

Evaluation of Genetic Diversity in Three Species of *Retama* Genus: *R. monosperma*(L) Boiss, *R. raetam* (Forssk) Webb and *R. sphaerocarpa* (L) Boiss. (Fabaceae) Based on SDS-PAGE

Belmokhtar Zoubir and Kaid- Harche Meriem

Laboratoire des Productions Valorisations Végétales et Microbiennes (L2PVM)
Department of Biotechnology; Sciences faculty; University of Sciences and Technology,
Mohamed Boudiaf (USTOMB), P.O. Box. 1505, El Mnaouar, Oran, 31000, Algeria

Abstract: Genetic interpretation of protein polymorphism in *Retama* genus has not been well established until now. The aim of this study was to investigate interspecific and intraspecific polymorphism in three Species of *Retama* Genus: *R. monosperma* (L) Boiss, *R. raetam* (Forssk) Webb and *R. sphaerocarpa* (L) Boiss, by sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE). In this study twelve populations collected from different regions of Algeria have been studied for the analysis of seed storage protein profiles to examine their relationship. Based on electrophoresis bands spectra, Jaccard's Similarity Index was calculated and cluster analysis was performed using unweighted pair group method with arithmetical means (UPGMA), to check the polymorphism among populations. The results from the present study indicate that the low polymorphism between *R. monosperma* and *R. raetam*. The cluster analysis indicated that the twelve populations constituted two clusters. Cluster A comprised from all population of *R. monosperma* and *R. raetam*, and Cluster B comprised of 2 populations from *R. sphaerocarpa*. The SDS banding proteins pattern showed that four bands are monomorphic in all populations of three species with molecular weights of 31.761, 63.767, 92.363 and 82.832 KDa.

Key words: Electrophoresis, polymorphism, SDS-PAGE, *R. monosperma*, *R. raetam*, *R. sphaerocarpa*

INTRODUCTION

Retama genus commonly called R'tem, belongs to the family Fabaceae, endemic of the Mediterranean regions, represented in Algeria by three species: *Retama monosperma* growing on the coast and coastal dunes stabilized, she protect beaches from erosion, *Retama raetam* and *Retama sphaerocarpa* that span arid and Saharan areas where they plays an important ecological role in the fight against desertification by stabilized sand dunes Quezel and Santa (1962).

They are symbiotic associations with *Rhizobia* which give them an environmental interest. Their physical and edaphic effects on their surroundings they provide habitat for herbaceous species adapted to environmental conditions very different from those of the open areas, increasing their abundance and the overall species richness (Villar-Salvador *et al.*, 2008; Boulila *et al.*, 2009).

Phytochemical studies have shown that these species are very rich in flavonoids, triterpenoides, saponins, alkaloids, phytosterols, cellulose, hemicellulose and lignin. (Loprz Lazaro *et al.*, 1997; Martin-Cordero *et al.*, 1998; Moubasher, 1998; Kassem *et al.*, 2000; Cheriti

et al., 2004). These molecules justify their various applications in traditional medicine which we quote: antinflammatoire, antitumor, cicatrizant, sédatif and Vomitif activities (Bellakhdar, 1997; Merzouki *et al.*, 2000).

Often, the determination of differences between those three species is difficult because of the similarity of phenotypic characters; they differ only by the color of the banners which are white in *Retama monosperma* and *Retama raetam* and yellow in *Retama sphaerocarpa*.

Over the years, the methods for detecting and assessing genetic diversity have extended from analysis of discrete morphological traits to biochemical and molecular trait. Therefore, the advent of the electrophoresis as an analytical tool provides indirect methods for genome probing by exposing structural variations of enzymes or other protein genome (Cooke, 1984; Gilliland, 1989).

Genetic interpretation of protein polymorphism in those three species has not been well established until now. The aim of the present study was to describe the genetic diversity of three species of *Retama* genus and possibilities of their identification by means of seed protein polymorphism evaluated under SDS-PAGE conditions.

Corresponding Author: Belmokhtar Zoubir, Laboratoire des Productions Valorisations Végétales et Microbiennes (L2PVM).
Department of Biotechnology, Sciences faculty; University of Sciences and Technology, Mohamed Boudiaf (USTOMB), P.O. Box. 1505, El Mnaouar, Oran, 31000, Algeria, Tel.: 00213772434234

Table 1: Regions of the studied populations

Species	<i>R. monosperma</i>	<i>R. raetam</i>	<i>R. sphaerocarpa</i>
Stations	Cape falcon`	Sfessefa	Oued chellala
	Genet	Chellala	Oued benhandjir
	Kristel	Kheiter	Djelfa
	Mactaâ	Djelfa	
		Naâma	

MATERIALS AND METHODS

Plant materials: Seeds of 12 populations from the three species used were collected from its natural habitat from diverse regions of Algeria. 4 populations of *Retama monosperma*, 5 populations of *Retama raetam* and 3 populations of *Retama sphaerocarpa*. Table 1 lists the regions about the 12 populations used in this study.

The morphological and phenological observations of the three species under the natural conditions do not show difference at the vegetative stage. The distinctions relate to the stages of flowering and fructification (Table 2).

Electrophoresis of proteins: The extraction of total proteins has been realized according to the method of Payene and Corfield (1979). 400 µL of the solution of extraction (Tris-HCl: 0.125 M. pH: 6, 8. SDS: 4%, glycerol: 20%, β mercaptoéthanol: 10% and Bromophenol blue: 0.1% as tracking dye to watch the movement of protein in the gel) The homogenate samples were incubated at 60°C during 2 h to accelerate the denaturation of proteins by the SDS. To purify extraction, the homogenate samples were mixed thoroughly by vortexing and centrifuged at 3600 rpm for 10 min.

The extracted proteins were separated by SDS - PAGE using the method described by Laemmli (1970). The separation gel concentration was 2.8% and the stacking gel concentration was 12.8%. 12 µL of each sample were loaded to each slot. The electrophoresis was performed at room temperature for 4 h at 80 mA constant until the blue marker reached the bottom of the gels. The gels were stained in the staining solution (9% methanol, 2% acetic acid and 0.05% coomassie brilliant R-250) for 4 h. Destaining was done in a solution (9% methanol, 2%

acetic acid) until the background color disappeared, and protein bands were clearly visible. Staining, destaining was conducted on shaker at the speed of 40 r/min.

The molecular weights of dissociated proteins were estimated by using molecular weight marker (14.4, 20.1, 30.0 , 45.0 , 66.0 and 97.0 KDa).

Statistical analysis: The gel was scored as present (1) or absent (0) of each band noted. Presence and absence of bands were entered in a binary data matrix, and pair wise comparisons between individuals were made to calculate the Jaccard's coefficient Similarity Index (JSI) by the formula Sneath and Sokal (1973).

$$S = W/(A+B-W)$$

where 'W' is the number of bands of common mobility, 'A' the number of bands in type 'A' and 'B' is the number of bands in type 'B'. Cluster analysis was performed to produce a dendrogram using unweighted pair group method with arithmetical means (UPGMA).

RESULTS AND DISCUSSION

SDS-electrophoretic was used to find out the phylogenetic relationships among some populations of three species of *Retama* genus: *Retama monosperma*, *Retama raetam* and *Retama sphaerocarpa* Fig. 1 and 2.

Intraspecific diversity: The SDS banding proteins pattern of *Retama monosperma* produced 12 bands distributed in all populations with molecular weights ranging from 18.420 to 100.762 KDa, with polymorphism percentage reached to 16.66%. The similarity coefficient indicate that the different populations of *Retama monosperma* of different stations are similar, only one (Kristel station's) who present two bands in addition with molecular weight of 24.991 and 100, 762 KDa.

Seed storage proteins of *Retama raetam* were resolved 13 detectable polypeptides on SDS-PAGE. Molecular weights ranged from 18.420 to 105.244 KDa, with polymorphism percentage reached to 15.38%.

Table 2: Distinctive parameters of the three species

	<i>R. monosperma</i>	<i>R. raetam</i>	<i>R. sphaerocarpa</i>
Flowers	White color, 14-15mm Banner slightly veined of purple, shorter than the keel. Very scented Flowering from February to May	White color, 8-10 mm. Banner slightly veined of purple, equal keel. Scented. Flowering from April and May	Yellow color, 5 to 6mm. Flowering May.
Seeds	Chestnut-brown or green-olive Colored.5 mm 1 to 4 seeds per legume	Color chestnut-yellow. 3 to 6 mm. 1 to 3 seeds per legume	Black, green-olive colored.4-5mm 1 to 2 seeds per legume
Legumes	Straw- yellow colored, ovoid form with a beak dilated on the ventral side 12 to 15 mm length	Straw-yellow colored, ovoid form with a beak. 8 to 15 mm length	Yellow-brown colored, Reniform7 to 8 mm
Region/climate	Mediterranean coastal. Continental mediterranean	High plateaus. Arid	High plateaus. Arid

Table 3: Similarity coefficient of the twelve *Retama* populations

	R.m1	R.m2	R.m3	R.m4	R.r1	R.r2	R.r3	R.r4	R.r5	R.s1	R.s2	R.s3
R.m1	1											
R.m2	0.83	1										
R.m3	1	0.83	1									
R.m4	1	0.83	1	1								
R.r1	0.69	0.64	0.69	0.69	1							
R.r2	0.76	0.66	0.76	0.76	0.92	1						
R.r3	0.75	0.64	0.75	0.75	0.91	0.84	1					
R.r4	0.83	0.71	0.83	0.83	0.84	0.92	0.91	1				
R.r5	0.69	0.60	0.69	0.69	1	0.92	0.91	0.84	1			
R.s1	0.50	0.42	0.50	0.50	0.53	0.50	0.46	0.42	0.53	1		
R.s2	0.53	0.47	0.53	0.53	0.66	0.62	0.60	0.56	0.60	0.61	1	
R.s3	0.28	0.25	0.28	0.28	0.42	0.40	0.35	0.35	0.42	0.33	0.5	1

R.m: *Retama monosperma*: 1: Cap falcon, 2: Kristel, 3: Genet, 4: Mactaâ; R.r: *Retama raetam*: 1: Sfëssifa, 2: Chellala, 3: El kheiter, 4: Djelfa, 5: Naâma; R.s: *Retama sphaerocarpa*: 1: Oued Benhandjir, 2: Oued Chellala, 3: Djelfa

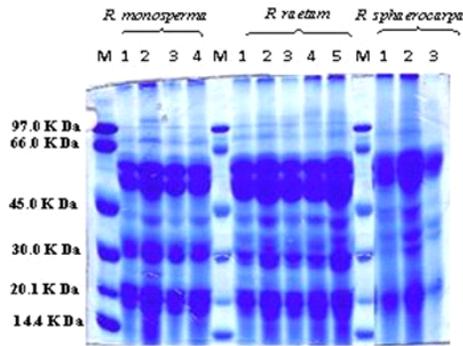


Fig. 1: Electrophorégram identified by SDS PAGE of seeds proteins present in a diferents populations of the three species of *Retama* genus. M: Markers; *R. monosperma*: (1: Cap falcon, 2: Kristel, 3: Genet, 4: Mactaâ); *R. raetam*: (1: Sfëssifa, 2: Chellala, 3: Kheiter, 4: Djelfa, 5: Naâma); *R. Sphaerocarpa*: (1: Oued Benhandjir, 2: Oued Chellala, 3: Djelfa)

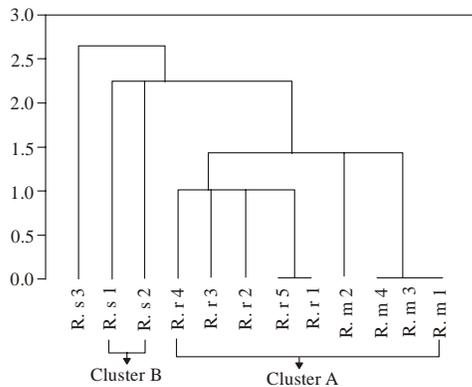


Fig. 2: Clustering analysis according to storage protein bands. R.m: *Retama monosperma*: 1: Cap falcon, 2: Kristel, 3: Genet, 4: Mactaâ; R.r: *Retama raetam*: 1: Sfëssifa, 2: Chellala, 3: kheiter, 4: Djelfa, 5: Naâma; R.s: *Retama sphaerocarpa*: 1: Oued Benhandjir 2: Oued Chellala, 3: Djelfa

Polymorphism was observed in 2 variable bands ranged from 31.761 and 31.074 KDa. The similarity coefficient showed the highest similarity value, it is between (0.92)

and (0.84), and moreover, *Retama raetam* of Sfëssifa and Neama stations are similar (Table. 3).

The SDS banding proteins pattern of *Retama sphaerocarpa* produced 14 bands distributed in all populations with molecular weights ranging from 20.100 to 112.356 KDa. 10 bands are polymorphic with polymorphism percentage reached to 71.42 %. The similarity coefficient ranged from (0.61) to (0.33), showed that the highest similarity value (0.61) was between Oued Benhandjir and oued Chellala populations. While the lowest value of (0.33) was between Oued Benhandjir and Djelfa populations, and between oued Chellala and Djelfa was (0.5). Phenotypical, those populations have different color of seed tegument: black for Djelfa, and green-olive for Oued Benhandjir and oued Chellala.

Interspecific diversity: A total of 19 bands were obtained, distributed in all population of three species with molecular weights ranging from 18.420 KDa to 112.356 KDa. 4 bands are monomorphic with molecular weights of 31.761, 63.767, 92.363 and 82.832 KDa. but the other bands show some variation.

Genetic similarity coefficients grouped the 12 populations into two clusters. Cluster A comprised of 9 lines from all population of *R. monosperma* and *R. raetam*. Phenotypical, the key for the distinction between these two species is only the size of the flowers, 14-15 mm for *R. monosperma* and 8-10 mm for *R. raetam*. Cluster B comprised of 2 lines from *R. sphaerocarpa*.

CONCLUSION

In natural conditions, the species of these two clusters are morphologicly different only on flowering phase which are white at *R. monosperma* and *R.raetam* and yellows at *R. sphaerocarpa*.

Low genetic diversity revealed insignificance of SDS-PAGE for studying intraspecific diversity in *R. monosperma* and *R. raetama*, and suggest a strict autogamous of the population's studied. This approach will set the stage for improving our knowledge of the evolution and taxonomy of the group. Therefore

isoenzyme electrophoresis and 2D-electrophoresis are needed for varietal identification. Further DNA markers are also suggested to investigate intraspecific and interspecific genetic diversity of *Retama* genus.

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