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GENETIC DIVERSITY OF BULGARIAN *PHASEOLUS VULGARIS* L. BASED ON PHASEOLIN TYPE AND SEED-COAT COLOUR

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Abstract

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Knowledge of genetic diversity enhances the efficiency of germplasm conservation and improvement. Previous studies, using phaseolin seed protein as a molecular marker, have revealed four groups (T, Ca, C and S) of Bulgarian common bean accessions (*Phaseolus vulgaris* L.). The present study examines seed-coat colour diversity of 292 *P. vulgaris* accessions and the relation between phaseolin type and seed-coat colour. A previous study focused on phaseolin type and the present study adds an analysis of seed-coat colours and patterns and their relationships. The results identify subgroups within the major Andean and Mesoamerican genetic groups. Seed-coat colour and pattern comparisons by accessions, complemented with biochemical data, increase the discriminating power of genetic diversity analyses.

Key words: Common bean, germplasm conservation, molecular marker, seed storage protein

Introduction

The neotropical genus Phaseolus contains fifty to sixty wild species. Most of them are distributed in Mesoamerica and only five have been fully domesticated: *P. vulgaris* L., *P. dumosus* Macfady, *P. coccineus* L., *P. acutifolius* A. Gray and *P. lunatus* L. (Gutiérrez Salgado et al., 1995; Caicedo et al., 1999; Delgado-Salinas et al., 1999). Genetic reservoirs of common bean (*P. vulgaris* L.) derive from two major origin centres, Mesoamerica and the Andes. Their gene pools are distinguished based on morphological (Evans, 1980; Poryazov, 1989; Apostolova et al., 2005; Chacyn et al., 2005; Negahi et al., 2014) and molecular data (Gepts, 1988; Koenig et al., 1990; Beebe et al., 2000; Myers and Davis, 2002; Logozzo et al., 2007; Kwak and Gepts, 2009; Tomlekova, 2012). The pathways of bean distributions into and across Europe seem to be very complex, with several introductions from the New World that were combined with direct exchanges between Mediterranean and other European countries. Beans from the primary domestication centers in the Americas were transported and distributed to the Balkan Peninsula, including Bulgaria, which is considered a secondary diversity center (Gepts et al., 1986). In the past, phaseolin and morphological traits were extensively used to analyze the structure of the European common bean germplasm and the presence of the two gene pools. Homogeneous phaseolin patterns in cultivated landraces were reported by Gepts (1993), unlike heterogeneous phaseolin patterns among wild bean accessions. Crossing experiments have shown that the genes coding for different polypeptides of each phaseolin pattern are tightly linked and inherited as single unit with co-dominant alleles (Brown et al., 1981). Therefore analy-

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sis of variation of the electrophoretic patterns of the major seed storage protein phaseolin is useful in establishing intra-species relationships (Gepts, 1993).

Various studies have examined molecular genetic diversity in the common bean (Ocampo et al., 2002; Rodiño et al., 2003; Rodiño et al., 2006; Logozzo et al., 2007) and lima bean (Fofana et al., 1997; Fofana et al., 2001; Motta-Aldana et al., 2010, Serrano-Serrano et al., 2010) but few have specifically studied snap beans. Based on phaseolin type, snap beans are derived from the Andean origin centre (Brown et al., 1982; Gepts et al., 1986). However, in some contemporary varieties, the divisions between Andean and Mesoamerican origin centers have been blurred due to crossing between the groups, as shown by molecular marker analysis (Skroch et al., 1992; Skroch and Nienhuis, 1995). Diversity in phaseolin patterns was compared among over four hundred *P. vulgaris* L. dry and snap bean accessions from Bulgaria and four types were found: T, Ca, C and S (Tomlekova et al., 2009). The phaseolin analysis of these accessions showed that varieties and recombinant inbred lines could be classified into the two major gene pools, even though some accessions showed an intermediate set of markers (Tomlekova, 2012).

The present study was to build on a previous knowledge of phaseolin markers. The objectives were (1) to expand knowledge of *P. vulgaris* germplasm diversity by describing the seed-coat colour of 292 Bulgarian *P. vulgaris* samples and (2) to establish the relationship between phaseolin type and seed-coat colour and pattern.

Materials and Methods

A representative sample of 292 accessions of Bulgarian dry and snap bean germplasm was assessed according to seed-coat colour following the IBPGR descriptor list (Kluwer and Dordrecht, 1982). They were collected from 1970 to 2000 and classified as breeding lines, local accessions or introduced samples. They were maintained at the Maritsa Vegetable Crop Research Institute in Plovdiv, the Dobrudza Agricultural Institute in General Toshevo and the Institute of Plant Genetic Recourses in Sadovo, Plovdiv. Basic seed-coat colours were beige to brown, red to purple, white and black, in addition to a variety of colour combinations and patterns.

As determined by Dimitrov (1973), regions where beans are traditionally grown in Bulgaria cover the following climatic zones: temperate continental, transient continental, transient Mediterranean, Black Sea and highland.

Results

Tables 1, 2 and 3 show the distribution of the Bulgarian common bean genotypes according to the two main gene pools, seed-coat colours, patterns and climatic zones.

The collection included 115 samples of snap beans and 177 samples of dry beans. *P. vulgaris* genotypes in Bulgaria belonged mainly to the Andean wild gene pool (63%), in which snap beans were the most common. Skroch and Nienhuis (1992) reported that snap bean accessions were predominantly of Andean origin using

Table 1
Distribution of Bulgarian common bean collection among the primary gene pools

Common bean genotypes	Total number of samples	Andean gene pool		Mesoamerican gene pool	
		numbers	%	numbers	%
Total number of samples	292	183	62.7	109	37.3
Snap beans:	115	80	69.6	35	30.4
Dry beans:	177	103	58.2	74	41.8

Table 2
Distribution of the phaseolin types according to seed coat colours and patterns in the Bulgarian common bean collection

Phaseolin type			Basic seed coat colour								Additional Patterns	
			Beige-brown		White		Black		Red-violet			
	No. of access.	%	No. of access.	%	No. of access.	%	No. of access.	%	No. of access.	%	No. of access.	%
S	119	40.8	14	11.8	93	78.2	10	8.4	2	1.7	5	4.2
Ca	89	30.5	17	19.1	65	73.0	3	3.4	4	4.5	8	9.0
T	64	21.9	17	26.6	30	46.9	16	25.0	1	1.6	19	29.7
C	20	6.8	15	75.0	2	10.0	2	10.0	1	5.0	7	35.0
Total	292	100	63	21.6	190	65.1	31	10.6	8	2.7	39	13.4

* No. of access. – Number of accessions

Table 3
Distribution of accessions from different phaseolin types by climatic zones within Bulgaria

Accessions studied		Climatic zones				
Phaseolin type	Total number	Temperate continental, number	Transient continental, number	Transient Mediterranean, number	Black Sea, number	Highland, number
S	119	41	60	3	1	13
Ca	89	16	62	1	0	9
T	64	32	29	1	0	3
C	20	2	16	0	1	2
Total amount	292	91	167	5	2	27

cluster analysis of data obtained from RAPD (random amplified polymorphic DNA) markers. The Mesoamerican gene pool was represented by 37% of the Bulgarian accessions; of these, 30% and 41% were snap and dry beans, respectively.

The S phaseolin was present at the highest frequency (40.8%), followed by the Ca type (30.5%), the T type (21.9 %), and the C type, with only 6.8% (Table 2). Among S phaseolin genotypes from Mesoamerica, white-seeded accessions predominated (78.2%), followed by beige-seeded (18%), black-seeded (13%) and red-violet-seeded ones (only 1%) (Figure 1). In addition, 4% of the S phaseolin genotypes presented brown or violet stripes over the basic beige colour of the seed coat (Figure 2).

The S phaseolin accessions were distributed mainly in transient continental (n = 60) and temperate continental zones (n = 41). Among all phaseolin types, S phaseolin genotypes were most frequent in the highland climatic zone (Table 3). The Ca type was the second largest group and is from the Andean wild gene pool. Seed-coat colour was mostly white (73%) in addition to beige-brown (19%), red-violet (4.5%) and black (3%). Most of the Ca phaseolin accessions were from the transient continental climatic zone. Among the T phaseolin, the white-seeded genotypes were most common (47%) and some had black spots. One accession had white and red seeds. Black seeds were observed in 25% of the accessions and two were a mixture of black and white. Beige to brown colours were observed in 27% of the accessions; in most cases this colour was not homogeneous, with seeds showing spots or stripes in addition to the basic coat colour. The T type was represented in 64 genotypes, most of them from temperate continental (n = 32) and transient continental zones (n = 29) with a few from the highland zone. The C type, of Andean origin, was identified in 20 genotypes and accessions were mostly beige to brown (73%), but also white (12%), red-violet (8%) and black (8%). The beige-brown accessions with the C type were the second most-frequent colour (35%). Most of the accessions from C type were local landraces from the transient continental climatic zone.

Fig. 1. Seeds with single colour of the coat

Fig. 2. Seeds with additional pattern to the basic colour of the coat

White-seeded genotypes dominated the collection (65%). They were in every phaseolin type and the most frequent in the Ca and T phaseolin types.

Discussion

Consumer preferences in Bulgaria for white-seeded beans are well known. Bulgarian farmers have selected white-seeded accessions for many generations which have resulted in the predominance of white seeds among local populations and landraces. The white colour was described as typical for the Ca type 'nuña' bean from the Mediterranean region (Tohme et al., 1995). In previous studies, the Ca type was identified in Bulgarian collections as predominant in the recombinant breeding lines (Tomlekova et al., 1999; Tomlekova, 2012). However, in the present study, beige colour with violet stripes was identified in 19% of Ca phaseolin accessions. According to seed-coat colour, the Ca and C groups were the most homogeneous while T groups were the most heterogeneous, which were mostly from local landraces. The S type was the most distinct group, well separated from the other three groups, the most common in the study and mostly from local landraces. Contrary to expectations, some accessions with black, beige-brown and red-violet seeds were also observed in the S phaseolin group.

Common bean accessions from the secondary diversity centre in the Balkan Peninsula and Bulgaria in particular belonged to Mesoamerican and Andean gene pools, according to both morphological and molecular data. The Andean group included 183 accessions with the Andean phaseolin types (T, C, Ca) and the Mesoamerican group included 109 accessions with only one phaseolin type (S). There was considerable variation in seed-coat colour and climatic zones distribution within the phaseolin groups. Accessions with phaseolin types of one gene pool and one or more morphological and allozyme traits of the other gene pool were classified as inter-gene pool recombinants by Rodiño et al. (2006).

Snap beans have been domesticated in different locations and this seems to have happened often (Myers and Davis, 2002). Introgression between Andean and Mesoamerican gene pools may have been facilitated by occasional outcrossing as well as cultivating both types together in home gardens or small farms in Bulgaria. Since the 20th century, breeding efforts have been mainly aimed at developing varieties for the canning industry with bush-growth patterns conducive to mechanized cultivation and harvest, usually by combining white seeds and improving productivity. With the goal of combining these traits with disease resistance, hybrids were made between accessions from Bulgaria, Western Europe and the United States (Poryazov et al., 1983; Poryazov, 1989). The Bulgarian varieties developed for commercial markets and the canning industry seem to be derived from separate lineages.

Conclusions

Our study was based on two levels: marker-based analysis using phaseolin, and seed-coat colour and pattern. The results suggest the existence of subgroups within the two major Andean and Mesoamerican groups. The subgroups have distinctive seed morphology and intermediate traits. Information on gene pool origins, domestication syndrome traits, molecular diversity and evolutionary significance of recombinant accessions would be worthwhile to undertake pre-breeding research. Further complementary studies using other morphological and agronomical traits (such as days to maturity, seed shape, size and weight, reaction to biotic and abiotic stresses) as well as molecular markers will be required to develop improved, well-adapted varieties.

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