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ASSESSMENT OF GENETIC DIVERSITY IN LENTIL (*LENS CULINARIS* MEDIK.) AS REVEALED BY RAPD MARKERS

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Abstract

The genetic diversity in nine varieties of lentil (Lens culinaris Medik.) - six cultivars obtained from ICARDA, Syria, one cultivar developed in Romania and two landraces from Germany and France - was evaluated using RAPD (Random Amplification Polymorphic DNA) markers. Ten primers were assayed and among them only a few (4) have presented the polymorphic bands. Genetic distances were calculated using Nei&Li (1979) similarity coefficient, displayed in a dendrogram (UPGMA method). Cluster analysis based on RAPD amplification products divided genotypes in two main groups according to their geographical origin and their maturity. The similarity values found were higher than 70%, suggesting that genetic diversity between lentil genotypes analyzed are relatively low.

INTRODUCTION

Lentil is a self-pollinated diploid (2n = 14 chromosomes) annual cool season grain legume, with a relatively large genome of 4,063 Mpb (Arumuganathan and Earle 1991). Lentil seeds are valued as a food source of both high quality plant proteins and fiber, in addition, the remaining plant residues can be used as animal feed and fodder. This ancient pulse crop was domesticated in the Fertile Crescent where it has been cultivated since at least the seventh century B.C. (Ladizinky, 1979), and its cultivation area expanded around the Mediterranean Basin, Middle East, Ethiopia and the Indian Subcontinent.

The International Centre for Agriculture in Dry Areas (ICARDA) has a global mandate for research on lentil improvement. As such, ICARDA houses the world collection of *Lens*, totaling 10,509 accessions.

The ICARDA collection includes 8789 accessions of cultivated lentil from 70 different countries, 1146 ICARDA breeding lines, and 574 accessions of 6 wild *Lens* taxa representing 23 countries.

A comprehensive understanding of the genetic variation within any lentil breeding program is important for the efficient selection of parents, for introgresion o genetic material into superior cultivated lines and for the implementation of an effective genetic conservation program for the cultivated species.

The genetic diversity of *Lens culinaris ssp. culinaris* has been studied with many DNA-based molecular marker systems including: restriction fragment length

polymorphism (RFLP) analysis of nuclear DNA (Havey&Muehlbauer, 1989) and chloroplast DNA (Muench et al., 1991; Mayer and Soltis, 1994), amplified fragment length polymorphism (AFLP) analysis (Sharma et al., 1996) and random amplified polymorphic DNA (RAPD) analysis (Abo-Elwafa et al., 1995; Sharma et al., 1995; Ford et al., 1997).

The objective of this research was to evaluate the level of genetic diversity in the lentil genotypes, using random amplified polymorphic DNA (RAPD) analysis.

MATERIAL AND METHODS

Plant material and DNA extraction

The study was conducted in greenhouse and Molecular Genetics laboratory from University of Agronomic Sciences and Veterinary Medicine Bucharest, Romania in 2008. The materials used in this work are presented in Table 1. They include seven improved varieties and two landraces.

The genomic DNA was isolated from young leaves of greenhouse – grown plants according to the CTAB procedure (Saghai-Maroof et al.,1984) with minor modifications.

Table 1

Characteristics of lentil varieties used in the study

No.	Varieties	Origin / Pedigree	Phenotypic seed color	Type varietal ***	Maturity
1	Idlib 1	Siria-ICARDA*	Gray	m	Early
2	Idlib 2	Siria-ICARDA/ Single-plant selection from a Jordanian landrace, 74TA14	Reddish	m	Early
3	Idlib 3	ILL 99♀Moroccan landrace x ILL5588♂ elite line from Jordanian landrace population	Brown with patterns in black spots	m	Early
4	Idlib 4	Siria-ICARDA / ILL5879♀ x ILL5714♂	Gray	m	Early
5	Hurani	Siria -ICARDA / local cultivar	Brown	m	Early
6	Kurdi	Siria- ICARDA / local cultivar	Gray	M	Late
7	Oana	USAMV** Iasi Romania / Mutagenesis and selection from local landrace	Gray	m	Late
8	Lt m	Germany /landrace	Gray	M	Late
9	Lt n	France / landrace	Black	m	Late

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*** m – microsperma (small to medium-sized seed); M – macrosperma (large seed)

Random amplified polymorphic DNA (RAPD) analysis

The DNA obtained was amplified by the RAPD procedure with 4 decamer random primers from "Operon Technologies" (California, USA), that identified polymorphisms: OPA-17, OPA-12, OPG-05, OPG-06.

Polymerase Chain Reaction (PCR) were achieved in a final volume of 25 µl, containing: 25 ng DNA, 0.1 mM of each dNTP, 2.0 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.4 mM of one primer decamer and one unit of Taq DNA polymerase (Williams et al., 1990). The amplification was performed using a M.J. Research thermal cycler, programed for 45 cycles, each consisted of: one denaturation step at 94°C for 1 min., one annealing step at 36°C for 1 min and one extension step at 72°C for 2 min. The extension step in the last cycle was 7 min at 72°C. RAPD amplification products were separated on 1.2% agarose stained with ethidium bromide and scored for presence or absence of bands.

Statistical analysis

RAPD markers were scored as present (1) or absent (0). Distance genetics was computed after Nei&Li (1979) formula, using TREECON 1.3 b software package. A cluster analysis was performed using the unweighted pair-group method using arithmetic average (UPGMA) and the dendogram was obtained in order to visualize the relationship among lentil cultivars. The genetic similarity (Sij) was estimated using the Nei & Li coefficient (1979), by the expression: $Sij = 2\ N_{ij} / (N_i + N_j)$, where N_{ij} - the number of bands in common between accessions i and j; N_i and N_j - the number of bands for accession i and j, respectively.

RESULTS AND DISCUSSION

RAPD analysis

Four decamer random primers were used to differentiate between the nine lentil varieties (Tables 2). A total of 43 bands were amplified in lentil genotypes taken in study. Of the total bands 27 (P = 62.7%) were polymorphic. On the average, each primer amplified 10.75 bands, of which 6.75 were polymorphic. The percentage polymorphic loci varied from 58.3% (OPG – 06) to 69.2% (OPA-17) with an average of 62.5% bands / primer (Table 2).

Genetic distance and similarity

Pair–wise comparisons between the tested genotypes were used to calculate the genetic similarity (table 3). The highest similarity value (99.1%) was recorded between the lentil cultivars Idlib 3 and Idlib 4, indicating that these were closely related to each other (genetic distance = 0.090). Meanwhile, the lowest similarity value (70.4) was found between Idlib 1 and landraces Lm and Ln, indicating that these were distantly related genotypes (genetic distance = 0.296).

Table 2

Primers used in RAPD analysis of *Lens culinaris*, the number of scored and polymorphic loci

S/ No.	Primer name	Nucleotide sequence 5‘- 3‘	No. of loci		P*
			Total	Polymorphic	
1	OPA - 17	CACCGCTTGT	13	9	69.2
2	OPA - 12	TCGGCGATAG	10	6	60.0
3	OPG - 05	CTGAGACGGA	8	5	62.5
4	OPG - 06	GTGCCTAACC	12	7	58.3
	Average		10.75	6.75	62.7
	Total		43	27	62.7

*P – percentage of polymorphic loci

Table 3

Genetic similarity (below diagonal) and genetic distance values (above diagonal) in lentil varieties

Variety	Idlib 1	Idlib 2	Idlib 3	Idlib 4	Hurani	Kurdi	Oana	Lt m	Lt n
Idlib 1	***	0.143	0.067	0.067	0.134	0.173	0.215	0.296	0.296
Idlib 2	85.7	***	0.072	0.072	0.143	0.120	0.231	0.200	0.200
Idlib 3	93.3	92.8	***	0.090	0.134	0.104	0.143	0.186	0.186
Idlib 4	93.3	92.8	99.1	***	0.134	0.104	0.143	0.186	0.186
Hurani	86.6	85.7	86.6	86.6	***	0.173	0.286	0.260	0.260
Kurdi	82.7	88.8	89.6	89.6	82.7	***	0.112	0.077	0.077
Oana	78.5	76.9	85.7	85.7	71.4	88.8	***	0.120	0.120
Lt m	70.4	80.0	81.4	81.4	74.0	92.3	88.0	***	0.084
Lt n	70.4	80.0	81.4	81.4	74.0	92.3	88.0	91.6	***

The high similarity values found between the lentil genotypes tested indicate that the genetic diversity between them is narrow and due to their common origin in the breeding program. Similar results were also found by Sharma et al. (1996) used AFLP and RAPD marker techniques to evaluate and study the diversity and phylogeny of 54 lentil asscessions.

Cluster analysis

The clustering obtained by UPGMA method is shown in Figure 1. Cluster analysis revealed two main groups of lentil genotypes studied. The first cluster included five lentil cultivars obtained from ICARDA, with early maturity and microsperma

type: Idlib 3, Idlib 4, Idlib 1, Idlib 2 and Hurani, while the second cluster included genotypes with late maturity, from Europe except for Kurdi: Oana, Ltm, Ltn and Kurdi. RAPD analysis detected in the branch **A** other two categories grouping Idlib 3, Idlib 4, Idlib 1 and Idlib 2 improved lentil cultivars in **1** group and Hurani cultivar in **2** group (figure 1). Branch **B** is divided too in two categories, grouping romanian lentil cultivar – Oana in one group (**cluster 3**), and two lentil landraces Lt m, Lt n and Kurdi genotype in another group (**cluster 4**) (figure 1).

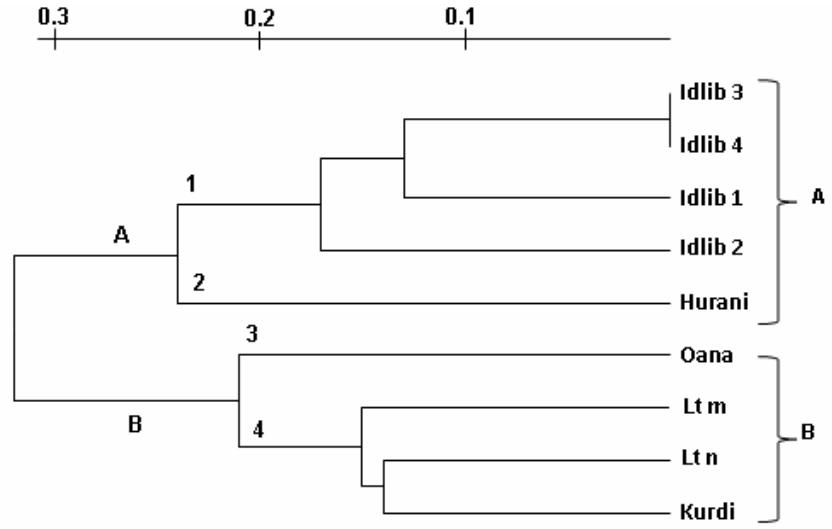

Fig. 1. Cluster analysis dendrogram of nine lentil genotypes (*Lens culinaris* Medik.) using RAPD data and UPGMA method

CONCLUSIONS

1. The analyses performed in this study indicate that investigated lentil accessions are genetically distinct. The nine lentil varieties formed two distinct groups. In the future breeding programs, crosses between lentil varieties from these two major groups (A and B) might lead to high heterosis, despite the theory that in autogamous species that have undergone evolution under domestication the dominant and additive alleles prevail, reducing the advances of F1 heteros
2. The RAPD technique contribute with a significant number of polymorphic markers wich could be useful in identifying lentil cultivars. It was concluded that RAPD markers could be exploited as alternative or supplementary tools to already established methods for the evaluation and classification of lentil genetic resources.

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