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Molecular characterization of *Phaseolus vulgaris* L. genotypes included in Bulgarian collection by ISSR and AFLP™ analyses

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Abstract

Seventy-eight (33 Bulgarian and 45 foreign) common bean (*Phaseolus vulgaris* L.) genotypes included in Bulgarian collection were screened for ISSR (Inter Simple Sequence Repeats) and AFLP™ (Amplified Fragment Length Polymorphism) markers. ISSR analysis was performed with 13 primers, and 55 (36.7 %) out of the 150 bands observed were polymorphic. One hundred and sixty-four AFLP™ fragments were obtained with three primer combinations, of which 54 (32.9%) were polymorphic. UPGMA (Unweighted Pair Group Method Arithmetic Averages) analysis was performed using DICE coefficient and dendrograms were constructed using either the data from each method (ISSR and AFLP™) separately or combined in a single joint matrix. Despite some genetic heterogeneity observed in both pools (Middle American origin: M and Andean origin: A) the genotypes were separated in to main groups: one gathering genotypes mainly from pool M and the other more genotypes from pool A. Moreover, Bulgarian genotypes were spread over the two groups suggesting that they are not subject to genetic erosion and that the *Phaseolus* genetic diversity is conserved.

Keywords: Common bean; Genetic similarity; Molecular markers; Germplasm

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is an important economic food legume widely grown in many countries in the world. However, there is evidence that its domestication has induced a strong reduction in diversity at the molecular level (Gepts et al., 1986; Schinkel and Gepts, 1988). This reduction contrast with the increase in diversity observed for morphological traits during and after domestication (Gepts et al., 1992) because of different consumer and breeding purposes.

Comparison of wild and cultivated beans indicate that the crop was domesticated several times and in distant regions (Tohme et al., 1996). Molecular, physiological and morphological analyses, carried out on common bean, strongly support the existence of two distinct centers of genetic diversity commonly known as the Middle American and Andean gene pools (Gepts and Bliss, 1985; Gepts et al., 1986). Each of these gene pools has been subdivided into races (Singh et al., 1991a).

The necessity of preserving important germplasm has led to the construction and maintenance of very large germplasm collections. The biggest collection of common bean in the world, is that one at CIAT and currently it contains over 24,000 accessions (Tohme et al., 1995). It was subdivided on core and reserve collections, in order to facilitate the study and understanding of the existing diversity (Skroch et al., 1998).

Different ways were chosen to investigate common bean diversity. Isozyme (Bassiri and Adams, 1978; Koenig and Gepts, 1989a; Singh et al., 1991b) and morphological (Koenig and Gepts, 1989b; Singh et al., 1991c) analyses were performed. Phaseolin protein type (Gepts et al., 1986; Gepts, 1990) was also used.

For more accurate determination of the genetic relationships, DNA markers have advantages over morphological traits, such as distinguishing among accessions with similar morphology and discriminating polymorphism over far more loci than isozymes and seed proteins (Beebe et al., 2000).

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Becerra Velasquez and Gepts (1994) revealed genetic differences among common bean races of the Middle American gene pool applying RFLP (Restriction Fragment Length Polymorphism) analysis to genomic DNA, while Khairallah et al. (1992) used mitochondrial RFLPs in wild beans for understanding the domestication processes.

The genetic structure of a core collection of wild *P. vulgaris* L. was characterized by AFLP™ (Amplified Fragment Length Polymorphism) (Tohme et al., 1996). The advantage of RAPD (Random Amplified Polymorphic DNA) markers as a rapid, cost effective tool for the indirect selection of economic traits was immediately recognized (Kelly and Miklas, 1998). In addition to their value in mapping and gene 'tagging' studies (Park et al., 1999; Schneider et al., 1997; Haley et al., 1994; Johnson et al., 1997), RAPDs have been deployed extensively in germplasm identification and characterization (Alzate-Marin et al., 1996; Beebe et al., 1995; Cattan-Toupance et al., 1998; Duarte et al., 1999; Freyre et al., 1996; Schneider et al., 1997). RAPD markers overcome the limited number and undesirable pleiotropic effects of many morphological markers, the limited number and difficulties associated with the tissue- and development-dependent isozyme markers, and the high labor requirements of RFLP analyses (Miklas et al., 1996). However, RAPD has been known for its sensitivity to many factors that limit reproducibility mainly among different laboratories (Penner et al., 1993). The use of SCAR (Sequence Characterized Amplified Region) produces genetic markers that are highly specific, which may overcome this problem (Sartorato et al., 2000). The effectiveness of RFLP, DAMD-PCR (Direct Amplification of Minisatellite Region DNA), ISSR and RAPD markers in assessing polymorphism and relationships between commercial lines of *P. vulgaris* L. was evaluated by Métais et al. (2000).

The aim of the present study was to analyse DNA fingerprints from a Bulgarian common bean collection utilizing ISSR and AFLP™ methods to determine the genetic structure of the populations and to compare Bulgarian with foreign genotypes.

2. Materials and methods

2.1. Plant material

Seventy-eight (33 Bulgarian and 45 foreign) genotypes of common bean (*P. vulgaris* L.), included in Bulgarian collection were analysed (Table 1). Foreign genotypes were received by exchange of germplasms between Bulgarian and foreign research institutes. Young trifoliate leaves were taken for analysis from three plants of each genotype.

2.2. DNA extraction

After grinding the plant material in a mortar with a pestle in the presence of liquid nitrogen, the resulting fine powder was resuspended in buffer containing: 200 mM Tris–HCl pH 8.5; 25 mM EDTA; 255 mM NaCl; 0.5% SDS and 2% PVP. The extract was further purified by a Rnase-Atreatment (20 μg/ml) performed at 37 °C for 30 min and followed by a ''classical''-phenol:chloroform extraction (Sambrook et al., 1989). After precipitation with isopropanol the DNA was washed with cold ethanol (75°), dried, and resuspended in TE buffer (10 mM Tris–HCl pH 8.0, 1 mM EDTA). The DNA concentration was assessed spectrophotometrically and by 1% agarose gel electrophoresis.

2.3. ISSR analysis

ISSR markers were synthesized using 18 bp primers (GIBCO BRL Custom Primers). ISSR analyses were performed using a Biometra T-Gradient thermal cycler programmed for an initial step of 4 min at 94 °C followed by 40 cycles at 94 °C for 30 s, 46 °C for 45 s, 72 °C for 2 min and finally a 7 min extension step at 72 °C. Reactions were performed in a 20 μl volume, using 40 ng of template DNA, 1 μM of primer, 1 U of Taq DNA polymerase (Pharmacia, Biotech), and 0.2 mM of each dNTP (100 mM dNTP Set, Life Technologies) in reaction buffer containing 10 mM Tris–HCl pH 9.0, 50 mM KCl and 1.5 mM $MgCl_2$. The amplification products were separated by electrophoresis in 2% agarose gels and visualised by ethidium bromide staining. A molecular weight marker ladder of 100 bp scales was used in electrophoresis. Gels were photographed using a Kodak EDAS 120 system.

2.4. AFLP™ analysis

AFLP™ analyses were performed using standardised procedures (Vos et al., 1995) with minor modifications, as described by Monte-Corvo et al. (2000). Primers EcoRI (5′-GACTGCGTACCAATT-3′) and MseI (5′-GATGAGTCCT-GAGTAA-3′) with three selective nucleotides at the 3′ end were used. The EcoRI primers were 5′ labelled using γ^{33}P [ATP] for detection by autoradiography. Reagents required were obtained from Life Technologies Inc. as kits, except for Taq DNA polymerase (Pharmacia Biotech) and γ^{33}P [ATP] (NEG). AFLP™ products were separated in denaturing 6% poliacryilamide gels (19:1 ratio acrylamide:bisacrylamide, 7.5 M urea, 1 × TBE buffer), at 45 W constant power for about 2 h 30 m. Gels were transferred to 3 MM (Whatman) paper, dried on vacuum (80 °C, 60 min), and exposed to X-ray film (Kodak X-OMAT AR5), at room temperature, for 24–72 h, depending on signal intensity. Molecular weight marker ladders of 10 bp and a 100 bp scales (Life Technologies Inc.) were used in electrophoresis. Two replicated cuts of the DNA sample from the same genotype were analysed.

2.5. Data analysis

Band profiles generated by ISSR or AFLP™ were completed onto a data matrix on the basis of the presence (1) or absence (0) of selected bands. Data were statistically analysed by the software program NTSYSpc 2.01b (Numerical Taxonomy and Multivariate Analysis System, Applied Biostatistic Inc., 1986–1997.) (Rohlf, 1989). Dendrograms were