Genetic Diversity in the Dwarf Dry Bean (Phaseolus Vulgaris l.) Populations Grown in Konya

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Abstract : This research was conducted to determine the genetic differences among the dwarf dry bean (*Phaseolus vulgaris* L.) populations collected from the Konya province of Turkey. Seedlings were grown in a controlled greenhouse of Selçuk University, Faculty of Agriculture during the year 2007. A total of 38 populations were gathered from the city center, the towns and the villages. Genetic differences were determined via ISSR method. By using 10 primers a total of 85 DNA bands were obtained of which 71 were found to be polymorphic.
Our results have demonstrated that the populations used were clustered into 3 major groups. Similarity degrees were in between 0.48 – 097. Knowledge about the genetic characteristics of a population is fundamental in breeding programs and it involves the local farmers. This strategy allows to improve and, at the same time, safeguard the genetic reliability of landrace genetic resources.

Keywords: Dry bean, ISSR, *Phaseolus vulgaris*, polymorphism.

Introduction

Common bean (Phaseolus vulgaris L.) is an annual, diploid (2n=22) species derived from wild ancestors distributed from Northern Mexico to Northwestern Argentina. Common bean (Phaseolus vulgaris L.) is an important economic food legume widely grown in many countries in the world.

Konya ranks first in Turkey in terms of the bean cultivation areas with a total area of 14.869 ha and a production level of 27.818 tons (Anonymous 2008).

The necessity of preserving important germplasm has led to the construction and maintenance of very large germplasm collections.

For 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.

Many molecular marker techniques have been developed in recent years. They started to be used successfully in molecular genetics laboratories. Those that are PCR (Polymerase Chain Reaction) based include SSR (Simple Sequence Repeat), AFLP (Amplified Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA) and ISSR (Inter Simple Sequence Repeats). Cultivar lines that are morphologically similar could be separated and defined with the use of markers (Lowe et al. 1996). The relationship degree that is found in between genetic materials can be used in selection programs in order to improve the agricultural material. Polymorphism is much higher in DNA-based systems when compared to classical and biochemical markers.

According to research on wheat, ISSR provides results similar to RAPD and RFLP in determining genetic relationships and diversity. Additionally, ISSR markers were found to be more reliable (Nagaoka and Ogihara 1997). Like RAPDs, ISSRs are quick and easy to handle, but they seem to have the reproducibility of SSR markers because of the longer length of their primers (Bornet and Branchard 2001). Kantety et al. (1995) reported that ISSRs can be used to determine the polymorphism among species and for plant improvement. Using ISSR in maize was found to be easy and cheap, taking short time and giving more polymorphic bands when compared to RAPDs.

ISSR technique have been widely applied in various plant varieties in recent years. It was successfully used in bean (Galvan et al. 2003; Marotti et al. 2007), chickpea (Iruela et al. 2002; Rajesh et al. 2002; Sudupak 2004), pea (Kuznetsova et al. 2005), lentil (Duran et al. 2004) and barley (Hou et al. (2005).

It is amazing to see that farmers obtain high yields from the populations without using certified lines.

There are a numerous genotypes in the hands of the farmers. Usually the farmers give different local names for the same seeds. This study aims to demonstrate both the applicability of ISSR-PCR method and to reveal the DNA fingerprints belonging to the populations involved.DNA level identification of genetic diversity among dwarf dry bean (Phaseolus vulgaris L.) populations, which are grown in Konya province was also targetted.

Material and Method

Plant Material and DNA Isolation

Each of 10 samples used were collected from 38 dry bean seed populations that were grown widely in the center, county and villages of Konya (Table 1).
Seeds were germinated in a glasshouse on wet sand under controlled conditions (20 C^0 of temperature and 12 h photoperiod). After 21 days, the primary leaves were harvested and DNAs were extracted. For DNA isolations 2xCTAB method was used.

Table 1. The codes, origins and local names of the dry bean populations used in DNA isoloations

Code	Origin	Local name
1	Başarakavak County	Horoz
2	Başarakavak County	Sarıkız
3	Başarakavak County	Kanada
4	Çumra (Center)	Şeker (Bıyıklı)
5	Çumra (Center)	Kırgız Çalısı
6	Çumra (Center)	Horoz
7	Çumra (Center)	Beyşehir Çalısı
8	Çumra (Center)	Bombay (Bomba)
9	Çumra (Center)	Kanada
10	Altınekin (Center)	Amerikan Kollu Fasulye
11	Altınekin (Center)	Sarnıç
12	Altınekin (Mantar Village)	Amerikan Çalısı
13*	Çumra (Center)	Niğde Barbunyası
14	Konya (Center)	Gina
15	Ereğli (Center)	Dermason
16	Ereğli (Center)	Horoz
17	Kadınhanı (Center)	Welhung
18	Kadınhanı (Center)	Kanada
19*	Kadınhanı (Center)	Akman - 98
20	Derbent (Center)	Amerikan (Beretta)
21	Derbent (Center)	Sarhoş (Washington)
22	Derbent (Center)	Şeker
23	Beyşehir (Göçü Village)	Horoz
24	Seydişehir (Center)	Sıra
25	Ilgın (Beykonak Village)	Beyaz Horoz
26	Ilgın (Center)	Horoz (Kırk Günlük)
27	Sarayönü (Center)	Kanada
28	Sarayönü (Center)	Amerikan Çalısı
29	Yunak (Center)	Üveynk (Veynk)
30	Yunak (Center)	Kanada
31	Çumra (Center)	Kırgız Yuvarlak (Kollu) Barbunya
32	Derbent (Center)	Yuvarlak Barbunya
33	Akşehir (Center)	Dermason
34	Akşehir (Sorkun Village)	Ayşe Kadın
35	Akşehir (Center)	Horoz (Oturak)
36	Akşehir (Center)	Dermason (Oturak)
37	Kazım Karabekir	Kanada (Kara Yaprak)
38	Kazım Karabekir	Dermason (Kırgız)

* Certified line

Figure 1 shows the geographical origins of the common beans used.

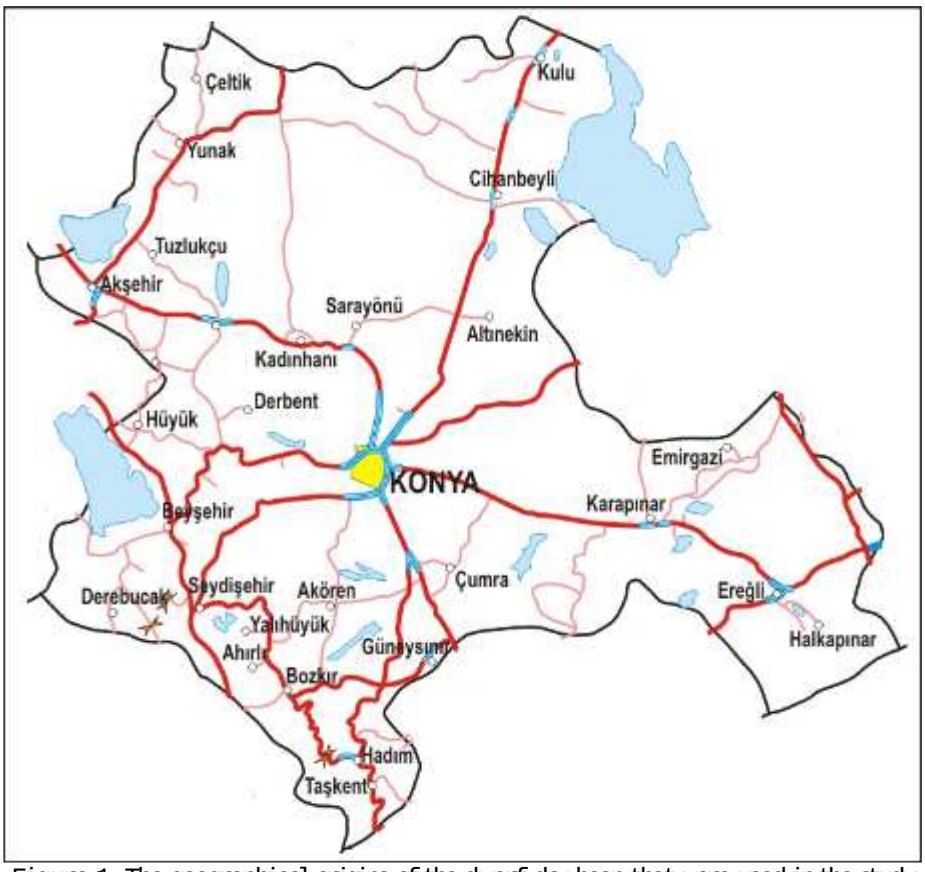

Figure 1. The geographical origins of the dwarf dry bean that were used in the study

PCR Amplification

Genomic DNAs were extracted from leaf samples using 2xCTAB mini-prep (cetil three metil amonyum bromid) method (Hulbert and Bennetzen, 1993).Sequences of ISSR markers used are listed in Table 2. Concentrations of DNA samples that were dissolved in 100 ul TE buffer were read by using a spectrophotometer. DNA concentrations were equalized to 20 ng/ul with sterile pure water. PCR mixture was consisted of 2.5 µl 10 X Taq buffer (Bioron), 2.5µl 25 mM $MgCl_2$, 0.4 µl dNTPs (25 mM of each) (Lavron), 0.5 µl Primer (50 pmol/µl) and 0.3 µl Taq DNA Polymerase (Bioron 5 U/µl), 14.8µl distilled water and 20 ng of genomic DNAs. The final reaction volume was 25 µl.

PCR conditions

The PCR conditions included initial denaturation step for 1 min at 95°C, followed by 20 cycles with denaturation at 94°C for 1 min; annealing at 55°C for 2 min; extension at 72 °C for 2 min and final extension for 10 min at 72°C. Amplification products were fractioned in 2% agarose gel, stained using ethidium bromide and viewed under UV light.

Scoring and analysis of ISSRs

ISSR analysis of 38 dwarf dry bean (Phaseolus vulgaris L.) populations was conducted with 10 primers (Table 2). DNA bands were scored for their presence (1) or absence (0) in the ISSR profile of populations used. from all populations. UPGMA dendogram and Principal Coordinate Analysis (PcoA) was obtained using a commercial software named as NTSYS-PC.

Results

In this study, ISSR markers were used for quick, easy to practice and economic application possibilities. ISSR fragments from 38 dwarf dry bean (Phaseolus vulgaris L.) populations were obtained using 10 primers. The primers used in the study and the total fragment numbers, number of polymorphic fragments and polymorphism ratios are given in Table 2.

Primers	Base Sequence (5'-3')	Melting Tempeture Tm (oC)	Length of the primer (bp)	G/C (%)	Fragment numbers	Polymorphic fragment numbers	Polymorphism ratios (%)
F1	$GAG(CAA)_5$	49.1	18	38.9	7	4	57.14
F2	$CTC(GT)_8$	56.7	19	52.6	6	6	100
F3	$(AG)_8CG$	56.0	18	55.6	5	5	100
F4	$(AG)_8TG$	53.7	18	50.0	6	2	33.33
F5	$(AG)_8$	49.2	16	50.0	15	15	100
F6	$C(CAC)_4CA$	53.3	15	66.7	6	4	66.67
F7	$(AC)_8$	49.2	16	50.0	9	7	77.78
F8	$(GCC)_5$	67.0	15	100	10	7	70
F9	$(GAA)_5$	39.6	15	33.3	10	10	100
M1	$(AGC)_5G$	63.1	16	68.4	11	11	100
Total and Mean		53.7	16.6	56.6	85	71	83.53

Table 2. Primers used in the study and the total number of fragments, number of polymorphic fragments and polymorphism ratios

As it can be seen from Table 2, in total 85 DNA fragments were obtained, 71 of which were polymorphic, the remaining 14 being monomorphic. While fragments obtained from all populations, F5 primer was the one which yielded the highest number of fragments (15), and F3 was the one that yielded the lowest number of fragments (5). F2, F3, F5, F9 and M1 primers gave the highest polymorphism ratios (100%) while F4 primer had the lowest polymorphism ratio (33.33%). Taken into consideration all of the primers, the overall ratio of polymorphism obtained in the study was identified as 83.53%. Genetic similarity values of 38 bean populations were in between 0.48 and 0.97 based on the results of applied 10 ISSR markers.

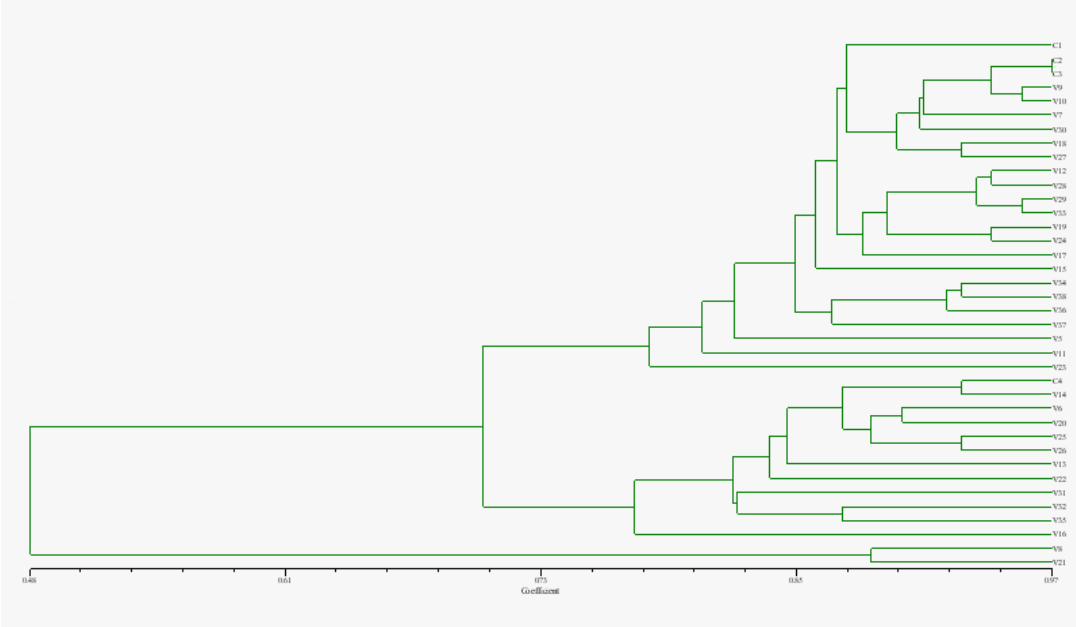

Figure 2. The UPGMA dendogram of the 38 dwarf dry bean (Phaseolus vulgaris L.) populations based on 10 ISSR markers

In the study NTSYS-pc 2.0 software was used in order to identify the genetic relationship and genotypic diversity of the populations through UPGMA method. As it can be seen in Figure 2, the populations were divided into three main groups, one of which were constituted of V8 and V21 populations that were genetically different from the other populations. The populations that constituted the other groups were also divided into sub-groups. C2 and C3 which were genetically the closest populations.
The populations C2 and C3, which are found to be closest to each other in genetic constitution on the dendogram

were also found to be genetically related in the Principal Coordinate Analysis (PCoA) (Figure 3). In addition, genetically the most distant individuals were found to be the populations V8 and V21.

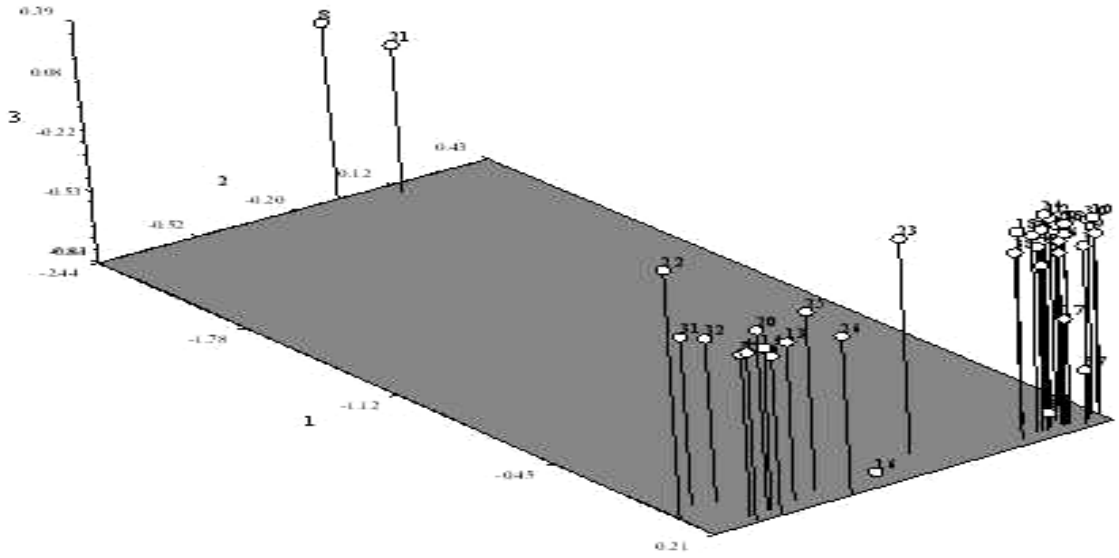

Figure 3. PCoA analysis of the 38 dwarf dry bean (Phaseolus vulgaris L.) in terms of ISSR markers

Discussion

According to the results it can be said that the ISSR markers present high level of polymorphism in dry bean populations that were applied. Thus, they can serve as markers to be used in determination of genetic relationships. They are attractive since it is easy to apply them and they are very sensitive tools in molecular studies. They also have high level of reproducibi,lity. (Bornet and Branchard 2001; Reddy et al. 2002; Galvan et al. 2003; Duran et al. 2004; Sudupak 2004; Gonzalez et al. 2006; Kuznetsova et al. 2005; Hakkı, E. E. et al. 2007; Marotti et al. 2007).

A previous study has considered the utility of ISSR markers for the genetic diversity and to determine the relationships among thirteen cultivars of common bean, eight of the nine primers that were used were same with this study (Galvan et al. 2003). In that research primers ($F_{1\text{-}2\text{-}3\text{-}4\text{-}5\text{-}7\text{-}8\text{-}9}$) provided 85 amplified bands and 71 of them were polymorphic.

Inter simple sequence repeats (ISSRs) may be usable and reliable genetic markers to provide accurate information for the identification of germplasms.

As a result, it can be said that these dwarf dry bean (Phaseolus vulgaris L.) populations have a heterogenous nature and they could be used for purposes of genetic improvement in Konya province. At the same time, with a more comprehensive study with other ISSR primers and different molecular markers the power of the analysis may be improved. Additionally, the number of populations may be extended to include additional genetic material, so that a larger genetic background may be used in further breeding programs.

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