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Genetic diversity among the Turkish common bean cultivars (*Phaseolus vulgaris* L.) as assessed by SRAP, POGP and cpSSR markers

Ahmet Ceylan, Necip Öcal, Mikail Akbulut*

Erciyes University, Faculty of Sciences, Department of Biology, 38039 Kayseri, Turkey

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ABSTRACT

The genetic diversity among the Turkish cultivars of common bean (*Phaseolus vulgaris* L.) was estimated by studying the Sequence Related Amplified Polymorphism (SRAP), Peroxidase Gene Polymorhism (POGP), and Chloroplast Simple Sequence Repeats (cpSSR) markers. The unweighted pair group method arithmetic average (UPGMA) and Neighbor joining (NJ) algorithm resulted in a dendrogram representing the genetic relationship among major common bean cultivars grown in Turkey. The dendrogram generated two groups possibly representing two different major gene pools. By using three different marker systems, 194 alleles were detected and 118 were found to be polymorphic. For SRAP, POGP and cpSSR, 64, 64 and 26% polymorphism ratio were obtained, respectively. Principal Component Analysis (PCA) was also carried out to determine genetic variation among common bean genotypes and three different groups were generated. The individuals were placed into three different populations in structure analysis. Three populations created in structure analysis were exactly corresponded to the three groups in PCA. Analysis of Molecular Variance (AMOVA) was used to partition the genetic variations. The percentage of the variance was approximately 59%, 3%, and 38% among groups, among populations within groups and, within populations, respectively. The percentages of variation were found to be significantly high within the populations and among the groups.

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important staple crop which is extensively cultivated and used in human feeding in many countries in the world. Genetic diversity studies using molecular and morphological approaches suggest the existence of two distinct centers of genetic diversity, namely Andean and Mesoamerican (Gepts et al., 1986). Accessions of those two gene pools can be distinguished by several characteristics such as the seed size in the first instance and some other characteristics. Accessions from Mesoamerican bean germplasm contains as either small or medium seeds, while the accessions from Andean germplasm contains larger seeds (Angioi et al., 2010). Genetic diversity studies using DNA and protein markers demonstrated the existence of common bean genotypes representing both Andean and Mesoamerican gene pools and also their hybrids in Europe (Angioi et al., 2010; Sicard et al., 2005).

* Corresponding author. Tel.: +90 3522076666/33061, +90 5305617412 (mobile); fax: +90 3524374933.
E-mail addresses: aceylan@erciyes.edu.tr (A. Ceylan), necipocal@gmail.com (N. Öcal), akbulut@erciyes.edu.tr (M. Akbulut).

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Having higher genetic diversity provides a strong base for selecting superior genotypes for plant breeding. There is indication that breeding of common bean has caused a considerable decrease in genetic diversity (Svetleva et al., 2006). Discrimination of plant genotypes is essential in plant breeding, genetic diversity and population structure studies. Genetic diversity and population structure of *P. vulgaris* were generally analyzed based on different types of molecular markers such as RFLPs (Becerra-Velásquez and Gepts, 1994), RAPDs (Martins et al., 2006; Tiwari et al., 2005), AFLPs (Sustar-Vozlic et al., 2006; Pallottini et al., 2004) and SSRs (Kwak and Gepts, 2009; Burle et al., 2010; Mercati et al., 2012). SSRs were considerably more efficient among the markers used to characterize bean germplasm collections from both America and Europe (Blair et al., 2009; Angioi et al., 2010) in recent studies. In addition to nuclear SSR, cpSSR were also used to determine the origin and genetic structure of common bean accessions in various studies (Angioi et al., 2010).

Characteristic primer design makes SRAP markers reproducible, simple, and efficient in detecting genetic diversity in different plant materials SRAP have been used for genetic diversity and phylogenetic studies in many legume species such as vicia (Alghamdi et al., 2012), Pea (Esposito et al., 2007), lentil (Smutkupt et al., 2006), and Soybean (Baloch et al., 2010).

Considering higher level of sequence diversity in peroxidase gene sequences among the plant genotypes (Zhang et al., 2001), POGP markers have efficiently been used to assess the genetic relationship among the accessions of different plant species (Gulsen et al., 2007, 2009).

Dry bean is one of the important staple crop in Turkey with 200.000 tonnes of annual production (FAO stat, 2012). There is very little information about genetic diversity of Turkish common bean and few articles about molecular characterization of Turkish dry bean cultivars and accessions. Recently, Khaidizar et al. (2012) used SSRs for molecular characterization of 38 landrace and 12 registered cultivars. The aim of the present study was to investigate the level of genetic variation among nationally registered Turkish common bean cultivars by analyzing the polymorphism of POGP, SRAP and cpSSR molecular markers. To the best of our awareness, POGP has not been used for molecular characterization of common bean genotypes.

2. Materials and methods

2.1. Plant materials

Of the 14 registered Turkish bean cultivars used in this study, 13 were obtained from Eskişehir Transition Zone Agricultural Research Institute and 1 local cultivar Kırıkkale (unregistered cultivar) was obtained from a farmer. Characteristics of the cultivars are provided in Table 1.

2.2. DNA extraction

Total DNA was extracted from 300 mg fresh leaf tissue obtained from dark germinated bean seedlings. Modified CTAB DNA extraction procedure was used for DNA extraction (Doyle and Doyle, 1990). DNA concentrations were checked using 1% agarose gel and diluted to 10 ng μl^{-1} for further use in PCR amplifications.

2.3. SRAP markers

13 combinations of seven forward and 6 reverse SRAP primers were used in this study (Table 2). The reaction mixture (15 μl) comprised of 30 pmol of each of primer pairs, 200 μM of each of dNTPs, 2.5 mM of $MgCl_2$ as a final concentration, and 1 unit of Taq polymerase (FC, Biotek, Turkey) and 25 ng of template DNA. Sensequest DNA thermal cycler was used and cycling parameters were as follows; one cycle of 2 min at 94 °C, 34 cycles of 1 min at 94 °C, 1 min at 35 °C annealing, 1 min at 72 °C, for extension, and one cycle 5 min at 72 °C. PCR products were separated on 2.5% agarose gel at 90 V for 5 h and visualized with Vilber Lourmat gel documentation system.

Table 1
Phaseolus cultivars and landraces used for the analysis of diversity.

Cultivar	Growth habitus	Flower color	Number of seeds per pod	Grain morphology	100 grain Weight
Akdağ	Dwarf-Erect	White	3–5	Rectangular-white	50.5–55.5 gr
Akman-98	Semi-climbing	White	3–5	Reniform-white	34.0–35.0 gr
Aras-98	Dwarf-Erect	White	4–5	Rectangular-white	45.0–47.0 gr
Eskişehir-855	Erect	White	4–5	Rectangular-white	61.5 gr
Göynük-98	Dwarf-Erect	White	3–5	Rectangular-white	53.5–55.5 gr
Karacaşehir-90	Semi-climbing	White	6–7	Mini reniform white	18.0–19.0 gr
Kırıkkale	Semi-climbing	White	3–5	Reniform white	34.0–35.0 gr
Noyanbey-98	Dwarf	White	3–5	Rectangular-white	45.0–52.0 gr
Önceler-98	Dwarf-Erect	Light lilac	3–5	Pale-cream, redstriped, cuboid	40.5–41.0 gr
Şahin-90	Erect	White	4–5	Rectangular-white	45.0–50.0 gr
Şehirali-90	Erect	White	4–5	Rectangular-white	45.0–47.0 gr
Yakutiye-98	Dwarf-Erect	White	3–5	Rectangular-white	43.0–45.0 gr
Yunus-90	Dwarf-Erect	White	4–5	Rectangular-white	41.0–43.0 gr
Zülbiye	Dwarf-Erect	White	3–5	Rectangular-white	49.5–51.5 gr