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Assessment of genetic diversity in *(Lens culinaris M.)* using ISJ marker

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ABSTRACT

Lentil((Lens culinaris M.), as one of the most important members of the fabaceae plays fundamental role in the nutrition of the people in developing countries due to its high protein contant.Therefore, diversity is very important for the management of breeding programs. Hence evaluation of genetic diversity for exploitation of variation is essential for breeding projects and also helps breeding to select favorite parents in hybridizations. In this study, the genetic variation of 23 genotypes was assessed using ISJ markers. Based on molecular data,165 bands were detected and 117 bands were polymorph. The mean number of bands was 9.1 bands per primer using ISJ, the fragment size varied withen a significantaly narrower range(150-2500bp). The similarity matrix was subjected to cluster analysis by the unweighted pair-group method(UPGMA). The PIC value was ranged 0.201 to 0.433 and the MI index averaged(1.93). In the ISJ marker the UPGMA cluster diagram have showed 4 major clusters. According to similarity matrix, the least similarity belonged to gachsaran and F2006-6L and the highest similarity belonged to F2004-53L and F200456L. The results showed that intron-exon splice junction (ISJ) markers is an effective method for analysis of genetic variation among lentil germplasm.

Key words: Lentil, geneytic diversity, ISJ marker, polymorph.

INTRODUCTION

Lentil*(Lens culinaris Medik)* is a grain legume originated in the Near East [11,20]. widely cultivated in the world because largely appreciated by consumers. This legume crop is well suited for low input cultivation in marginal areas and productes seeds with a high protein content (up to 26-27%); nevertheless, its value is often compromised by low levels of grain yield [3]. Lentil is self-polinated, diploid (2n=2x=14) with a large genome size of approximately 4Gb [2]. To understand better the genetic structure of such a large genome, The genome needs to be characterized and mapped by molecular markers. Molecular markers are important tools for generating genetic linkage maps and have provided a significant increase in genetic information of plant species [14]. Determination of genetic diversity in plans is the first step when developing breeding programmes. Conventional analysis of morphological traits is insufficient to precisely estimate plant diversity.With rapid developments in biotechnology, molecular markers have been widely employed in plant diversity research [17]. Isozyme and DNA marker analysis enables accurate identification of species and varieties, determination of kinship and specification of their place in the systematics [5,12]. One molecular system, semi specific PCR and the use of primers with partial homology to sequences of intron-exon junctions was developed by weinig and langrage (1991) and RAPD system which developed by (Williames *et al*., 1990). The RAPD system is useful for many cropes, but has low rate of polymorphism, along difficulties with the reproducibility of the results. ISJ seems to be an alternative to RAPD and order tedious and expensive methods such as RFLP and AFLP. These allow the generation of great diversity of markers without any additional sequence information [6]. It has been widely used in assessments of genetic diversity [8,16]. used RAPD, and ISSR, markers for assessment of genetic variation in a collection of lentil, they revealed the

dendrogram generated from ISSR data was quite different from the generated from RAPDs ones [10]. In this study, we employed diversity assessment system based on polymerase chain reaction(PCR)-based method (ISJ).

MATERIALS AND METHODS

Plant material and DNA extraction

Twenty three lens genotypes were used in the study obtained from the Lorestan Agriculture and Natural Resources Research center (Table 1). Young-leaf tissue (0.4g) from each lentil line was ground in liquid nitrogen to a fine powder and total genomic DNA was extracted using the [4] method with minor changes. DNA was treated with RNAse and proteinase K. The quality and quantity of DNAs were determined in 0.8% agarose gel and Biophotometer (model Biorad).

Plant analysis

Primers were used in this study listed in the (table 2). The details of the intron-splice-Junction primers were previously described by Weining and Langridge (1991). PCR was carried out in a 25µl volume containing 10X PCR buffer, 2 µl of dATP, dCTP, dGTP and Dttp, 15mM Mgcl2, 10mM Tris-Hcl (PH 8.3), 50Mm kcl and 1 units of Taq polymeras (Bioran). Subsequently the first 7 cycles were at 94º C for 1min, 40º C 1.8min and 72º C 2min. This was followed by a futher 28 cycles of 94º C 1 min, 58º C 1.5 min and after the last cycle an extension step of 72º C for 8 min was performed. The PCR products were resolved on 1.5% agarose gel in 1X TAE buffer at 100 to 120 volts for 2 to 2.5 h. The gel were stained with EtBr and visualized under ultraviolet light.

Statistical Analysis

ISJ markers were scored as presence (1) or absence (0) of a band, and the data obtained were used in rectangular matrix. The data matrix was then used to generate a genetic distance Index ‘ Nei’. The polymorphic information content (PIC) value was calculated for both molecular marker systems using formula PIC=[$\sum$ 2 Pi(1-Pi)] [7]. Where Pi is frequency of the I band. MI was calculated as MI= PIC×n β [13], where PIC is the average PIC- value , n is the number of band detected and β is proportion of polymorphic bands. Cluster analysis was arried out based on genetic distance, using unweighted pair-group method using arithmetic average(UPGMA) through the software NTSYS-pc program [9].

RESULTS AND DISCUSSION

In the present study, 20 ISJ primers belonged to two groups of Intron Targeting (IT) and Exon Targeting (ET) that showed a high level of polymorphism and a high number of clearly amplified bands (Figure1). Semi random primers produced a total of 165, 117 bands (71) were polymorphic bands. The bands were characterized based on size and ranged from approximately 150-1800 bp . the mean number of bands 9.1 per primer. For Semi random primers average PIC-values ranged from 0.201 to 0.433 with mean of 0.292, the MI ranged from 0.80 to 4.42 and averaged 1.93. In study were used to estimate the similarity between lentil lines by the Jaccard similarity Index. The highest number of polymorph band was abtained with primers IT10-2 and IT35-15, while the lowest number was abtained with primers ET28-12 and ET30-12. Generaly,IT primers in comparison with the ET primers generated polymorphic fragments with higher resolution. the finding are in accordance with the results of [16] in plantago. Similarities ranged between 0.33-0.79, the lowest genetic similarity was found between genotypes F-2003-4L and Gachsaran, with the highest(0.79) was observed between genotypes F2004-53L and F2004-56L. Genetic similarities among the genotypes are presented as a dandrogram (Figure2). If the cutting is done on the 0.54 similarity coefficient, genotypes will devided into 4 major groups. Cluster-I comprised 19 genotypes, cluster-II contained two genotypes (F2005-4L and P1297799), cluster III contained genotypes F2005-1L and IV comprised cultivar Gacsaran. Number of amplified bands varied from 15 (IT10-1) to 6 (ET26-12).

Table 1. Characteristics of lentil varieties used in the study

NO	Varieties	Origin	NO	Varieties	Origin
1	Flip2004-52L	ICARDA*	13	ILL 6037	Icarda
2	Flip 2006-6L	Icarda	14	Flip 2004-26L	Icarda
3	X96S14K26	Icarda	15	Flip 2003-2L	Icarda
4	Flip 2003-9L	Icarda	16	Flip 2005-4L	Icarda
5	Lc960254	USA	17	X 96147K8	Icarda
6	Flip2004-53L	Icarda	18	Flip 2004-55L	Icarda
7	Flip2004-56L	Icarda	19	AKM397	Icarda
8	local cultivar	Iran- Lorestan	20	Cabralinta	Icarda
9	Flip 2006-9L	Icarda	21	Syrian	Syria
10	Ghachsaran	Iran	22	Flip 2005-1L	Icarda
11	Flip 2003-4L	Icarda	23	P 1297799	Icarda
12	Flip 92-12 L	Icarda	-	-	

**International Center for Agricultural Research in the Dry Areas, Allepo, Syria*

Table2. The result of cultivars lentil using ISJ marker

Semi random Primer	Sequence (5'-3)	Annealing temperature	P%*	PIC**	MI***
IT10-1	ACGTCCAGAC	63	83	0.322	1.6
IT10-2	ACGTCCAGGT	63	87.12	0.341	4.42
IT10-3	ACGTCCAGCA	63	78.16	0.243	1.7
IT10-4	ACGTCCACCA	63	80	0.268	2.14
IT10-6	ACGTCCATCC	63	100	0.433	2.59
IT31-15	GAAGCCGCAGGTAAG	58	70	0.267	1.86
IT34-15	ACCTACCTGGCCGAG	58	75	0.217	1.95
IT35-15	CGAAGCCAGGTAAG	58	83	0.412	4.12
IT36-15	ACCTACCTGGGGCTC	58	62.5	0.381	1.91
ET1-18	ACTTACCTGCTGGCCGGA	63	60	0.241	1.44
ET4-18	ACTTACCTGCCTGCCGAG	63	50	0.201	0.80
ET31-15	ACTTACCTGGGCCAG	58	75	0.282	1.69
ET35-15	ACTTACCTGCCGCAG	63	90	0.317	2.85
ET26-12	AGCAGGTGGACT	58	55.57	0.241	1.2
ET27-12	AGCAGGTCCTAG	58	69.7	0.250	1.75
ET28-12	AGCAGGTCGAAG	58	50	0.289	0.861
ET29-12	AGCAGGTCGTGA	63	44.31	0.267	1.061
ET30-12	AGCAGGTGGTAC	63	42.8	0.289	0.867

: Polymorphism percentage;:PIC – polymorphic information content; ***:MI: Marker index*

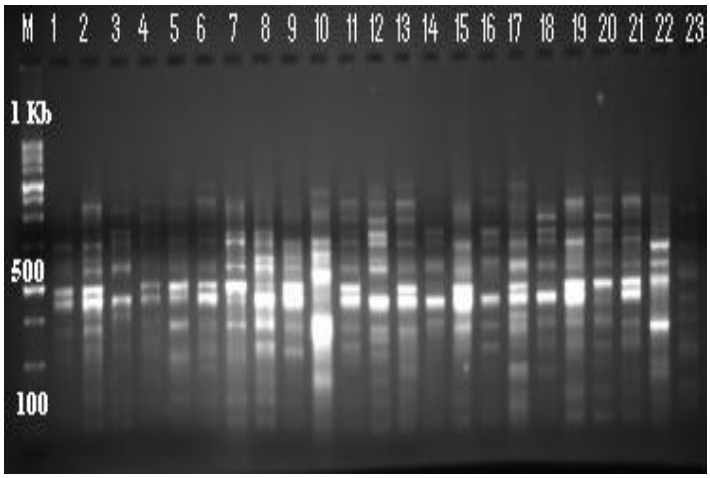

Figure1. Banding pattern amplified DNA using IT34-15 primer in agaros gel

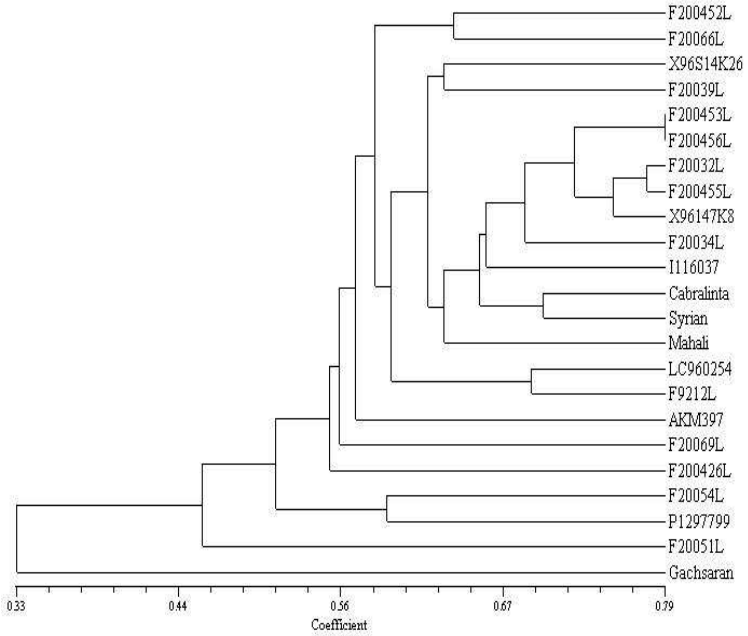

Figure2. Dandogram based on UPGMA, using semi random primers in the lentil lines

The ISJ primers are valuable in detecting and mapping polymorphisms between Wheat, barley, faba, triticale and tritipyrum [1,15,18]. It is important that understand they also detect polymorphism between lines and cultivars of lentil. (Figure 1) shows an axample of the products abtained when the primer is used to amplify band from 23 lines of and one local cultivar. The estimation of the degree of differentiation between materials included in a crossing program is useful, since it can help in selecting the different parents. Local cultivar has high yield stability and adaptation to environmental unusable conditions, but has low yield. Molecular study of genetic resources collection has prepared identification and select of favourable parents for crossing programs.

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