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Molecular polymorphism in dentate lavender from littoral Algerian

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Abstract
Overexploitation of plant species leads to their reduction which requires strategies for improvement and management of resources. This study aims to seek a genetic polymorphism between the different dentate lavender populations collected in the Algerian littoral. Populations of Lavandula dentata were sampled in different ecoregions. The genotypic difference between the six populations was measured by the RAPD-PCR method using 8 RAPD primers and molecular phylogeny was performed by cluster analysis. The RAPD electrophoretic diagrams of the 6 populations of Lavandula dentata showed a rate of 50.49% of polymorphic bands. The dendrogram obtained via cluster analysis allowed us to group the populations into 2 clusters with a similarity index> 68% which showed that they are genetically close despite their geographical distance.
Keywords: Lavandula dentata, RAPD primer, PCR, Cluster. Genetic diversity

Introduction

Overexploitation of plant species can lead to their extinction. The development of strategies for improvement or preservation of plant resources must be carried out by assessing genetic variability through analyzing agronomic traits and at the molecular level such as; isozymes, denatured proteins and restriction fragment length polymorphism (PLFR) thus the use of various mathematical tools to process and explain the collected data(Lefort-buson et al., 1988).A better understanding of genetic diversity and its distribution is essential for its conservation and use. It will help us in determining what to conserve as well as where to conserve and will improve our understanding of the taxonomy and origin and evolution of plant species of interest. Knowledge of both topics is essential for collecting and use of any plant species and its wild relatives (Rao et Toby Hodgkin, 2002).

The estimation of genetic polymorphism and its distribution within populations and species is made possible today by the use of molecular markers, which serve to specify their evolutionary mechanisms. RAPD molecular markers are essential tools for mapping genetic resources and in plant breeding programs. Their principle consists of a hybridization with a DNA sequence which are complementary to them and if there is no complementary sequence, there will be no amplification and a polymorphism of presence and absence of bands will be detected. After visualization of the PCR products on agarose gel (Santoni et al., 2000).

"The Mediterranean region has been long considered as an important source of medicinal plants, and

one of the most interesting families that provides a greater number of species to the catalog of popular herbal medicine for the treatment of inflammatory disorders is Lamiaceae (Algieri et al., 2016)".

The family of Lamiaceae has several species whose lavender is the most popular in the Mediterranean basin. For decades, lavender has been known for its flavor, smell and biological properties. It has been traditionally used as an aromatic, culinary, cosmetic and medicinal plant(Lauren et al., 2016). Algeria has several lavender species known locally as El khouzama or Halhal El Djebl. These species occupy a priori the coastline which has been our collection site of the toothed lavender. The objective of this study is to search for a genetic polymorphism between different populations of Lavandula dentata using RAPD molecular markers to establish a molecular phylogeny of this species.

Materials and methods

Plant material

Six populations of the *Lavandula dentata* species are taken from wild forms from different stations: five in the town of Chlef (Tenes, Beni Houa, Maïnis and El Marsa) and one in the Ain Témouchent (El Amria) during the period from from March to April (Figure 1). *The latter were identified by Dr. Medjahed, a professor in the Department of Biology at the University of Chlef (Algeria).*

Figure 1*: Flowers of the three phenotypes of L. dentata L studied.*

Methods

DNA extraction

DNA was extracted by a commercial extraction kit. Before extraction of the DNA, the working chamber and the scalpel must be cleaned and sterilized with 70 ° alcohol. The leaves were cut into small pieces 0.5 to 0.7 cm in diameter and put in Eppendorf tubes containing 100 µl of the extraction solution. After incubation in a hot block at 95 ° C for 10 min; we added 100 µl of the dilution solution. The DNA is stored at a temperature of 2 to 8 ° C.

Evaluation of the quality and quantity of extracted DNA

The presence and the quality of the DNA obtained were evaluated by electrophoresis on 0.8% TAE agarose gel, stained with ethidium bromide, and observed under UV light and then by measuring the optical density (OD) at 230 ° C. nm, 260 nm, 280 nm.

The purity of the extracted DNA was evaluated by the ratio of the absorbance at 260/280 nm (Sambrook & Russell, 2001) or polyphenols was determined by measuring the UV absorbance ratio of A260 / 230 (Abu-Romman, 2001). The concentration of the extracted DNA was evaluated by measuring the OD at 260.

DNA amplification procedure by PCR / RAPD

We tested 8 different primers (Table 1). The RAPD reactions were carried out in a final volume of 25 µl. Reagents are introduced in order and handled in ice to maintain their storage temperatures. A master mix was prepared for each primer to be divided into 6 labeled Eppendorf tubes: P1; P2; P3; P4; P5; P6: 35 µl 5 X buffer; 17.5 µl MgCl 2; 1.4 µl of each dNTPs; 1.4 µl of Taq, 17.5 µl of primer and 84 µl of water for PCR. In each tube; the reaction mixture (23 µl of the mix + 2 µl DNA: 64.46ng for P1, 58.9ng for P2, 60ng for P3, 52.6ng for P4, 60.73ng for P5 and 65.86ng for P6) is covered with vaseline oil to limit evaporation.

PCR / RAPD amplification program used is: 1) an initial denaturation 95 ° C to 1min, 2) 45 cycles each cycle of which comprises denaturation of the DNA 95 ° C to 1min, hybridization of the primer 29 ° C at 1min, polymerization of the primer 72 ° C at 2min. and 3) the program ends with a final extension of 72 ° C to 7 min. The products of the PCR / RAPD test were verified in the TAE and visualized under UV.

Table 1: List of RAPD primers used and their hybridization T ° C

N°	Primer	Sequence 5'-------3'	hybridization T ° C
1	OPA-01	CAGGCCCTTC	34°C
2	OPA-03	AGTCAGCCAC	32°C
3	OPA-04	AATCGGGCTG	32°C
4	OPA-05	AGGGGTCTTG	32°C
5	OPA-07	GAAACGGGTG	32°C
6	OPA-08	GTGACGTAGG	32°C
7	OPA-10	GTGATCGCAG	32°C
8	OPA-12	TGGGCAGAAG	32°C

Data analysis

For PCR / RAPD gels, the size of the fragments is determined using a DNA ladder marker. The polymorphic bands of each profile are designated by their presence (1) or absence (0). The matrices thus established for the different fragments generated by the different primers are analyzed by Cluster Analysis using the Jaccard similarity index, resulting in the construction of a phylogenetic dendrogram.

Results

Evaluation of the quantity and quality of extracted DNA

The DNA extracted by Kit Extract-N-Amp ™ plant PCR, is of good quality as shown in Figure 3. The extracts made by the kit seem to provide DNA of good quality but also in appreciable quantity (Tableau 2). Indeed, we find that, for the 6 populations of L. dentata, the quantities of DNA obtained are high and vary between 1578 and 1976 ng / µl. The 260/280 ratios are between 1.78 and 2.09 indicating a high level of purity of our DNA except for the P4 and P6 populations where this ratio is below 1.8.

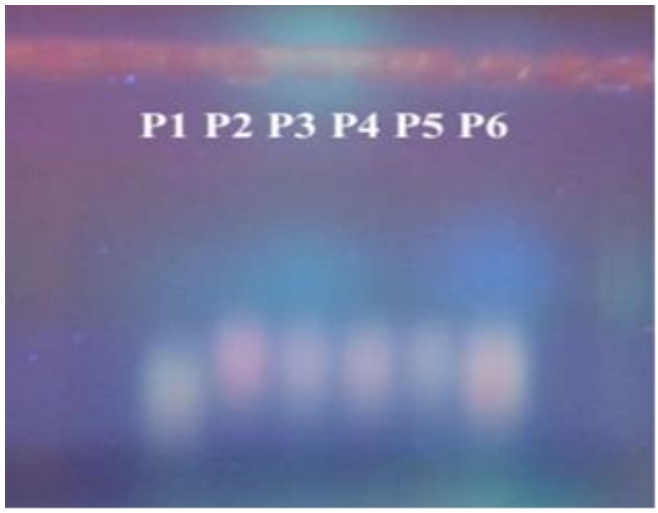

Figure 2: Evaluation of DNA quality on agarose gel for 6 populations of *L dentata*

Table 2: Evaluation of the quality and purity of the DNA extractedfrom leaves of the 6 *L. dentata* populations by a commercial kit.

Population Code	DO 230	DO 260	DO 280	260/280	260/230	Concentration DNA ng/µl
P1	0.519	0,967	0.469	2.06	1.86	1934
P2	0.450	0.884	0.422	2.09	1.96	1768
P3	0.505	0.9	0.495	1.78	1.81	1800
P4	0.887	0,789	0.519	0.90	1.52	1578
P5	0.472	0.911	0.463	1.97	1.93	1822
P6	0.757	0.998	0.696	1.41	1.30	1976

P1: BeniHoua 1 ; P2 : BeniHoua 2; P3: El Marsa ; P4: Maïnis; P5: Ténes ; P6: El Amria.

Analysis of PCR / RAPD products

A set of 8 primers was tested on the DNA samples from the 6 *L. dentata* populations; P1; P2; P3; P4; P5 and P6. The eight primers have been shown to be effective and have legible profiles. Figure 4 illustrates the RAPD genomic profiles for the 6 populations of *L. dentata* generated by the 8 primers. The different primers produce a number of bands ranging from 3 to 10. The size of all the amplified fragments is between 100 and 1500 bp. In total, 53 fragments, including 10 fragments obtained by the OPA-05 primer, were counted, whereas the OPA-01 and OPA12 primer provided only 3 fragments.

Primers that have generated monomorphic bands

The results of the amplification showed that some primers generated fragments of different sizes that were present in all populations tested. The primers that produced these monomorphic bands are three in number: OPA-01, OPA-04 and OP12. The number of generated bands varies from one primer to another.

The OPA-01 primer reacts with all studied genotypes. The size of the generated fragments ranged between 180 bp and 350 bp (180, 270 and 350 bp) with a level of 0%polymorphism.The three monomorphic amplicons obtained are present in the 6 populations which are of an identical bioclimatic stage except for the population of El Amria(P6; Figure 4A).

Primer OPA-04 generated four fragments of different sizes (150, 200, 300 and 400 bp). The four bands are common to the 6 populations tested a null polymorphism (Figure 4B).

OPA-12 Primer: A total of three bands of different sizes (150, 250 and 400 bp) were generated using this primer. They are present in the six tested populations (Figure 4C).

Primers generated polymorphic bands

The results of the amplification show that the majority of tested primers (five out of eight) generated polymorphic bands but also monomorphic bands. It varies according to the populations. The primers are:OPA-03, OPA-05, OPA-07, OPA-08 and OPA-010.

The OPA-03 primer resulted in nine fragments ranging in size from 200 to 1200 bp (200, 250, 300, 380, 450, 580, 700, 900 and 1200 bp) with a polymorphism rate of 88.88% (8 polymorphic bands).

The results showed that some populations have bands in common. This is the case of populations P2, P3 and P4 that share 7 identical bands or populations P5 and P6. With certain population like the P1, it is provided with a number of intermediate bands but also of a specific band of 1200 bp (Figure 4D).

A total of 10 bands were generated by primer OPA-05 and their sizes ranging from 210 to 1250 bp (210, 350, 390, 430, 500, 600, 700, 800, 900 and 1250 bp). With this primer the percentage of polymorphism has reached 33%. The results showed that some populations like P2, P3 and P4 have identical bands (figure 4E).

The rate of polymorphic bands recorded with primer OPA-07 reached 66.66%. The sizes of the six bands generated are: 150, 300, 380, 480, 700 and 850 bp. It has been noticed that three bands are common to all populations and a specific band for P1(Figure 4F)**.**

The amplification products resulting from primerOPA-08 showed a 77.77% polymorphism with specific bands for populations P2 and P4 whose sizes are1500, 850 and 750 bp, respectively with two common bands (290 and 390) for the 6 populations (Figure 4G).

The OP10 primer generated 6 bands including 3 polymorphs. We also note that the P2 population was distinguished by the presence of a specific band of 850 bp (Figure 4H).

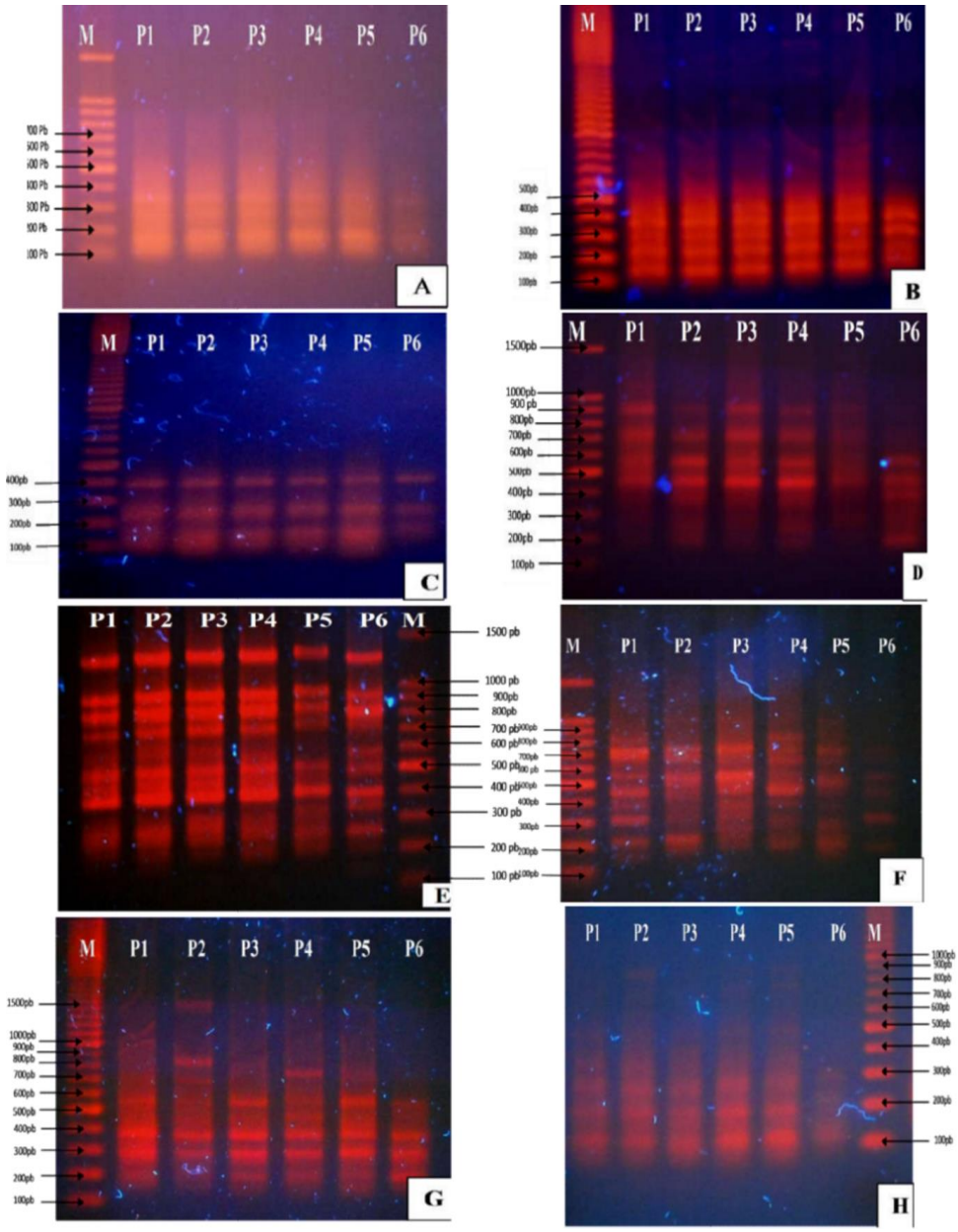

Figure 3: RAPD electrophoretic diagrams of the 6 populations of *L. dentata* generated by 8 primers.
BeniHoua 1; P2 :BeniHoua 2; P3: El Marsa; P4: Maïnis; P5: Ténes; P6: El Amria and M: Molecular marker.

In addition, the P2 population has the highest polymorphism (39.02%) with a total of 16 polymorphic bands, of which two are specific (850 bp with primer OPA-08 and 1500 bp with OPA-10). P1, P3 and P4 populations revealed polymorphism levels of 35 to 36.58%.As for the populations P5 and P6; they recorded lower polymorphism rates (25.71 and 25.77%, respectively) knowing that these two populations in addition to their different phenotypes, they are geographically distant. Recall also that the polymorphism rate generated by the 8 primers is 50.94%, which leads us to say that the intraspecific variability in *L. dentata* species is moderate (Table 3).

Table 3: Population Polymorphism of the species *L. dentata* .

	Nt de B	Nt de B mono	Nt B poly	% poly	% mono
P1	41	26	15	36,58	63,41
P2	41	25	16	39,02	60,97
P3	40	26	14	35	65
P4	44	28	18	36,36	63,64
P5	35	26	9	25.71	74.28
P6	35	26	9	25.77	74.28
8 OPA	53	26	27	49.06	50.94

P1:BeniHoua 1, P2 : BeniHoua 2,P3: El Marsa, P4: Maïnis, P5: Ténes, P6: El Amria

Molecular phylogeny

The dendrogram conducted for the 6 populations studied via cluster analysis allowed us to visualize the relative position of each population within the species in the 6 ecoregions studied (Figure 5). The hierarchical classification of the RAPD molecular markers makes it possible to distinguish two large groups with a similarity index higher than 68% (Table 4).

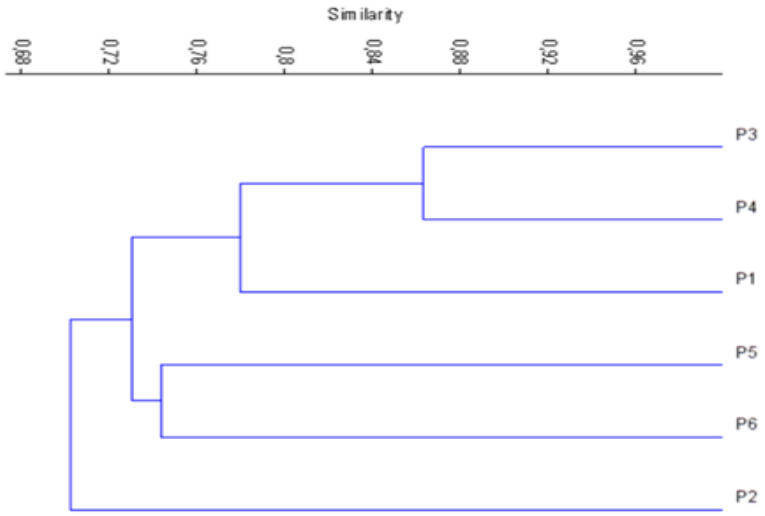

Figure 4: Dendrogram of RAPD markers based on the Jaccard similarity index of the 6 populations of*L. dentata*. P1: Benihoua, P2: Benihoua, P3: El Marsa, P4: Maïnis, P5: Ténes and P6: El Amria.

Table 4: Similarity matrix of 6 *L. dentata* populations.

	P1	**P2**	**P3**	**P4**	**P5**	**P6**
P1	1	0,70213	0,75556	0,80435	0,7619	0,68182
P2	0,70213	1	0,71739	0,76596	0,64444	0,68182
P3	0,75556	0,71739	1	0,86364	0,69767	0,78049
P4	0,80435	0,76596	0,86364	1	0,71111	0,75
P5	0,7619	0,64444	0,69767	0,71111	1	0,74359
P6	0,68182	0,68182	0,78049	0,75	0,74359	1

Jaccard's index

Each cluster groups genetically close populations. The first cluster is formed by the population of BenihouaP2 (intermediate green foliage) while the second cluster comprises two subgroups; one is constituted by the populations Ténes and El Amria with a similarity index of 75% whereas the other subgroup is represented by the populations Maïnis, El marsa and Beni Houa (dark green foliage) with a similarity index of 78%.

Discussion

Molecular characterization of Lavender populations using RAPD markers to assess genetic diversity revealed an important polymorphism indicative of an exploitable gene pool for the enhancement of these resources (Ghariani et al. 2004).

The polymorphism generated by the RAPD markers in our study may be due to the mutations that are the main ones responsible for the existence of the polymorphism. Indeed, the environment can alter the expression and function of the gene of a character through spontaneous mutations. It can produce a wide range of phenotypes are referred to as genotype–environment interactions (Baye et al. 2011).

According to Renny et al. (2017), the evolutionary history of species as well as their geographical distributions and environmental conditions may explain their genetic diversity. "Understanding the natural history of model organisms is important for the effective use of their genomic resources (Ansell et al.2010)".

Mable and Adam, (2007), report that there is no significant relationship between genetic distance and range of populations of Arabidopsis lyrata to multiple breeding systems using microsatellite markers

Welsh & Mohamed, (2011), have deduced that there is a correlation between geographic and genetic distance because genetic differences between Striga hermonthica populations in Ethiopia have been attributed to their range, which was the main determinant of the structure of the population.

"The low levels of genetic diversity within populations and the relatively high levels of genetic diversity among populations suggest that strong moist habitat preferences, clonal reproduction, low level of gene flow among populations, genetic drift, and historical events may have played roles in the genetic structuring of the species (Chung, 1995)".

On the other hand, according to Chevallier &Borgel, 1998, the reproductive system plays a determining role in genetic diversity. Many species that are allogamous, leading to high intrapopulation variability. Indeed, Level of genetic variation differ significantly among species with different geographic ranges, life forms, taxonomic affinities, seed dispersal modes, breeding systems and patterns of environmental heterogeneity (Loveless,1992).

Conclusion

The conservation of spontaneous plant species in situ and ex situ following the human, animal and even climatic destruction must pass essentially by a program of study of their genetic diversity in order to select the most performing populations. The RAPD markers enabled us to detect a low genetic diversity with a high similarity, hence the need to test other molecular markers namely ISSR and RFLP by collecting bioclimatic and ecopedological data in the natural sites of these populations.

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