Plant Omics Journal POJ

POJ 4(4):220-227 (2011) ISSN:1836-3644

Genetic variation in common bean landraces efficiently revealed by Td-DAMD-PCR markers

Ayse Gul Ince[1], Mehmet Karaca[2]

[1]Department of Horticulture, Faculty of Agriculture, Akdeniz University, 07059 Antalya, Turkey
[2]Department of Field Crops, Faculty of Agriculture, Akdeniz University, 07059 Antalya, Turkey

***Corresponding author: mkaraca@akdeniz.edu.tr**

Abstract

Some farmers still grow common bean (*Phaseolus vulgaris* L.) landraces for self-consumption in developing countries although many landraces are being replaced with modern cultivars. An effective and cheap method to reveal the genetic diversity in landraces is important. In this study, Td-DAMD-PCR, Td-SSR and CAPS-microsatellite techniques were compared using the same PCR amplification profile and reagents. Comparison analyses revealed that Td-DAMD-PCR markers amplified with 13 minisatellite primers, which were selected from 22 primers based on resolutions and reproducibility, were as reliable as Td-SSR and CAPS-microsatellite markers and produced more polymorphic markers, differentiating all the 24 common bean landraces. This study also showed that common bean landraces grown in Turkey contain great genetic variations. Td-DAMD-PCR markers amplified with selected 13 minisatellite primers can be effectively used in identification and preservation of common bean landraces that Td-SSR and CAPS-microsatellite markers could not reveal polymorphisms.

Keywords: common bean landraces, DNA markers, CAPS-microsatellites, SSRs.
Abbreviations: AFLP- Amplified Fragment Length Polymorphisms; CAPS- Cleavage Amplified Polymorphic Sequence; cDNA-Complementary DNA; DALP- Direct Amplification of Length Polymorphisms; ISSR- Inter Simple Sequence Repeats; PCR-Polymerase Chain Reaction; PIC- Polymorphism Information Content; RAPD- Random Amplified Polymorphic DNA; RFLP-Restriction Fragment Length Polymorphism; SNP- Single Nucleotide Polymorphisms; SSCP- Single Strand Conformational Polymorphism; SSR- Simple Sequence Repeats; Td-DAMD-PCR- Touch-down Direct (Directed) Amplification of Minisatellite-region DNA Polymerase Chain Reaction; Td-PCR- Touch-down PCR; TRAP- Targeted Region Amplified Polymorphism.

Introduction

Phaseolus genus contains approximately 70 species and within this genus, common bean (*Phaseolus vulgaris* L.) is an excellent food choice with its nutritional composition includes carbohydrates, proteins, vitamins, minerals and antioxidants (Sousa and Delgado, 1993; Svetleva et al., 2006). Common bean is a diploid ($2n = 2x = 22$) and predominantly self crossing species although 3% or more out crossing ratio has also been observed (Ibarra-Perez et al., 1997). Studies using molecular, physiological and morphological analyses in common bean strongly support the existence of two distinct centers of genetic diversity known as the Mesoamerican or small-seeded type and Andean or large seeded type gene pools (Blair et al., 2007; Burle et al., 2010). Today common beans are grown in many countries but widely cultivated in the tropics, subtropics, and temperate regions (Ibarra-Perez et al., 1997; Burle et al., 2010). Although a large number of common bean varieties have been developed by modern plant breeders, landraces are also grown by small farmers for their self-consumption and/or to sell on local markets in many countries (Sousa and Delgado, 1993; Burle et al., 2010). A landrace as defined by Zeven (1998) "a variety with a high capacity to tolerate biotic and abiotic stress, resulting in high yield stability and an intermediate yield level under a low input agricultural system". Modern cultivars provided by global seed companies are more productive and more uniform, especially with reference to maturity and seed size started to replace the landraces by these cultivars causing erosion on landraces. Therefore, it is important to identify and preserve landraces as genetic resources for the future generations (Lioi et al., 2005; Mienie et al., 2005; Blair et al., 2007; Marotti et al., 2007; Kumar et al., 2009). Up to date many different ways were chosen to investigate common bean genetic diversity. Many studies have used morpho-agronomic traits, isozymes and protein markers in common bean improvement and diversity studies (Delaney and Bliss, 1991; Vallejos and Chase, 1991; Park et al., 2000; Gonzalez et al., 2010; Perez-Vega et al., 2010). However, the use of morpho-agronomic traits, isozymes and protein markers has several drawbacks including i) a few number of loci available, ii) existence of intermediate phenotypes causing difficulties in identification and classification, iii) subject to environmental influences, iv) subject to age, organ and development influences, v) subject to gene interaction and, vi) require expertise on crop and species for characterization (Santalla et al., 2002; Karaca et al., 2004; Lioi et al., 2005; Blair et al., 2007; Ceolin et al., 2007; Faschiani et al., 2009). DNA-based molecular markers have been widely used to assist common bean breeding programs including the studies on the origin and diversity. RFLP, RAPD, AFLP, SSR, cDNA-AFLP, DALP, TRAP, SSCP and SNP have been used in common beans (Miklas et al., 2006; Marotti et al., 2007; Galeano et al., 2009; Faschiani et al., 2009; Hanai et al., 2010; Galvan et al., 2010; Szilagy et al., 2011; Mishra et al., 2011). Among the most widely used DNA markers in common bean and in many other crops is SSRs, also known microsatellites. Microsatellites are one of the most variable types of tandem repeated DNA sequences found in all organisms studied so far (Bilgen et al., 2004;

Mishra et al., 2011). Repeated DNA motifs in microsatellites can consist of a single base to six bases which are repeated several times. The repeats can be either perfect tandem repeats (exact) or interrupted by several non-repeat nucleotides (inexact) or compound repeats (Bilgen et al., 2004). Polymorphism is detected when insertion or deletion occurred in the microsatellite domains or between the flanking regions which could be detected using CAPS-microsatellite technique (Ince et al., 2010). Microsatellite are considered markers of choice in many plant species including common bean, because they are i) PCR-based markers, ii) usually inherit as co-dominant, iii) often multiallelic and hyper variable, iv) randomly dispersed throughout the genome, and v) publically available via published flanking primer sequences (Bilgen et al., 2004; Blair et al., 2007; Kumar et al., 2009; Faschiani et al., 2009; Burle et al., 2010; Mishra et al., 2011). Direct or directed amplification of minisatellite-region DNA polymerase chain reaction (DAMD-PCR) is another DNA marker method used in plant genetic studies (Heath et al., 1993; Bhattacharya and Ranede, 2001; Ha et al., 2002; Ho et al., 2006; Karaca and Ince, 2008). However the use of DAMD-PCR markers is not common in common bean landraces. In comparison to RAPD markers, which are one of the most commonly used marker types, DAMD-PCR markers are more reproducible and reliable due to the effectively amplification carried out at relatively high PCR stringencies (Heath et al., 1993; Ho et al., 2006; Karaca and Ince, 2008; Mihalte et al., 2011). The use of touch-down polymerase reaction conditions could also increase the reproducibility of this technique. Annealing temperature, length and specificity of primers along with the amount, type and quality of PCR reagents used in the development of PCR-based DNA markers are important. Annealing temperature is dependent on the nucleotide sequences of primer or primer pairs. The use of high stringency Td-PCR profile (higher annealing) could reduce the occurrence of PCR artifacts and it may increase the reliability of DNA markers. In the present study high stringency Td-PCR profile was used to compare Td-DAMD-PCR, Td-SSR and CAPS-microsatellite techniques to identify an effective method for genetic diversity studies in common bean landraces. Polymorphism information content (PIC) analysis was used to evaluate marker types used in the present study.

Results

In our unpublished previous studies it was found that some of the 12 cultivars grown in Turkey could not be differentiated based on SSR markers. On the other hand, initial studies of Td-DAMD-PCR markers showed higher level of polymorphisms than SSR markers and could differentiate all the 12 common bean cultivars (data not shown). Previous studies undertaken in other research groups in different countries and regions revealed that landraces have the best adaptation to restricted geographical areas and some of them have high nutritional value, short cooking time, thin coat and good yield (Marotti et al., 2007; Zhang et al., 2008). However, modern cultivars replace the landraces in many part of the world including Turkey. Landraces are valuable genetic resources and should be protected from genetic erosion. In this study instead of using known cultivars, landraces collected from different cities were used in one hand to compare the three different PCR-based DNA markers and to investigate the presence of genetic diversity within 24 landraces. In all Td-PCR studies of this research, negative controls, which contained primer or primer pairs, enzyme and other PCR reagents but no DNA template, were used. Since there were no amplified products in negative controls it was an indication of polymorphisms detected did not come from the internal condition of PCR. This study did not use positive control but comparison of independent PCR repetitions was used. All the Td-PCR-SSR amplicons, CAPS-microsatellite and scored Td-DAMD-PCR markers were reproducible. Repeatability of markers in 3 different DNA methods was an indication of reliability of the markers used in the present study.

SSR and CAPS-microsatellite

In the SSR studies, depending on the primer pairs, one to three amplified products per primer pair were detected on 3% agarose gels. Selected patterns of Td-SSR markers are shown in Fig. 1. The number of markers (presence or absence or size differences) varied from one to six depending on the primer pairs. Among the 13 SSR primer pairs, 3 produced polymorphic markers in common bean landraces and PIC values of the all Td-SSR markers varied from 0 to 0.396 (Table 1). However, none of the 13 SSR primer pairs could be able to differentiate all the 24 landraces. SSR primer pair, PV23 with the highest PIC value, could divide the 24 landraces into 3 groups. However, there were no relationships between the groups and seed morphologies of landraces. In order to investigate the undetected polymorphism existed among the amplicons of the 10 primer pairs, which produced monomorphic markers, six different restriction enzymes were used based on the CAPS-microsatellite technique. Although majority of the restriction enzymes could cut the amplified products, no monomorphic amplicons could be converted into useful markers after the digestion studies. Results of this study confirmed that Td-SSRs and TD-CAPS-microsatellite were highly reproducible and easy to score but their level of polymorphism was very low (Table 1). Low level of SSR polymorphism has also found in other studies of common beans. For instance, Faschiani et al. (2009) reported that among the 23 SSR primer pairs, only 10 were useful in common bean landraces diversity studies. In the present study 3 SSR primer pairs (PV05, PV23 and PV32) among the 13 produced polymorphic markers (Table 1). Although SSR markers are considered the choice of marker type in many plant species, this study revealed that other marker types are also required in diversity and genetic studies in common bean landraces.

Td-DAMD-PCR

Amplification of minisatellite primers were performed in a touch-down polymerase chain reactions consisting of 40 cycles, ten of which used initial annealing temperature of 60^{o}C and reduced 0.5^{o}C per cycle and 30 of which used 55^{o}C constant annealing temperature. A total of eight selected common bean landraces were first screened using a total of 22 minisatellite primers (listed in Ince et al., 2009). In the present study combination of two minisatellite primer as reverse and forward was also used. But the number of amplicons was very high and they could not be easily scored due to the low resolution in agarose gels. Therefore, single primers were used in amplification of all Td-DAMD-PCR

Table 1. Sequences of SSR primer pairs, PIC, number of amplicons and motif types in common bean landraces used in this study

Primer[1]	Sequence (5'➔ 3')	PIC[2]	N[3]	Motif Type
PV02F	GGATGAGTCCTTTCCCTACCC	0	1	$(CTGTTG)_4(CTG)_4(TTG)_3(CTG)_3(CTG)_4$
PV02R	TGCAGACACAATTTATGAAGGC			
PV05F	CAGACATGCAAATTGGAAC	0.219	2	$(TA)_5$
PV05R	GGAGCACCAAAGATCATAGA			
PV06F	GGTTGGGAAGCCTCATACAG	0	2	$(AG)_5$
PV06R	TAGTCCTTGCTTTCTTTTGC			
PV10F	TGGAGCCATCTGTCTCTTACCCAC	0	2	$(AAAT)_3$
PV10R	GAGCACGAGTCACGTTTGCAAC			
PV12F	ACTTCTTTCATCATCCATCCATCC	0	2	$(ATCC)_3(AG)_2(TAC)_3T(CTA)_3$
PV12R	TATCTTGGCTCTCTTCCTCTTCC			
PV13F	ACGTACGAGTTGAATCTCAGGAT	0	2	$(AG)_8$
PV13R	GGTGTCGGAGAGGTTAAGGTTG			
PV16F	TCTCCATGCATGTTCCAACCAC	0	2	$(ATCC)_3$
PV16R	GGAGTGGAACCCTTGCTCTCATC			
PV17F	TTTACGCACCGCAGCACCAC	0	2	$(TTTC)_4$
PV17R	GGACTCATAGAGGCGCAGAAAG			
PV22F	TCGTAGCACTAAGATGGAAGA	0	2	$(AC)_6$
PV22R	GTTTTGTGAACTGTTGAATGTG			
PV23F	TTATACGAGGTTAGCCTAAATC	0.396	3	$(AT)_{12}$
PV23R	CATTCCCTTCACACATTCACCG			
PV30F	TTCATCCTCTCTCCCGAACTT	0	1	$(CT)_7$
PV30R	CTTTTGTGGCTGAGACATGGT			
PV32F	AGACATTGTTGATACGGGAGAT	0.109	2	$(GA)_3(GT)_2$
PV32R	CACCTTGACTTGCCTTTGAC			
PV20F	GCTGCATGTTTATCCACCTT	0	2	$(GT)_7$
PV20R	TTGTTACTCACCCCACCATAC			

[1]: primer pairs are randomly selected from several previous researches cited. [2]: polymorphism information content, [3]: number of amplicons per primer pair

Table 2. DAMD primer sequences, PIC and number of amplicons

Primer[1]	5'➔ 3' Sequence	PIC[2]	N[3]
URP1F	ATCCAAGGTCCGAGACAACC	0.162	11
URP2F	GTGTGCGATCAGTTGCTGGG	0.088	5
URP25F	GATGTGTTCTTGGAGCCTGT	0.195	8
URP30F	GGACAAGAAGAGGATGTGGA	0.290	11
14C2	GGCAGGATTGAAGC	0.234	8
6.2H1	CCCTCCTCCTCCTTC	0.201	14
6.2H2	AGGAGGAGGGGAAGG	0.239	9
HBV5	GGTGTAGAGAGGGGT	0.247	10
33.6	GGAGGTGGGCA	0.153	11
FVIIEX8	ATGCACACACACAGG	0.222	20
FVIIEX8C	CCTGTGTGTGTGCAT	0.198	6
YNZ22	CTCTGGGTGTGGTGC	0.243	9
M13	GAGGGTGGCGGCTCT	0.153	20

[1]: the source of these primers are given in Ince et al., (2009). [2]: polymorphism information content, [3]: number of amplicons per primer

studies. A total of 13 minisatellite primers were selected from 22 primers based on the reproducibility and resolutions on gels. Resolution means the number of distinct and countable markers. Twenty four common bean landraces were amplified using these selected primers listed in Table 2. Selected amplification patterns of Td-DAMD-PCR markers are shown in Fig. 1d-e. Amplified product size varied from more than 2 kb to 100 bp depending on the primer used (Fig. 1d-e). PIC values of the 13 DAMD-PCR primer varied from 0.088 to 0.29 (Table 2). The number of amplified products ranged from 5 to 20 depending on the minisatellite primers used. The highest number of 20 markers in Td-DAMD-PCR was obtained using M13 primer while URP2F primer produced 5 markers (Table 2). Comparison studies indicated that differentiation power of Td-DAMD-PCR markers was greater than Td-SSR and Td-CAPS-microsatellite markers. Repeatability of this technique was also equal to other two methods. Although scoring of Td-DAMD-PCR markers requires special care due to the low resolution in agarose gels, the results of the present study indicated that Td-DAMD-PCR markers could be reliably used in common bean genetic studies.

Discussion

Common beans introduced from Mesoamerican and Andean origins are the main sources of landraces grown in many

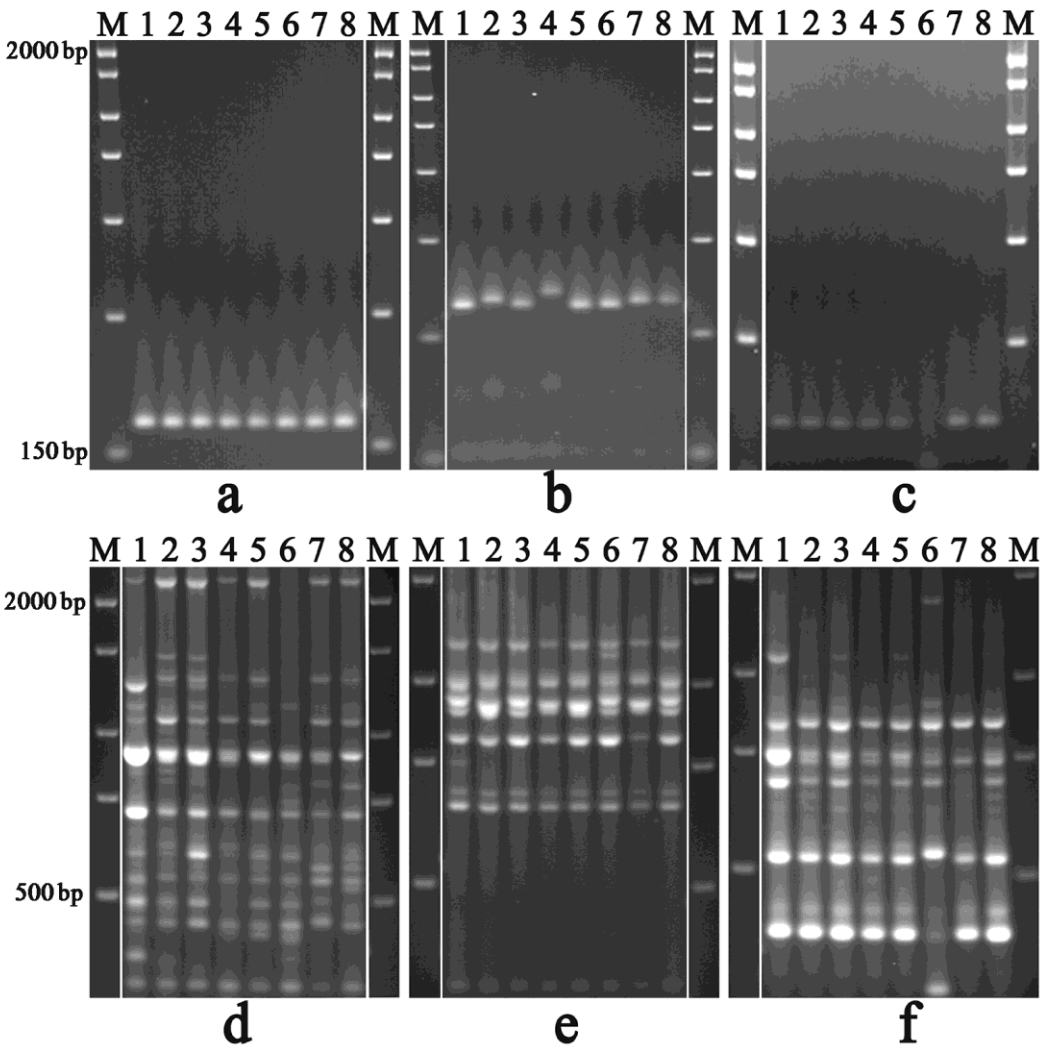

Fig 1. Amplification patterns of selected Td-SSR, CAPS-microsatellite and Td-DAMD-PCR markers. M: DNA size markers ranging from 2000 bp to 150 bp. Panel a: monomorphic SSR markers obtained using PV30 primer pair separated in 3% agarose gel. Panel b: polymorphic CAPS-microsatellite markers obtained using PV23 primer pair digested with *Hind* III separated in 3% agarose gel. Panel c: a polymorphic SSR marker obtained using PV05 primer pair separated in 3% agarose gel. Panel d, e and f are polymorphic Td-DAMD-PCR amplification patterns obtained using DAMD-PCR primers FVIIX8, 33.6 and YNZ22, respectively. Td-DAMD-PCR markers are separated in 2% agarose gels.

countries. Some countries pose landraces from Mesoamerican or Andean while some others have the two gene pools (Lioi et al., 2005; Marotti et al., 2007; Blair et al., 2007; Zhang et al., 2008; Asfaw et al., 2009). Some of the landraces have inter gene pool introgressions. Introgressions between the gene pools create landraces which may provide new adaptation capacity of common bean to the diverse agro-ecosystems and are valuable resources in common bean improvement studies (Martins et al., 2006; Svetleva et al., 2006; Faschiani et al., 2009). Standard SSRs with constant annealing temperature have been previously used in our laboratory on several common bean varieties (unpublished data). Densities and minor amplicons of standard SSR varied among the SSR primer pairs while there were less variation in amplicons of Td-PCR-SSR. In Td-PCR, annealing temperature was decreased in increments of 0.5 ^{o}C for 10 subsequent set of cycles. The primer or primer pairs annealed at 60 ^{o}C temperature which is least-permissive of nonspecific binding. Thus, the first sequence amplified was the one between the regions of greatest primer specificity and the target sequence. These fragments were further amplified during subsequent rounds at relatively lower temperatures (55 ^{o}C). The reliability of amplified products (SSR, CAPS-microsatellite and DAMD-PCR) markers were increased by the use of Td-PCR. Comparison studies of standard and Td-PCR-SSR also revealed that Td-PCR-SSR amplicons had no or less background and markers were reproducible between different thermal cycler runs (data not shown). This study revealed that the level of polymorphism detected using 13 SSR primer pairs was lower in common bean landraces grown in Turkey (Table 1). The lower level of polymorphism of SSR markers has also been reported in common bean (Kumar et al., 2009). Previous comparative studies revealed that gene-specific microsatellites proved to be less polymorphic than genomic microsatellites in terms of both number of alleles and PIC values (Kumar et al., 2009; Burle et al., 2010; Hanai et al., 2010; Blair et al., 2011). However, in this present study there were no clear differences between the polymorphism level of gene-specific and genomic SSR markers in Td-PCR condition. The number of amplicons per

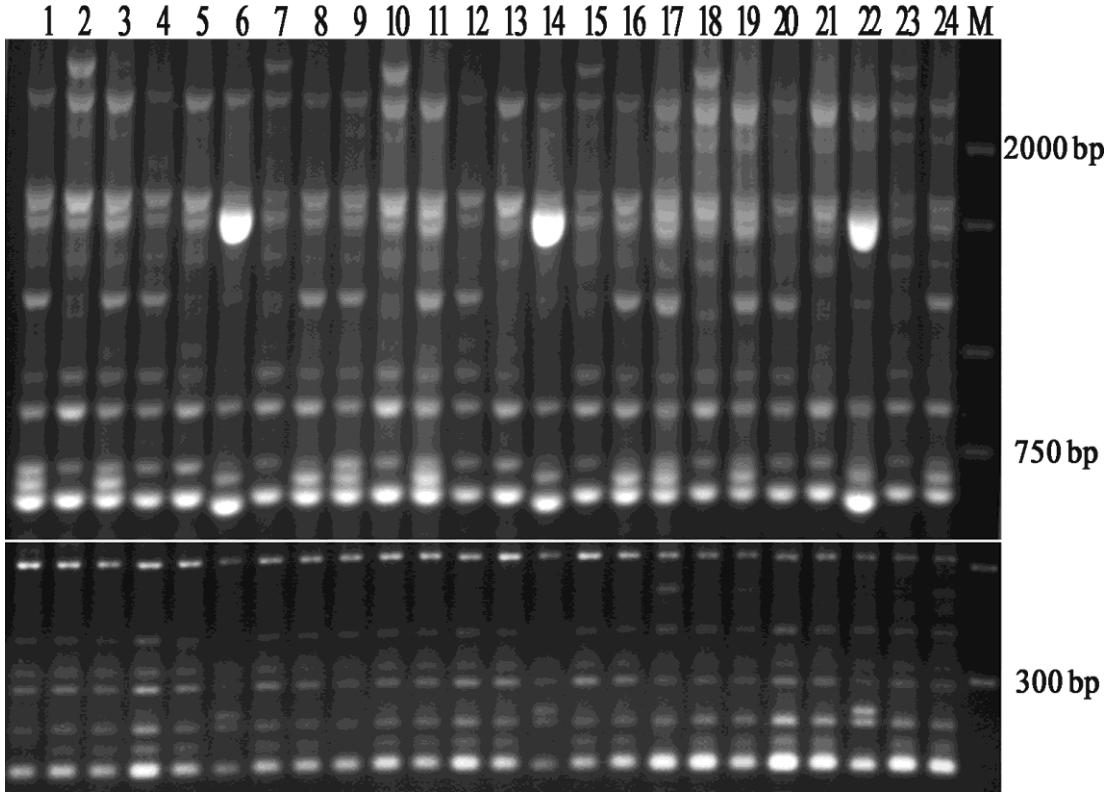

Fig 2. Td-DAMD-PCR markers obtained using M13 primer separated in 2% agarose gel. Lanes 1-24 are common bean landraces used in this study. M: DNA size markers ranging from 2000 bp to 300 bp. Note that upper part of the agarose gel is a picture taken after 14 h while lower part is a picture taken after 6 h of electrophoresis. Td-DAMD-PCR markers larger than 1 kb separated in 2% gels need longer electrophoresis while those markers with smaller size elutes from the gels.

primer pair obtained in Td-SSR technique of this study were according to the results of Kumar et al. (2009), Hanai et al. (2010), and Talukder et al. (2010) who used standard PCR. The level of polymorphisms detected using SSR markers in landraces were also found to be less in several studies including the papers described in Lioi et al. (2005), Kumar et al. (2009). Due to the low level of polymorphism detected using SSR technique, the use of other DNA markers such as DAMD-PCR is needed in common bean genetic studies. Minisatellites are tandem repeated DNA regions, which show high levels of allelic length variation due to differences in the number of repeated nucleotide units (Jeffreys et al., 1985). Minisatellite loci are highly informative genetic markers that have been used extensively in many plant species including yeasts and fungi (Bhattacharya and Ranade, 2001; Ha et al., 2002; Karaca et al., 2002; Ince et al., 2009). Minisatellite loci could be specifically amplified using DAMD-PCR technique (Heath et al., 1993; Karaca and Ince, 2008). In DAMD-PCR technique, polymorphism with the use of single primer flanking the minisatellite regions, repeat unit differences could be detected. However, annealing temperature, primers, type and quality of PCR reagents used in PCR may affect presence or absence of minisatellite in DAMD-PCR amplicons. For instance, a study of Karaca and Ince (2008) revealed that not all the DAMD-PCR products contained the minisatellite domains in DAMD-PCR markers amplified at 45^{o}C annealing temperature. In the present study Td-DAMD-PCR markers were obtained at 55^{o}C annealing which is considerable high stringencies than RAPD, ISSR and DAMD-PCR (Karaca and Ince, 2008). The use of higher annealing and selected universal minisatellite primers might have contributed to the reliability of Td-DAMD-PCR markers. However, reproducibility may also strongly dependent on the uniformity of PCR amplification conditions and brand of chemical reagents used between experiments. Hence, in this study, the same brand of chemical reagents as well as thermo cycler were used for all Td-DAMD-PCR amplifications to increase the data reliability.

The results of the present study indicated that the amplified Td-DAMD-PCR products with high fragment size may be not well resolved using 1.2-1.5% agarose gel. On the other hand, in the 2% agarose gels, markers larger than 1.5-3 kb require longer separation time than 15 h electrophoresis. In order to obtain and use the all the amplified Td-DAMD-PCR markers, it may be necessary to take more pictures of gels, one picture for those markers less than 500 bp before they elute from the gels and one picture for those markers greater than 750 bp and combine these picture for final scoring studies as shown in Figure 2.

The results of this study showed that the level of polymorphism of Td-DAMD-PCR markers detected in common bean landraces was higher than RAPD and ISSR markers as reported previously. For instance, Galvan et al. (2010) reported that among the 10 ISSR primers, four revealed polymorphisms in nineteen Argentinean common bean landraces and wild populations. Galvan et al. (2003) found that ISSR markers were better than RAPD markers to identify beans by gene pool of origin. Reproducibility of Td-DAMD-PCR markers was equal to SSR markers. Previous studies indicated that RAPD markers were not reproducible between different laboratories and even in the same laboratory (Martins et al., 2006; Karaca and Ince, 2008). Therefore, Td-DAMD-PCR markers could be replaced with RAPD markers in genetic studies.

Materials and methods

Plant material

Common bean seeds (*Phaseolus vulgaris* L.) used in the present study consisted of 24 landraces obtained from several farmers who sell their products obtained from their smallholder-farmer systems at open markets of different cities in Turkey. In these open markets, which are called "open folk markets" several products including the common bean landraces are sold under the name of "organic products" meaning that they are neither varieties nor genetically modified crops but have special taste and nutrition values.

DNA extraction

Three to four dry seeds per landrace sample were used for DNA extraction studies. Genomic DNAs were extracted using a protocol described in Ince and Karaca (2009) with following modifications. Seeds were powdered in a mortar using a pestle in liquid nitrogen. Powdered materials (0.2 g) were suspended in 3.9 ml of the MAGi solution, 0.1 ml 14.4 M β-mercaptoethanol (BME, Amresco), 0.5 ml 2 M sodium acetate (pH 7.0) and 1 ml phenol: chloroform: isoamyl alcohol (Amresco, 25:24:1 v/v). After centrifugation, the DNA was precipitated and prepared for Td-PCR studies according to the protocol (Ince and Karaca, 2009).

Touch-down polymerase chain reactions

Amplifications of Td-SSR and Td-DAMD-PCR markers were carried out in 25 µl reaction volume containing 80 ng genomic DNA as template, 0.5 µM of a primer pair for SSR or 2 µM minisatellite core primers listed in Table 1 and 2, respectively, 80 mM Tris–HCl (pH 8.8), 19 mM $(NH_4)_2SO_4$, 0.009% Tween-20 (w/v), 0.28 mM of each dNTP, 2 or 3 mM $MgCl_2$, and 2 units of *Taq* DNA polymerase (MBI Fermentas, Amherst, NY, USA). Amplification of Td-PCR profile for SSR and DAMD-PCR markers used in the present study was as follows: initial denaturation at 94 ^oC for 3 min, 10 cycles with denaturation at 94 ^oC for 30 s, annealing at 60 ^oC for 30 s in the first cycle, diminishing by 0.5 ^oC each cycle, and extension at 72 ^oC for 2 min in a 96-well GeneAmp PCR System 9700 (Carlsbad, California, USA). An additional 30 PCR cycles were run using the same cycling parameters with constant annealing at 55 ^oC. Denaturation and extension conditions of these 30 cycles were the same as indicated above. The amplifications finished with final extension at 72 ^oC for 8 min. Reproducibility of markers was determined using the same primer or primer pairs within the same PCR set or between different thermal cycler runs. In all Td-PCR studies a negative control which contained all the reagents necessary but missing template DNA was used to control the presence of primer dimmers or artifacts or cross-contaminations of the PCR reagents.

Agarose gel electrophoresis

Amplicons were separated using 2% (Td-DAMD-PCR) and 3% (Td-SSR) high resolution agarose gel (Serva, Heidelberg, Germany) electrophoresis according the procedures described in Ince et al. (2011). Amplified products in gels were visualized on an ultraviolet trans-illuminator to confirm amplifications, molecular mass and any observed length polymorphisms.

CAPS-microsatellites

Monomorphic SSR markers amplified using the above Td-PCR conditions were individually digested with *Hinf* I, *Cla* I, *Msp* I, *Bam* HI, *Hind* III and *Rsa* I restriction enzymes and analyzed according protocol described in Ince et al. (2010). Briefly 10 µl Td-SSR amplified products were digested with ten units of the above mentioned restriction enzymes separately (MBI Fermentas, Amherst, NY, USA). Products were visualized on an ultraviolet trans-illuminator to confirm digestions and any observed length polymorphisms.

Comparison analyses

Polymorphism information content (PIC) analysis is a powerful analysis that can be used to evaluate marker types so that the most appropriate ones can be selected for genetic mapping, phylogenetic analysis or diversity studies. PIC analysis of each primer or primer pairs, restriction enzymes with primer pairs along with number of amplicons, amplicon size and reproducibility of markers were used to compare Td-DAMD-PCR with other two methods. Amplicons were scored as presence (1) or absence (0) and data were recorded. PIC was calculated using formula $PIC=2P_iQ_i$ where P_i is the frequency of presence and Q_i is the frequency of absence of a particular amplicons obtained from primer (Td-DAMD-PCRs) or primer pair (Td-SSRs), primer pair with a restriction enzyme (CAPS-microsatellites). PIC values for a primer or primer pair, primer pair with a restriction enzyme were averaged according Rana and Bhat (2004).

Conclusion

Common bean is an important source of proteins in a great number of developing countries. However, the average yield of bean varieties grown in developing countries is still very low due to the narrow genetic base. The use of landraces and germplasm stored in gene banks as a source of genetic variability for breeding programs can certainly impact the development of higher yielding common bean cultivars. Based on the results of the present study it was concluded that Td-DAMD-PCR markers obtained from 13 selected core minisatellite primers could be useful in genetic diversity, conservation strategies and design breeding programs in common beans.

Acknowledgements

This work was supported by the Scientific and Technological Research Council and The Scientific Research Projects Coordination Unit of Akdeniz University of the Republic of Turkey. The authors thank to anonymous referees and associate editor for contributing valuable information to this manuscript.

Conflicts of interest statement

The authors declare that they have no conflicts of interest.

References

Asfaw A, Blair MW, Almekinders C (2009) Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) landraces from the East African highlands. Theor Appl Genet 120:1-12

Bhattacharya E, Ranade SA (2001) Molecular distinction amongst varieties of mulberry using RAPD and DAMD profiles. BMC Plant Biol 1:3

Bilgen M, Karaca M, Onus AN, Ince AG (2004) A software program combining sequence motif searches with keywords for finding repeats containing DNA sequences. Bioinformatics 20:3379-3386

Blair MW, Diaz JM, Hidalgo R, Diaz LM, Duque MC (2007) Microsatellite characterization of Andean races of common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 116:29-43

Blair MW, Hurtado N, Chavarro CM, Munoz-Torres MC, Giraldo MC, Pedraza F, Tomkins J, Wing R (2011) Gene-based SSR markers for common bean (*Phaseolus vulgaris* L.) derived from root and leaf tissue ESTs: an integration of the BMc series. Plant Biol 11:50

Burle ML, Fonseca JR, Kami JA, Gepts P (2010) Microsatellite diversity and genetic structure among common bean (*Phaseolus vulgaris* L.) landraces in Brazil, a secondary center of diversity. Theor Appl Genet 121:801-813

Ceolin ACG, Goncalves-Vidigal MC, Filho PSV, Kvitschal MV, Gonela A, Scapim CA (2007) Genetic divergence of the common bean (*Phaseolus vulgaris* L.) group Carioca using morpho-agronomic traits by multivariate analysis. Hereditas 144:1-9

Delaney DE, Bliss FA (1991) Selection for increased percentage phaseolin in common bean: 1. Comparison of selection for seed protein alleles and S1 family recurrent selection. Theor Appl Genet 81:301-305

Faschiani A, Miceli F, Vischi M (2009) Assessing diversity in common bean (*Phaseolus vulgaris* L.) accessions at phenotype and molecular level: a preliminary approach. Genet Resour Crop Evol 56:445-453

Galeano CH, Fernandez AC, Gomez M, Blair MW (2009) Single strand conformation polymorphism based SNP and Indel markers for genetic mapping and synteny analysis of common bean (*Phaseolus vulgaris* L.). BMC Genomics 10:629

Galvan MZ, Bornet B, Balatti PA, Branchard M (2003) Inter simple sequence repeat (ISSR) markers as a tool for the assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). Euphytica 132:297-301

Galvan MZ, Lanteri AA, Sevillano MCM, Balatti PA (2010) Molecular characterisation of wild populations and landraces of common bean from northwestern Argentina. Plant Biosyst 144:365-372

Gonzalez AM, Fuente MDI, Ron AMD, Santalla M (2010) Protein markers and seed size variation in common bean segregating populations. Mol Breeding 25:723-740

Ha WY, Shaw PC, Liu J, Yau FCF, Wang J (2002) Authentication of *Panax ginseng* and *Panax quinquefolius* using amplified fragment length polymorphism (AFLP) and directed amplification of minisatellite region DNA (DAMD). J Agr Food Chem 50:1871-1875

Hanai LR, Santini L, Camargo LEA, Fungaro MHP, Gepts P, Tsai SM, Vieira MLC (2010) Extension of the core map of common bean with EST-SSR, RGA, AFLP, and putative functional markers. Mol Breeding 25:25-45

Heath DD, Iwama GK, Devlin RH (1993) PCR primed with VNTR core sequence yields species specific patterns and hypervariable probes. Nucl Acids Res 21:5782-5785

Ho WS, Wickneswari R, Mahani MC, Shukor MN (2006) Comparative genetic diversity studies of *Shorea curtisii* (Dipterocarpaceae): an assessment using SSR and DAMD markers. J Trop Forest Sci 18:22-35

Ibarra-Perez F, Ehdaie B, Waines G (1997) Estimation of out crossing rate in common bean. Crop Sci 37:60-65

Ince AG, Karaca M, Onus AN (2009) Development and utilization of diagnostic DAMD-PCR markers for *Capsicum* accessions. Genet Resour Crop Evol 56:211-221

Ince AG, Karaca M (2009) The MAGi RNA extraction method: highly efficient and simple procedure for fresh and dry plant tissues. J Sci Food Agric 89:168-176

Ince AG, Karaca M, Onus AN (2010) CAPS-microsatellites: use of CAPS method to convert non-polymorphic microsatellites into useful markers. Mol Breeding 25:491-499

Ince AG, Karaca M (2011) Early determination of sex in jojoba plant by CAPS assay. J Agr Sci 149:327-336

Jeffreys AJ, Wilson V, Thein SL (1985) Individual specific 'fingerprints' of human DNA. Nature 332:278-281

Karaca M, Saha S, Zipf A, Jenkins JN, Lang D (2002) Genetic diversity among forage bermudagrass (*Cynodon* spp.): evidence from chloroplast and nuclear DNA fingerprinting. Crop Sci 42:2118-2127

Karaca M, Saha S, Callahan FE, Jenkins JN, Read JJ, Percy RG (2004) Molecular and cytological characterization of a cytoplasmic-specific mutant in pima cotton (*Gossypium barbadense* L.). Euphytica 187-197

Karaca M, Ince AG (2008) Minisatellites as DNA markers to classify bermudagrasses (*Cynodon* spp.): confirmation of minisatellite in amplified products. J Genet 87:83-86

Kumar V, Sharma S, Sharma AK, Sharma S, Bhat KV (2009) Comparative analysis of diversity based on morpho-agronomic traits and microsatellite markers in common bean. Euphytica 170:249-262

Lioi L, Piergiovanni AR, Pignone D, Puglisi S, Santantonio M, Sonnante G (2005) Genetic diversity of some surviving on-farm Italian common bean (*Phaseolus vulgaris* L.) landraces. Plant Breeding 124:576-581

Marotti I, Bonetti A, Minelli M, Catizone P, Dinelli G (2007) Characterization of some Italian common bean (*Phaseolus vulgaris* L.) landraces by RAPD, semi-random and ISSR molecular markers. Genet Resour Crop Evol 54:175-188

Martins SR, Vences FJ, Saenz De Miera LE, Barroso MR, Carnide V (2006) RAPD analysis of genetic diversity among and within Portuguese landraces of common white bean (*Phaseolus vulgaris* L.). Sci Hortic 108:133-142

Mienie CMS, Liebenberg MM, Pretorius ZA, Mikas PN (2005) SCAR markers linked to the common bean rust resistance gene Ur-13. Theor Appl Genet 111:972-979

Mihalte L, Sestras RE, Feszt G, Tamas E (2011) Assessment of genetic variation on four genera of *Cactaceae* using taxonomic, cytological and molecular markers methods. Plant Omics J 4:142-148

Miklas PN, Hu JG, Grunwald NJ, Larsen KM (2006) Potential application of TRAP (targeted region amplified polymorphism) markers for mapping and tagging disease resistance traits in common bean. Crop Sci 46:910-916

Mishra RK, Gangadhar BH, Yu JW, Kim DH, Park SW (2011) Development and characterization of EST based SSR markers in Madagascar periwinkle (*Catharanthus roseus*) and their transferability in other medicinal plants. Plant Omics J 4:154-162

Park SO, Coyne DP, Jung G, Skroch PW, Arnaud SE, Steadman JR, Ariyarathne HM, Nienhuis J (2000) Mapping of QTL for seed size and shape traits in common bean. J Am Soc Hortic Sci 125:466-475

Perez-Vega E, Paneda A, Rodriguez-Suarez C, Campa A, Giraldez R, Ferreira JJ (2010) Mapping of QTLs for morpho-agronomic and seed quality traits in a RIL

population of common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 120:1367-1380

Rana MK, Bhat KV (2004) A comparison of AFLP and RAPD markers for genetic diversity and cultivar identification in cotton. J Plant Biochem Biot 13:19-24

Santalla M, Rodino AP, De-Ron AM (2002) Allozyme evidence supporting southwestern Europe as a secondary center of genetic diversity for the common bean. Theor Appl Genet 104:934-944

Sousa SM, Delgado A (1993) Mexican Leguminosae: phytogeography, endemism and origins. In: Ramamoorthy TP, Bye RA, Lot J, Fa J (eds), pp. 459-511. Biological diversity of Mexico. Oxford University Press, New York

Svetleva D, Pereira G, Carlier J, Cabrita L, Leitao J, Genchev D (2006) Molecular characterization of *Phaseolus vulgaris* L. genotypes included in Bulgarian collection by ISSR and $AFLP_{TM}$ analyses. Sci Hortic 109:198-206

Szilagyi L, Tayyar S, Ciuca M (2011) Evaluation of genetic diversity in common bean (*Phaseolus vulgaris* L.) using RAPD markers and morpho-agronomic traits. Rom Biotech Lett 16:98-105

Talukder ZI, Anderson E, Miklas PN, Blair MW, Osorno J, Dilawari M, Hossain KG (2010) Genetic diversity and selection of genotypes to enhance Zn and Fe content in common bean. Can J Plant Sci 90:49-60

Vallejos CE, Chase CD (1991) Linkage between isozyme markers and a locus affecting seed size in *Phaseolus vulgaris* L. Theor Appl Genet 81:413-419

Zeven AC (1998) Landraces: A review of definitions and classifications. Euphytica 104:127-139

Zhang X, Blair MW, Wang S (2008) Genetic diversity of Chinese common bean (*Phaseolus vulgaris* L.) landraces assessed with simple sequence repeat markers. Theor Appl Genet 117:629-640