions; breeding lines and cultivars. 'T' type is prevalent in local accessions while 'Ca' type prevails over the 'C' and 'T' in breeding lines and cultivars. The 'C' type proportion is low, especially for the breeding lines and cultivars from the Andean gene pool (7%), while in the group of local forms, it represents 25%. The percentage of each identified phaseolin type in the two corresponding groups (local accessions: breeding lines and cultivars) has been estimated. 'S' phaseolin type covers 40% and 43%, respectively of the collection made of local accessions on one part, and of breeding lines and cultivars, on the other part. The remaining material belongs to the Andean gene pool. The accessions from the A predominate in both local accessions (64%) and breeding lines and cultivars (58%) (Table 2). Evaluation of standard normal distribution of the Andean representatives frequencies in the local accessions on one part, and in the breeding lines and cultivars on the other part, to the integer is $U_{1-p}=0.21$ (Table 2), which is considerable less than the tabular value of $N(0,1)=1.64$. Study the frequencies of distribution of the representatives from Andean gene pool there are no reasons to consider that the group of the local accessions is distributed in a different way from the group of the breeding lines and cultivars. The same distribution frequencies occur with the M representatives. The A and the M representatives are distributed similarly in the two groups, i.e. local accessions and breeding lines and cultivars, respectively. Therefore, the distribution of the phaseolin type frequencies was studied in the gene pool they belong to. In order to compare the types of phaseolin distribution frequencies, we used the predominating phaseolin types in the Andean group ('T' or 'Ca'). Percentage of types belonging to the Andean gene pool was examined in the two groups: the group of the local accessions and the second group of the breeding lines and cultivars. Percentage of the predominating phaseolin type is different between the two groups (Fig. 3). 'T' type predominates in local forms from the Andean gene pool, while 'Ca' type is the most frequent among breeding lines and cultivars from the same gene pool. Significant difference is obtained for the distribution of 'T' and 'Ca' types of phaseolin in the Andean group to which they belong. The calculated value $U_{1-p}=3.47$ (Table 2) is considerably higher than the tabular values, e.g. $N(0,1)=2.57$. In our case, 'C' phaseolin frequencies cannot be analyzed because of insufficient number of accessions corresponding to this type. The difference in the predominating phaseolin types is obvious and the difference of distribution frequencies of all the phaseolin types in the two groups (local accessions, and breeding lines and cultivars), reflects the preferences of farmers and breeders for genotypes bringing specific characters. The 'T' type is associated with the white seed coat colour that is preferred by the Bulgarian consumers.

Data from our study showed that 60% of the Bulgarian accessions came from the Andean gene pool (A) while 40% came from the Mesoamerican one (M). According to a preliminary study (Tomlekova, 1999), the domination of the Andean gene pool over the Mesoamerican gene pool was expected. However, our results showed a relatively fair proportion of accessions from the Mesoamerican group, which can be explained by the introduction of accessions characterized predominantly by 'S' phaseolin type (66%). Among the accessions introduced mostly during the last 15 years into our working collections, the Andean phaseolin types represented a lower proportion. Presence of 'S' phaseolin is frequently associated with resistances to important diseases which explain their introduction in the country for breeding purpose and hybridization with local materials (Koenig et al., 1989b).

On the basis of phaseolin determination, results showed a low variability level in

the analyzed Bulgarian accessions. The latter represent a very limited genetic diversity, by comparison with the total variability displayed among the material distributed in the primary centre of common bean, i.e., the New World. The low level of genetic diversity is a result of the significant 'founder effect', a term introduced by Ladizinsky (1985), in the secondary centres of *P. vulgaris*, such as Bulgaria. Common bean was introduced approximately 300 years ago in the country and this food legume was converted as a traditional crop. At the beginning, the level of genetic variability was low due to the limited number of introduced accessions. This reduction of genetic diversity was reinforced because of the lack of adaptation in some materials to the new ecological local conditions. Additional factors might have strongly restricted the genetic variability of Bulgarian accessions, such as a specific consumer preference (for large and white seeds), occurrence of diseases (*Bean Common Mosaic Virus*, *Xanthomonas* × *campestris* pv. phaseoli (Common Bacterial Blight), *Pseudomonas* × *syringae* pv. phaseolicola (Halo Blight) and insects, such as *Acanthoscelides* × *obtectus* (Bean weevil) and the breeding systems of the species (autogamous floral biology).

In this context, it might also be relevant to consider the domestication phases in *Phaseolus vulgaris*. First, there was a decrease in genetic diversity, but accompanied by an increase in morphological variability. This restricting effect was, on the basis of our own results, stronger among the Mesoamerican group represented by one phaseolin type. This compared with the Andean group represented by three different phaseolin types. This confirms the data published by Gepts (1990) for the primary domestication centre of the species, explaining the larger genetic diversity of the Andean Bulgarian accessions. In a second phase, there was a reduction in genetic diversity explained by the linkage occurring between the phaseolin locus and other loci, influencing the phenotype and submitting the genotype to breeding pressure (Maquet, 1995).

The accessions available in our collections were found in four different climatic zones. The most appropriate climatic zone, i.e., the Highlands zone, was represented by the lowest number of accessions in Bulgaria. This can be explained by a different adaptation of common bean accessions (only domesticated varieties) in a secondary diversity centre, compared to the adaptation of common bean (both wild and domesticated varieties) occurring in the primary diversity centre. The selection pressure (both natural and anthropic) is different between Bulgaria and the neotropical regions.

Farmer's preferences and consumer requirements, were the two reasons explaining the occurrence of specific seed trait. During the 20th century, breeding efforts concentrated on market cultivars, which were important to the region. Hybridizations were made between closely related genotypes resulting in homogeneous breeding lines. Gene pools and groups within them can be recognized in cultivated common bean on the basis of the biochemical marker. Our investigation confirmed that phaseolin, a useful indicator of genetic origin, has been a selection-neutral trait during the process of introduction and adaptation of bean accessions in Bulgaria.

CONCLUSIONS

Four phaseolin types are distinguished in the investigated material from the Bulgarian common bean collection: 'S', 'T', 'Ca' and 'C'. The accessions with 'S', 'Ca' and 'C' phaseolin types belong to the Andean gene pool group while the accessions with 'T', Mesoamerican (44%). 'T' phaseolin type is the most predominant (56%) over those from the local Bulgarian accessions, which represents 66% from the Andean gene pool. 'Ca' type is the most frequent in the breeding lines and cultivars, covering 79% from the Andean gene pool.

The introduced accessions have increased the genetic diversity of the national collection of common bean with their high proportion of 'S' type.

The marker-based analysis using phaseolin as an initial classification criterion, suggested the existence of subgroups within one of the two major groups, the Andean

one.

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Tables

Table 1. Distribution of *P. vulgaris* accessions according to phaseolin types and gene pools. Identity of 'S' and M (Mediterranean gene pool), 'S' being the unique representative of M group.

Number of investigated accessions (n)	Accessions grouped by Accessions grouped by phaseolin types of Andean group															
	A			M(S)			T			Ca			C			
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Local accessions	235	141	60	94	40	93	66	12	9	36	25					
Br. lines and cultivars	127	73	57	54	43	10	14	58	79	5	7					
Introductions	47	16	34	31	66	12	75	1	6	3	19					
Total	409	230	56	179	44	115	50	70	30	44	19					

Table 2. Distribution of Bulgarian *P. vulgaris* accessions according to their phaseolin types.

Accessions groups	Proportion (%) of Andean group according to the total number of accessions from the two gene pools	U _{1-p} of gene pools	Proportion (%) of 'T' and 'Ca' according to the total number of accessions of the Andean gene pool	U _{1-p} of phaseolin types
Local accessions	60.00		74.47	
Br. lines and cultivars	58.28	0.21	91.89	3.47 v.s.

Figures



Fig. 1. Representatives of the phaseolin types proteinograms for the Bulgarian *P. vulgaris* L. collection: 1a - MMW-1; 1b - 91E281 ('S' phaseolin); 1c - E5780 ('C' phaseolin); 1d - 'Ruets' ('T' phaseolin); 1e - E6087 ('Ca' phaseolin); 1f - MMW-2; The molecular weight of the standard protein bands indicated on the figure is in D - daltons.



Fig. 2. Identity of the phaseolin zones on the electrophoretograms of reference accessions with studied accessions. First of each pair is the referent accession, second - is taken from the studied collection : a-'S' phaseolin of ICA Pijao, b-'S' of 91E281, c-'Ca' of G2362, d-'Ca' of E6087, e-'C' of G21194, f-'C' of E5780, g-'T' of CDRK, i-'T' of cv. Ruets.



Fig. 3. Percentage proportion of phaseolin types in Bulgarian *P. vulgaris* collection.