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Determination of Genetic diversity of cultivated chickpea (*Cicer arietinum* L.) using *Medicago truncatula* EST-SSRs

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Abstract
Expressed sequence tags simple sequence repeats (EST-SSRs) are important sources for investigation of genetic diversity and molecular marker development. Similar to genomic SSRs, the EST-SSRs are useful markers for many applications in genetics and plant breeding such as genetic diversity analysis, molecular mapping and cross-transferability across related species and genera. In spite of low polymorphism, these markers show variation in the expressed part of the genome. In this study, *Medicago truncatula* EST-SSRs were used for investigation of transferability between *M. truncatula* and some chickpea (*Cicer arietinum* L.) genotypes, also genetic diversity between used chickpea genotypes was determined. In this research, 650 *M. truncatula* ESTs were searched to find simple sequence repeats (SSRs). A total of 131 EST-SSRs were contained di-and trinucleotide motif SSRs. In this study, thirty pairs of primers were designed to amplify over 10 chickpea genotypes. Thirteen primer pairs (43%) generated reproducible bands in at least one chickpea genotype that eight bands (61.5%) were polymorphic in the chickpea genotypes. A total of 24 alleles were amplified with an average of 3 alleles per primer. The average of polymorphism information content (PIC) was 78.75% and transferability across *M. truncatula* and *C. arietinum* was 43.32%. The results indicate that the developed EST-SSR markers from *M. truncatula* as a model plant are valuable genetic markers for legume species such as chickpea. In addition to suitability of EST-SSR markers for genetic diversity analysis, their broad range of transferability also proved their potential for comparative genomics studies.

***Keywords*:** EST-SSR markers, genetic diversity, chickpea (*Cicer arietinum* L.), *Medicago truncatula.*

Introduction

Chickpea is the third most important food legume in the world which is grown as a source of protein in more than 40 countries (Varshney *et al*., 2009). In Iran, the annual production of chickpea grains is 80000 tons and it has an important role in the population's diet as a source of protein (Tavakoli *et al*., 2002). According

to ILDIS[1] this self-pollinated diploid (2X=2n=16) crop is a member of Fabaceae family which includes 18,000 species in 650 genera. Small genomic size (around 740 Mb) and short life cycle (about three months) makes it an interesting plant for genomics investigations (Varshney *et al*., 2009).

Molecular markers are appropriate tools for assessing diversity and genetic relationships within populations. Microsatellites or simple sequence repeats (SSRs) are 1–6 bp iterations of DNA sequences. They are ubiquitous in all over the genomes and study of them has been facilitated by recent advances in PCR technology (Gupta *et al*., 1999). The microsatellites in transcribed sequences are now well discovered and are commonly known as EST-SSR markers (Morgante *et al*., 2002 ; Li *et al*., 2004). These EST-SSRs are considered as a cost-effective and valuable molecular marker (Choudhary *et al*., 2009). Extension of EST databases generated a valuable source to develop SSR markers for these expressed regions of the genome. In addition, EST-SSRs are more conserved than SSR across species and transferable among related species (Li-Bin *et al*., 2008). EST-SSR markers in genetic studies have been used for a number of plant species such as rice (Cho *et al*. 2000), wheat (Gupta *et al*. 2003), alfalfa (Eujayl *et al*. 2004), cotton (Qureshi *et al*., 2004), citrus (Chen *et al*. 2006), sesame, cotton and soybean (Li-Bin *et al*., 2008), tall fescue (Sharifi Tehrani *et al*., 2009) safflower (Barati and Arzani, 2012) and chickpea (Choudhary *et al*., 2009 ; Lichtenzveig *et al*. 2005). Compared to the other legume crops, there are limited amount of genomic resources to chickpea.

Recently, due to collaborative efforts of ICRISAT and UC-Davis USA, about 20162 Sanger ESTs (Varshney *et al*., 2009) and 48796 BAC (bacterial artificial chromosome) - end sequences (BESs) are become available (Hiremath *et al*., 2011). Some of EST-SSR arrays are three nucleotides and often they are as a motif of $(ACC)_n$ and $(CAT)_n$ (Gupta *et al*., 2003). Buhariwalla *et al*. (2005) used some EST sequences of chickpea for genetic studies as a rich source of genetic molecular markers. Out of the 106 EST markers developed, only 14 markers contain SSR motifs. In another study, 183 primer pairs were designed which 60 of them showed polymorphism.

Choudhary *et al*., (2009) reported that for inter-specific transferability studies, nine accessions belonging to the five wild annual *Cicer* species were used and for cross-genera studies across legumes, 28 accessions belonging to seven legume genera were used. Cross- species transferability ranging of used markers in this study was from 68.3% to 96.6% across five annual *Cicer* and 29.4% to 61.7% across seven legume genera (Choudhary *et al*., 2009). In this study we used *M. truncatula* (as a model plant for legume) ESTs for investigation of genetic

1- International Legume Database & Information Service [http://www.ildis.org/Leguminosae]

diversity and transferability across some chickpea genotypes.

Material and Methods

Plant material and DNA extraction

Plant material containing 10 cultivated chickpea genotypes and *M. truncatula* (NCBI taxonomy ID. 3880) was prepared from chickpea Collection of Ferdowsi University of Mashhad, Iran (Table1). Seeds were disinfected using 1% sodium hypochlorite for 10 min and were grown in soil. DNA was extracted from young, fresh leaf tissues using QIAamp DNA mini extraction kit (Qiagen, Germany). Integrity of extracted DNA was estimated by agarose gel electrophoresis (1%).

Table 1. List of genotypes used in this study (common name, Acc.no, type and their source).

Common name	Acc.no/name	type[1]	Source
Jam	MCC* 361	Macrosperma	IRAN
Korosh	MCC 397	Macrosperma	IRAN
Siahkaka	MCC 362	Microsperma	IRAN
Pirooz	MCC 395	Microsperma	IRAN
Hashem	MCC 950	Macrosperma	IRAN
ILC 482	MCC 252	Macrosperma	Turkey
Karaj 12-60-31	MCC 358	Macrosperma	IRAN
-	MCC 49	Microsperma	Unknown
-	MCC 736	Macrosperma	Unknown
-	MCC 737	Macrosperma	Unknown
M. trancatula 3880	-	-	native to the Mediterranean region[2]

MCC*: Mashhad Chickpea Collection

1- http://test1.icrisat.org/chickpea/taxonomy/frachc.htm
2- http://medtr.comparative-legumes.org/

In silico development of EST-SSR markers

The *M. truncatula* EST sequences used in this study were iterated from NCBI's database EST (http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucest&cmd=search&term=medicago%20truncatulla%20EST%20dadabase). From 1,309 chickpea EST sequences available in the NCBI nucleotide database (up to January 2007), we explored 650 ESTs for the presence of microsatellite motifs using Simple Sequence Repeat Identification Tool (SSRIT) downloaded from the Cornell University web site (http://www.gramene.org/gramene/search/ssrtool). This program was used for detection of di-, tri- and tetranucleotide motifs with different repeats (Table 2). CAP3 program was used for cluster analysis on microsatellite containing sequences (EST-SSRs) to reduce redundancy (Huang and Madan 1999). The identified EST-SSR sequences were deposited in the GenBank to obtain the accession numbers (see Table 2). The putative function of the developed chickpea functional markers was found by the BLASTX tool of NCBI.

Primer design and PCR reaction

Primer design for non-redundant EST-SSRs was done using Primer3 software (http://fokker.wi.mit.edu/primer3/input.htm). All the oligonucleotides were synthesized by Gene works Company (Urrbrae, SA). PCR reactions were carried out in 20 µl volume by Bio Rad termocycler (C1000 Touch ^{TM}). Each reaction included 10 ng of extracted DNA as template, 1µl MgCl2 (50 mM), 1µl dNTPs (200 mM), 2µl PCR reaction buffer 10X (pH=8.5), one unit of Hotstart DNA Taq polymerase (Bioneer, Korea) and 1µl of each primer (10 pMol). The thermal profile for a touchdown PCR was as: (1) Initial denaturation at 95°C for 5 min, (2) 10 cycles of 94°C for 30s, 56°C for 30s As annealing step, 72°C for 50s and reducing annealing temperature of 0.8°C/cycle, (3) 20 cycles of 94°C for 30s, 48°C for 30s and 72°C for 30s, (4) final extension at 72°C for 10 min. PCR products were loaded on agarose gel (1%) and after that to better distinction of bands they were separated on acrylamide gel (8%). Acrylamide gel staining was performed using silver nitrate for 30 min. All scorable bands were determined and according to presence or absence of EST-SSR bands, binary matrices (0 or 1) were formed. Data were analyzed using POPGENE software, version 1.32 and number of alleles (N_a), observed heterozygosity (H), Shannon's informative index and fixation index (F_{IS}) values were calculated using this software (Yeh and Boyle, 1997). dendrogram was constructed using UPMGA method in NTSYS-pc version 2.1 software. Transferability between chickpea and *M. truncatula* was calculated according to Choudhary *et al*., 2009.

Table 2. Characteristics of 30 chickpea EST-SSR markers used in this study.

S. No.*	Locus name	Primer sequence (5′→ 3′)	Motif	*Tm* (°C)	Expected size (bp)	GeneBank No.	Putative function (from NCBI)
1	EY-ESTSSR1	Forward:CGGAAGCTTTTGCTTCTGTT Reverse:GCGAAATACGTGCAAGATCA	$(AT)_3$	59	122	EY478355	cytochrome b6
2	EY-ESTSSR5	Forward:TGACGTGGACGATTTCATGT Reverse:ACAAACTCTTGGGGCAGAGA	$(TGA)_4$	59	603	EY478352	unknown
3	EY-ESTSSR7	Forward:GCTGCACGAACAGAACAGAA Reverse:GTGATGTGGGTCTCGGATTC	$(AG)_4$	60	368	EY478350	Conserved hypothetical protein
4	EV-ESTSSR15	Forward:TTTGCCGGAAACTTCAATTC Reverse:GGGCGCATGTAACAACTTTT	$(TCA)_3$	60	431	EV260523	unknown
5	EV-ESTSSR18	Forward:TGGGAACTCCCTTTTCACAC Reverse:ATGGCAGTGCATCGAACATA	$(CT)_3$	60	186	EV260519	predicted protein
6	EV-ESTSSR21	Forward:TGCAACAGCCAACTGGTATC Reverse:TTGACTTTACAGCGCAGGTG	$(TC)_3$	60	138	EV260518	hypothetical protein
7	EV-ESTSSR23	Forward:GCATCTCTGCTGGTGATGAA Reverse:ATGTCGCCCTGTCAACCTAC	$(CA)_3$	60	269	EV260517	glutamine synthetize
8	EV-ESTSSR27	Forward: CCACCCATCATACCAAAACC Reverse: GCTTCATGGGTTTCTTTCCA	$(AC)_3$	60	135	EV260515	heat-shock protein
9	EX-ESTSSR31	Forward: TGATCGGTGATTCCTTGTGA Reverse: TGGTCAACAGTTTGGGGATT	$(CA)_3$	60	460	EX526999	H+-transporting two-sector ATPase
10	EX-ESTSSR34	Forward: TTAATTGGGGTTGGACCAGA Reverse: ACCACTACCTCCACGACCAG	$(GTC)_3$	60	156	EX526998	hypothetical protein
11	EX-ESTSSR39	Forward: CAAATCCTCAGCTGCTTTCC Reverse: AGGCAACTTCCACATTGTCC	$(AAG)_3$	60	304	EX526995	unknown
12	EX-ESTSSR48	Forward: ACATTTGTGCGGACTGTGTC Reverse: ATTGATGCTCTGCCAGGAAC	$(TA)_3$	60	728	EX526990	hypothetical protein
13	EX-ESTSSR50	Forward: GCTATGGGGCTTAGTGACCA Reverse: AACAAGTGGGCGGAATACAG	$(CA)_3$	60	222	EX526989	unknown
14	DW-ESTSSR53	Forward: CGACCCATTTCCTCACAACT Reverse: TAGCAAGAAAAAGCCCCAGA	$(TC)_3$	59	321	DW017290	conserved hypothetical protein

*Sequence number

Table 2. continued.

S. No.	Locus name	Primer sequence (5′→ 3′)	Motif	*Tm* (°C)	Expected size (bp)	GeneBank No.	Putative function (from NCBI)
15	DW-ESTSSR58	Forward: GGATTCCCAAAGTTCAGCAA Reverse: CCCTCAATTGCATCCTCAAT	$(AAG)_3$	60	703	DW017287	heat shock protein
16	DW-ESTSSR62	Forward: TGTTGTTGCCGAGTGTTGTT Reverse:TCCGCCTGTAAGCTGAAACT	$(AT)_3$	60	482	DW017285	hypothetical protein
17	DW-ESTSSR67	Forward: GACCCCCTTGTTCATTTTCA Reverse: AGCAGCGATGAGTTTGTGTG	$(TC)_3$	59	275	DW017280	unknown
18	BQ-ESTSSR73	Forward: ACATTTGGGCTCCTGTTCAC Reverse: CACGGGGGTTTTATTCAATG	$(AT)_3$	60	383	BQ135603	mutant green fluorescent protein
19	BQ-ESTSSR78	Forward: CGCTTAGGTGCAGTTGATGA Reverse: TTGTATAAAGGCCCGGACAC	$(GC)_3$	60	371	BQ135601	unknown
20	BQ-ESTSSR82	Forward: CTAAACAAGCATGGGGGCTA Reverse: ATCCGGTTTCTTAGCCCTGT	$(GA)_3$	60	251	BQ135600	hypothetical protein
21	BF-ESTSSR93	Forward: CCTCACCCCTCACTTGAAAC Reverse: TATGCGTAGCTCCACGACAG	$(GAA)_4$	59	203	BF640580	O-methyltransferase
22	BF-ESTSSR95	Forward:CAAGCAGAGAAATGGAAGCA Reverse: GTTCCATGGGTTTGGATTTG	$(AT)_4$	59	235	BF640577	cytochrome P450 monooxygenase
23	ES-ESTSSR101	Forward: TGGCAATGAGTTTTGTCTCG Reverse: CACTCACAATGACCCACCAC	$(TA)_3$	59	363	ES612471	---
24	ES-ESTSSR107	Forward: ACGGACCCTCAAGTTGTCAC Reverse: CTCTTCCAATCCCAGAACCA	$(CT)_3$	60	311	ES612451	unnamed protein product
25	ES-ESTSSR108	Forward: GGCTCTTTTCCCCTATGCTC Reverse: CCAACATTGCAAGTCCTGAA	$(AT)_4$	60	263	ES612450	short-chain dehydrogenase/reductase
26	ES-ESTSSR111	Forward: TTGTACCCACCTCCCTTGAG Reverse: ACCTACGAGGTGGCAAATGA	$(GT)_3$	60	223	ES612449	YihY family protein
27	AW-ESTSSR115	Forward:TCCTTCTCATTCCAACCTTGTT Reverse: CCAGCCAAGATAAGCGAAAG	$(GA)_3$	59	444	AW692381	lysine/histidine transporter
28	AW-ESTSSR118	Forward: AGGAATGCCACAAAAATTGC Reverse: CCAACCCATAGATCCCAATG	$(CCT)_3$	60	208	AW697088	unknown
29	EY-ESTSSR125	Forward: GGGAAAGGATAAAGCGAAGG Reverse: AGCAACAGCAATTGGCTACC	$(AT)_3$	60	711	EY477513	conserved hypothetical protein
30	EY-ESTSSR130	Forward: GTGGGAAGATGTTTGGAGGA Reverse: GCCTCTTGGAGAGGTGTCAG	$(AC)_3$	59	239	EY478413	unknown

Polymorphism information content (PIC) for each primer was calculated by following formula: Equation:

$$PIC = 1 - \sum_{i=1}^{n} P_{ij}^2$$

In the above equation P*ij* is the frequency of the ith allele for marker *j* and summation extends over *n* alleles (Eujayl *et al*., 2004). Frequency of each allele was calculated by POPGENE software.
Genetic distances were calculated adopting the Nie's formula (Nei 1973).

Results & Discussion

Development of EST-SSR Markers

650 *Medicago truncatula* ESTs were explored for finding of ESTs containing simple sequence repeats (EST-SSR). A total of 131 EST-SSRs were used in searching for the best SSRs. 30 EST-SSRs that had 3 or more repeats of SSRs were selected. In this study, thirty primer pairs were designed for 30 EST-SSRs and explored over 10 chickpea genotypes. Approximately, in 30 EST-SSR sequences, 27 sequences (90%) had 3 replications and 3 sequences (10%) had 4 replications (table 2). Among these 30 EST-SSR sequences, 7 motifs were three-nucleotides (23.3%) and others were di-nucleotides (76.7%). Among all the motif, AT motifs were the most abundant (20%) followed by CA (10%) and TC motif (10%) (Figure 1).

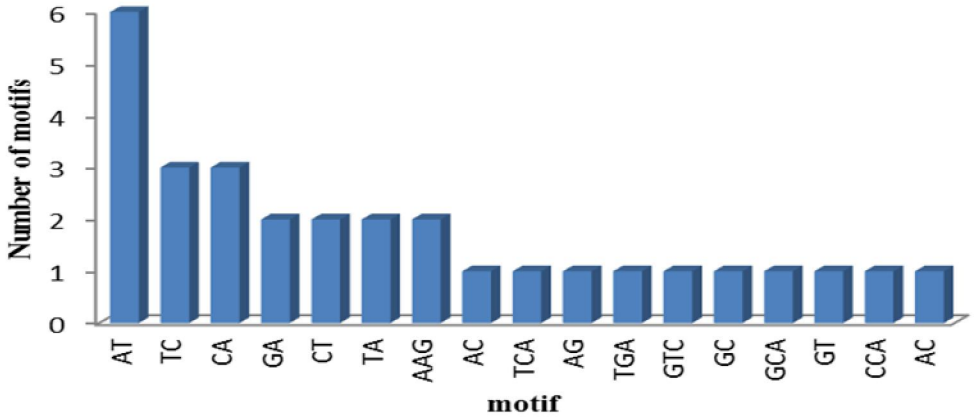

Figure 1. Distribution of the SSR motifs in the 30 selected EST-SSR.

Polymorphism of the EST-SSR markers

A total of 30 primer pairs were used for investigation of genetic diversity among chickpea genotypes. Thirteen primer pairs (43%) produced scorable SSR bands in at least one chickpea genotypes and eight out of 13 primer pairs (61.5%) were polymorphic in the chickpea genotypes (Table3). PIC of EST-SSR markers varied from 53% (EY-EST-SSR1) to 99% (EST-SSR107) with the average of 78.75% (Table 3). Transferability for *M. truncatula* EST-SSR markers across

chickpea genotypes was 43.3%. We previously found that using EST-SSR markers among chickpea accessions had a low level of polymorphism (16%) (Choudhary *et al*., 2009).

Although we attained a low polymorphism in EST-SSR markers, which is mostly attributable to using cDNA as template, the variation mirrors variation in genomic coding region which is highly valuable (Varshney *et al*., 2009). The surveying loci that exhibiting high polymorphism is so important in investigation of relationships among chickpea genotypes. EST-derived SSRs exhibit less polymorphism than SSR markers but they are cost-effective, valuable source for molecular markers generation and transferable across related genus. Number of alleles, Shannon's informative index (*I*), observed heterozygosity (*H*) and fixation index (F_{IS}) in eight polymorphic EST-SSR loci are presented in Table 4.

Table 3. Eight polymorphic EST-SSR loci. From thirteen primer pairs eight pairs showed polymorphism in used chickpea genotypes.

S. No.	Locus name	GeneBank No.	Primers Sequence (5′→ 3′)	Expected size (bp)	Motif	PIC (%)
1	EY-EST-SSR1	EY478355	Forward:CGGAAGCTTTTGCTTCTGTT Reverse:GCGAAATACGTGCAAGATCA	122	$(AT)_3$	0.99
2	EV-EST-SSR18	EV260519	Forward:TGGGAACTCCCTTTTCACAC Reverse:ATGGCAGTGCATCGAACATA	186	$(CT)_3$	0.96
3	EV-EST-SSR20	EV260518	Forward:TGCAACAGCCAACTGGTATC Reverse:TTGACTTTACAGCGCAGGTG	138	$(TC)_3$	0.73
4	EX-EST-SSR50	EX526989	Forward:GCTATGGGGCTTAGTGACCA Reverse:AACAAGTGGGCGGAATACG	222	$(CA)_3$	0.71
5	DW-EST-SSR53	DW017290	Forward:CGACCCATTTCCTCACAACT Reverse:TAGCAAGAAAAAGCCCCAGA	321	$(TC)_3$	0.60
6	DW-EST-SSR62	DW017285	Forward: TGTTGTTGCCGAGTGTTGTT Reverse:TCCGCCTGTAAGCTGAAACT	482	$(AT)_3$	0.53
7	BQ-EST-SSR78	BQ135601	Forward: CGCTTAGGTGCAGTTGATG Reverse: TTGTATAAAGGCCCGGACA	371	$(GC)_3$	0.99
8	ES-EST-SSR107	ES612451	Forward:ACGGACCCTCAAGTTGTCAC Reverse:CTCTTCCAATCCCAGAACCA	311	$(CT)_3$	0.79

Table 4. Number of alleles (N_a), observed heterozygosity (H), Shannon's informative index and fixation index (F_{IS}) values for eight polymorphic loci.

Locus	N_a	H	I	F_{IS}
EY-EST-SSR1	2	0.1653	0.3046	0.6900
EV-EST-SSR18	1	0.2975	0.4741	0.6842
EV-EST-SSR20	2	0.281	0.4453	0.6913
EX-EST-SSR50	3	0.1653	0.3046	0.6894
DW-EST-SSR53	5	0.4099	0.5981	0.6886
DW-EST-SSR62	2	0.4297	0.6207	0.6881
BQ-EST-SSR78	2	0.1653	0.3046	0.6884
ES-EST-SSR107	7	0.2644	0.4239	0.6907
Average	3	0.2723	0.4344	0.6888
SD		0.1401	0.1770	

Na= Observed number of alleles, H= (Nei 1973) gene diversity, I= Shannon's Information index and F_{IS}= fixation index (Lewontin 1972).

Genetic relationships between chickpea genotype

Genetic diversity among 10 cultivated chickpea genotypes was investigated using *Medicago truncatula* EST-SSRs markers. According to allelic banding pattern of eight primer pairs, the genetic distance was varied from 0 to 0.61. The highest genetic similarity was detected between genotypes MCC 358 and MCC 49 and the highest genetic distance was found between MCC 361 and MCC 737. Chickpea genotypes and *M. truncatula* were clustered in completely distinct branches (Figure 2). Constructed phylogenetic tree illustrated that these EST-SSR markers could not separate MCC 358 and MCC 49 base on these primer pairs and may be other primers could separate them. These results suggest that coding sequences of MCC 358 and MCC 49 may have been related to the same evolutionary pathway. However, it is required to compare them with other locus results. The observed heterozygosity values ranged from 0.140 to 0.429 with an average of 0.272 (Table 4). Generally, the diversity level of chickpea genotypes is low. Other studies verified a low level of sequence diversity that was observed in chickpea cultivated species (Gujaria, 2011).

Multiple alignment and sequence analysis

To evaluate the variation among chickpea genotypes, 5 different homologous alleles which amplified by DW-EST-SSR62 (MCC 49, MCC 252 upper and lower band, MCC 950 and *M. truncatula*), were sequenced (Figure 3). Multiple alignments of nucleotide sequences for locus DW-EST-SSR62 was carried out using BioEdit software and also dissimilarity of these sequences were detected in this software (Figure 4). A new sequence for this locus was identified and deposited in NCBI database with JF681971 accession number. Comparison between sequences exhibited the presence of a $(AT)_n$ repeat motif at position 34-40 bp. There is an additional nucleotide (T) in repetitive motif in MCC 49 at position 38 bp. Some transition mutations (T→C) at position 26 and 97 bp, (A→G) at position 42 and 45, (G→A) at position 57 in MCC 252 were observed. Also, transversion mutation (T→G) at position 28, (C→A) at position

59 and (A→C) at position 65 in MCC 252 were detected. Since SSRs in expressed regions are highly protected, increases or decreases of repetitive unit caused changes in length of gene and the EST-SSR markers can detect these polymorphisms very well (Sergio, 2003). Insertion/deletion mutations are the main cause of length size variation and cause allelic size variation in some of the genotypes. Several investigations have emphasized that microsatellites undertake expansion through evolution (Zhu *et al*., 2000; Peakall *et al*., 1998). In this study there was an expansion of A motif at position 102-104 bp in MCC 252 caused length size variation (Figure 4). Another study have reported that the GA motif was the most abundant motif in dicots ESTs like *Medicago* (Tian *et al*., 2004) but in this study dominance of AT motif was observed.

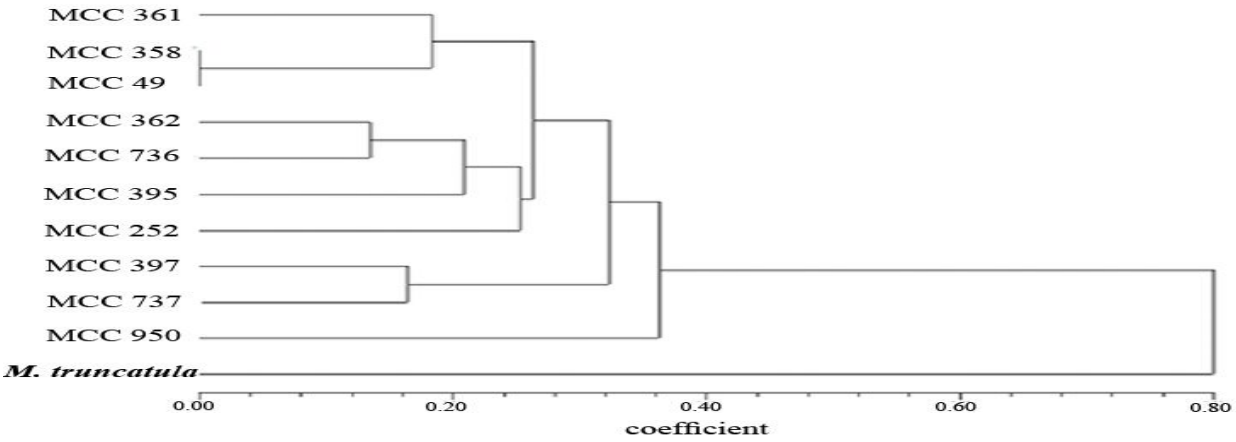

Figure 2. The dendrogeram of ten cultivated chickpea genotypes and *M. truncatula* using 8 EST-SSR primer pairs. The dendrogeram was constructed whit UPGMA method in mega4 software media.

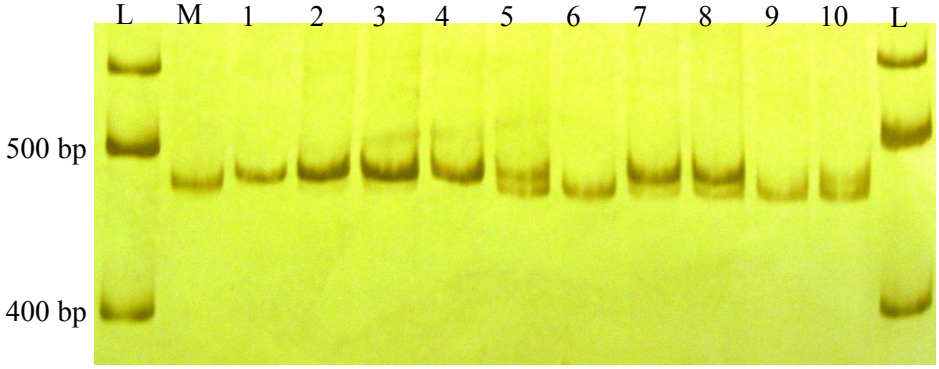

Figure 3. Electrophoretic patterns of SSR alleles at locus DW-EST-SSR62. 1) MCC 737, 2) MCC 736, 3) MCC 49, 4) MCC 358, 5) MCC 252,6) MCC 950, 7) MCC 395, 8) MCC 362, 9) MCC 397, 10) MCC 361, M) *M.trancatula* and L)1 k bp ladder. The acrylamaide gel was stained using silver staining.

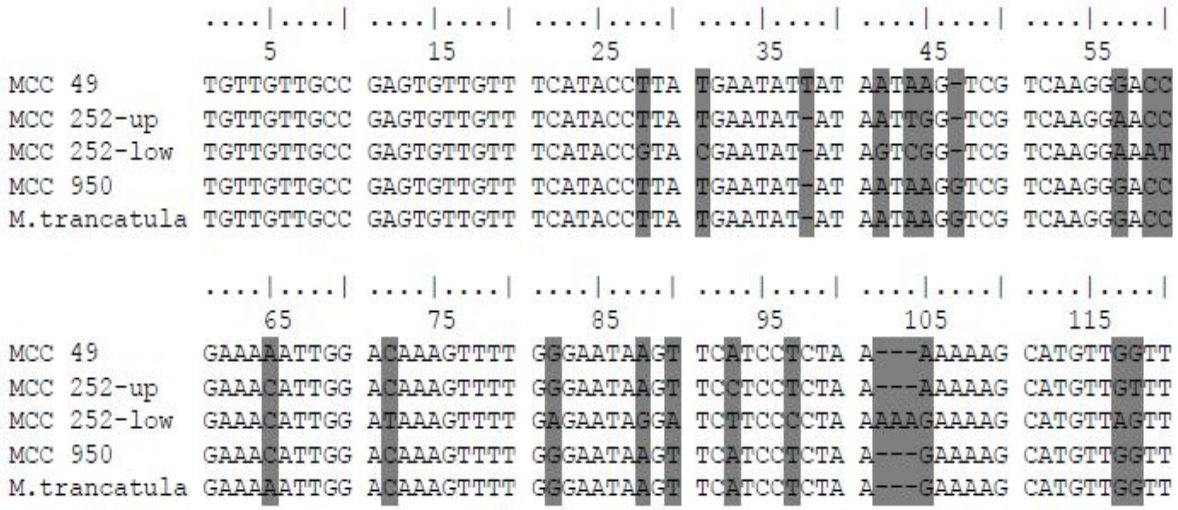

Figure 4. Partial sequence alignment for locus DW-EST-SSR62 across 5 different chickpea genotypes in comparison with *Medicago truncatula.* Gray parts show that these genotypes are different in these parts for this locus.

Advantages and deployment of EST-SSR markers

Model plants are important tools in many genetic studies. *M. truncatula* is a useful legume model plant which has been widely used for several genetic studies and a large number of molecular markers have been developed from this plant to other legumes.

Screening of EST sequences for finding hypervariable SSR motifs is the best and informative way for development of genetic molecular markers (Choudhary *et al*., 2009). To our best of knowledge, this was the first detailed report of application of EST-derived SSR markers in chickpea for Iranian accessions. There is a huge wealth of EST sequences in publicly available EST database of legumes (Fabaceae) which most of them are derived from *M. truncatula*, Lotus japonicus and *Glycine max* (Ramı´rez *et al*. 2005). Usefulness of EST-SSR *M. truncatula* markers in comparative mapping, construction of linkage maps and QTL discovery in *M. truncatula* have been reported (Danesh *et al*. 2002; Huguet *et al*. 2001). The high level of transferability and low polymorphism of these markers has also been demonstrated for legume species such as alfalfa (Eujayl *et al*., 2004). High level of transferability of these markers is useful for comparative genomic studies. EST is an appropriate and economic method for developing both co-dominant and polymorphic markers (Becher, 2007).

The number of gene-targeted molecular markers of chickpea are limited, since of the nature of self-polination they show a low level of polymorphism in molecular

markers (Choudhary *et al*.,2009). Other study provided evidence that using EST-SSRs marker in rice (Cho *et al*., 2000), sugarcane (Cordeiro *et al*., 2001) and wheat (Gupta *et al*. 2003) displayed lower levels of polymorphism rather than SSR markers. This study provides insights into EST-SSRs of a related species such as *M. truncatula*, can be useful for studying diversity in legumes like chickpea. The low level of polymorphism in EST-SSRs may be rewarded for by their ability of interspecies transferability (Thiel *et al*., 2003). The chickpea EST-SSR markers exhibited high transferability to six Cicer species and seven legume genera (P. *mungo*, *P. sativum*, *G. max*, *T. alexandrinum*, *L. esculenta*, and *C. cajan*,) (Choudhary *et al*., 2009). The transferability of EST-SSR markers has also been reported in some bean species (Gutierrez *et al*. 2005; Yu and Li, 2008). The SSR markers from *Medicago truncatula* showed transferability to faba bean, chickpea, and pea (Gutierrez *et al*., 2005). Transferability of *M. truncatula* EST-SSRs in 19 accessions of forage legumes comprising 11 genera was 40.6% (Chandra, 2011). Some of Chickpea SSR markers were designed from genomic sequence and do not show diversity of coding region and are not useful for functional genomics (Gujaria 2011). Functional markers such as EST-SSR ones are transferable more than other markers. Here, the EST derived SSR which has known gene functions can be used for identification of traits of plant, thus it will due to explore genes related to traits.

In conclusion, our study was the first attempt at characterization of some EST-SSRs of the several Iranian chickpea genome. This study not only contributed to strengthening the chickpea EST database but also provided some set of functional SSR markers to evaluation of chickpea germplasms. In the present study, it was established that the chickpea EST-SSRs were highly transferable across *M. truncatula* and chickpea thereby providing ample opportunity for mining of superior alleles and development of candidate gene markers for use in gene introgression programs and comparative genomics in legumes.

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بررسی تنوع ژنتیکی برخی ارقام نخود زراعی با استفاده از مارکرهای EST-SSRs برگرفته شده از *Medicago truncatula*

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چکیده

از رهیافت‌های مناسب برای تشخیص تنوع درون جمعیت و بررسی قرابت‌های ژنتیکی استفاده از نشانگرهای مولکولی است. نشانگرهای مولکولی EST-SSR از منابع مهم مورد استفاده در بررسی تنوع ژنتیکی به شمار می‌آیند. آغازگرهای EST-SSR از روی مناطق بیان شونده طراحی شده و با وجود ایجاد تنوع کم تنوع نسبتاً واقعی تری نسبت به دیگر ریزماهواره‌ها نشان می‌دهند. این نشانگرها همچنین دارای قابلیت انتقال‌پذیری بالایی در بین گونه‌های یک جنس می‌باشند. با توجه به مدل بودن گیاه یونجه یکساله (*Medicago truncatul*a) برای گیاهان لگومینوز، در این پژوهش از توالی‌های EST این گیاه برای بررسی تنوع ژنتیکی بین برخی ارقام نخود زراعی استفاده شد همچنین قابلیت انتقال‌پذیری بین این گیاه و نخود مورد ارزیابی قرار گرفت. بر این اساس ۶۵۰ توالی EST برای یافتن توالی‌های کوتاه تکراری مورد بررسی قرار گرفت که از این بین ۱۳۱ توالی EST حاوی SSR با دو یا سه تکرار تشخیص داده شدند. در این تحقیق از ۳۰ جفت آغازگر برای بررسی تنوع ژنتیکی بین ۱۰ رقم نخود زراعی استفاده شد. ۱۳جفت (۴۳٪) از این آغازگرها برای حداقل یک ژنوتیپ نخود تکثیر باند را نشان دادند که ۸ جفت (۶۱/۵٪) از این تعداد دارای چند شکلی بودند. به طور کلی تعداد ۲۴ آلل تشخیص داده شد که به طور متوسط ۳ آلل در هر پرایمر قابل تشخیص بود. میانگین PIC محاسبه شده ۷۸/۷۵٪ بود و قابلیت انتقال بین نخود زراعی (C. arietinum) و M. truncatula ۴۳/۳۲٪ محاسبه شد. نتایج نشان داد که با توجه به مطرح بودن گیاه M. truncatula به عنوان یگ گیاه مدل برای خانواده لگومینوز می‌توان از این گیاه به عنوان یک منبع غنی از اطلاعات برای بررسی های ژنتیکی دیگر لگوم ها استفاده گرد. وجود تنوع با ارزش ایجاد شده توسط نشانگرهای EST-SSR باعث توسعه روز افزون این آغازگرها شده است از این رو استفاده از توالی‌های EST موجود برای بررسی تنوع در گیاهان خویشاوند می‌تواند ابزاری با توجیه اقتصادی مناسب در این راه باشد.

کلمات کلیدی: نشانگرهای EST-SSR، تنوع ژنتیکی، نخود زراعی (*Cicer arietinum L.*)، *Medicago truncatula*