RFLP ANALYSIS OF cpDNA OF *SALVIA OFFICINALIS*. L OF NORTHERN ALBANIA CAN SERVE TO ELUCIDATE GENETIC DIVERSITY AMONG CLOSE NATURAL POPULATIONS

Stela PAPA[1*]& Ariola BACU[2]
[1,2] Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania, Blv "Zogu I".
TIRANA, ALBANIA

ABSTRACT

In higher plants in the plastid takes place the non-mevalonic acid pathway, which synthesizes among others the monoterpenoids, with specific roles in plant defense against biotic and abiotic stresses, or treated as signal molecules to attract the insects of pollination. Restriction analysis of cpDNA fragments amplified with universal primers has been used in a variety of plant species identification, genetic diversity and phylogenetic studies. In present study the genetic diversity of 43 genotypes of *Salvia officinalis* of Northern Albania were analyzed based on a methodology, which used the RFLP-PCR on the inter-genic region trnL-F of the cpDNA (Walker *et al*., 2004). PCR products were separately digested with two restriction enzymes (AluI and TaqI), which were used to cut the amplicons, and NTSYS software to build dendrograms of similarity among genotypes based on binary matrices (presence/non-presence of restriction bands). Results show that 43 genotypes could be grouped in 6 main clusters, and that genotypes of Kruja and Torovec are quite distinctive from the rest. The most unique genotypes will be used to further study possible diversity of monoterpene synthase coding genes, which are located in the cpDNA.

Keywords: cpDNA, monoterpene synthases, RFLP-PCR.

INTRODUCTION

Salvia L., composed of annual herbs evergreen or deciduous shrubs (Clebsch, 2003), is the largest genus in the *Lamiaceae* family with approximately 1000 species. It is mainly distributed in Central and South America (500 spp.); Central Asia/Mediterranean (250 spp.); and East Asia (90 spp.; Walker *et al*., 2004). Usually *Salvia* species produce various types of secondary metabolites, many of which have been subsequently exploited by humans for their beneficial roles in a diverse array of biological functions (Balandrin *et al*., 1985). The terpenoids, which constitute the largest class of natural products are extensively applied in the industrial sector as flavors, fragrances, spices and are also used in perfumery and cosmetics. Many terpenoids have biological activities (in plant defense against biotic and abiotic stresses, or they are treated as signal molecules to attract the insects of pollination (Bharat Singh and Ram A.Sharma, 2015), and are also used for medical purposes.

This huge class of secondary metabolites in higher plants are produced through the conventional acetate-mevalonic acid pathway which, operates mainly in the cytosol and mitochondria and synthesizes sterols, sesquiterpenes and ubiquinones mainly. In the plastid, the non-mevalonic acid pathway takes place and synthesizes hemi-, mono-, sesqui-, and diterpenes along with carotenoids and phytol tail of chlorophyll (Bharat Singh and Ram A.Sharma, 2015).

On the basis of the phylogeny, the gymnosperm terpene synthases have been subdivided into three distinct clades—TPS-d1 to TPS-d3. The TPS-d1 subclade are (−)-α/β-pinene synthases, (−)-linalool synthases and (*E*)-α-farnesene synthases; in TPS-d2 clade are longifoline synthase and in TPS-d3 clade are levopimaradiene/abietadiene synthases and isopimaradiene synthase (Martin *et al*., 2004). Many terpene synthase genes (*TPSd*) of terpenoid metabolism, appear to be expressed as single copy genes (Bohlmann *et al.*, 1999). These primary metabolism terpene synthase genes are basal to the specialized metabolism genes and are the descendants of an ancestral plant diterpene synthase similar to the non-vascular plant as *Physcomitrellapatens* (Hayashi *et al*., 2006; Keeling *et al*., 2010, 2011; Bharat Singh and Ram A.Sharma, 2015).

The monoterpenes are formed in plastids and the nucleus-encoded monoterpene synthases are targeted by N-terminal transit peptides of approximately 40–70 amino acids which reside upstream of the conserved RRx8W motif, and are cleaved during import from the nucleus (Williams *et al*., 1998; Turner *et al*., 1999; Bharat Singh and Ram A.Sharma, 2015). Chloroplast genomes as well as other organelle genomes evolve slower than nuclear genomes (Palmer, 1987).

This conservation phenomenon of organelle was exploited to design universal primers that were capable of amplifying polymorphic non-coding regions of cpDNA of some algae, bryophytes, pteridophytes, gymnosperms and angiosperms (Taberlet *et al*., 1991; Ibrahim *et al*., 2012) non-coding regions of mitochondrial and cpDNA (Demesure *et al*., 1995), coding regions of cpDNA (Badenes and Parfit, 1995; Tsumura *et al*., 1996) as well as whole chloroplast genomes (Dhingra and Folta 2005; Ibrahim *et al*., 2007; Rashid *et al*., 2012). Currently restriction analysis of fragments amplified with universal primers has been used in a variety of species identification, genetic diversity and phylogenetic studies in different plant species (Gielly and Taberlet 1994; Badenes and Parfitt *et al*. 1995; Demesure *et al.*, 1996; Tsumura *et al.*, 1996; Parducci and Szmidt 1999; Huang and Sun 2000; Parani *et al*., 2000, 2001; Wang *et al*., 2000; Xu *et al*., 2001). In the past few years, PCR amplication of particular cpDNA and mtDNA regions followed by subsequent restriction analyses (PCR-RFLP) was also widely used to analyze the intra-specific organelle genome variation in some species (Boscherini *et al.*, 1994; Dumolin *et al*., 1995; Vicario *et al*., 1995; Demesure *et al.*,1996; Parducci and Szmidt, 1999; Parani *et al*., 2000, 2001; Xu *et al*., 2001).

Walker *et al*., 2004 used the inter-genic region trnL-F and rbcL gene sequences of the cpDNA to study Salvia and related genera, and Ibrahim *et al*., 2012; Ibrahim *et al*., 2006 explored a 15kbp region of the cpDNA of *Salvia*. The nuclear ribosomal internal transcribed spacer region and three chloroplast regions (rbcL, matK and trnH-psbA) were used from Li *et al*., (2013) to describe the phylogenetic relationships among Salvia (*Lamiaceae*) in China. Possible monophylety of *Salvia* genus and its relations with other members of tribe *Menthae* were studied by Walker *et al*., 2004, who used cpDNA regions rbcl and trnL-F. Man Zhang *et al*., (2013), in a study that confirms the ecological role of terpenoids and provides new insights into their metabolic engineering in transgenic plants, characterized a novel nerol synthase gene located to chloroplasts at soybean.

Populations of *Salvia officinalis* L. of Albania have been exploited for a long time for trade purposes mainly, and lately have been object of study of their genetic diversity (Bacu *et al*., 2005; Bacu *et al*., 2011; etc). Present work considers the populations of Northern Albania, which display a lower morphological diversity, thus the best way to analyze their genetic diversity, remains the use of molecular tools. In order to define the most unique populations

regarding the total genomic DNA, we used the RFLP-PCR on cpDNA templates. The results will be used to continue the work on the identification of novel monoterpene coding genes from the chosen genotypes.

METHODOLOGY

Plant material: Fresh young leaves from 43 genotypes of *Salvia officinalis* of Northern Albania were used to extract total genomic DNA. The complete list of genotypes is described at Table 1.

Isolation of genomic DNA: Equal amounts (0.1 g) of leaf tissue were placed in a mortar chilled with liquid nitrogen and were ground to fine powder. Total genomic DNA was extracted as described by Doyle and Doyle (1987). Quality and quantity of DNA was measured according to Sambrook *et al*., 1989.

Table 1. Sage genotypes of Northern Albania analyzed by PCR-RFLP

Nr (acc to restriction with Alu1)	Population	Nr (acc to restriction with Taq1)	Source
1	Postriba 1	1	Lohe 1
2	Postriba 2	2	Lohe 2
3	Postriba 3	3	Lohe 3
4	Postriba 4	4	Lohe 4
5	Postriba 5	5	Lohe 5
6	Rubik(A) 1	6	Balldren 1
7	Rubik (A) 2	7	Balldren 2
8	Rubik (A)3	8	Balldren 3
9	Rubik (A) 4	9	Balldren 4
10	Rubik (A) 5	10	Balldren 5
11	F. Milot 1	11	Rubik(B) 1
12	F. Milot 2	12	Rubik(B) 2
13	F. Milot 3	13	Rubik(B) 3
14	F. Milot 4	14	Rubik(B) 4
15	F. Milot 5	15	Rubik(B) 5
16	Lohe 1	16	Kruje 1
17	Lohe 2	17	Kruje 2
18	Lohe 3	18	Kruje 3
19	Lohe 4	19	Kruje 4
20	Lohe 5	20	Kruje 6
21	Balldren 1	21	Kruje 8
22	Balldren 2	22	Shkoder 4
23	Balldren 3	23	Shkoder 5
24	Balldren 4	24	F.Milot 5
25	Balldren 5	25	Rubik(A) 1
26	Rubik (B) 1	26	Rubik(A) 2
27	Rubik (B) 2	27	Rubik(A) 3
28	Rubik (B) 3	28	Rubik(A) 4
29	Rubik (B) 4	29	Rubik(A) 5
30	Rubik (B) 5	30	F.Milot 1

31	Kruje 1	31	F.Milot 2
32	Kruje 2	32	F. Milot 3
33	Kruje 3	33	F. Milot 4
34	Kruje 4	34	Torovec 1
35	Kruje 6	35	Torovec 2
36	Kruje 8	36	Torovec 3
37	Shkoder 4	37	Torovec 4
38	Shkoder 5	38	Torovec 5
39	Torovec 1	39	Postriba 1
40	Torovec 2	40	Postriba 2
41	Torovec 3	41	Postriba 3
42	Torovec 4	42	Postriba 4
43	Torovec 5	43	Postriba 5

Isolation of genomic DNA: Equal amounts (0.1 g) of leaf tissue were placed in a mortar chilled with liquid nitrogen and were ground to fine powder. Total genomic DNA was extracted as described by Doyle and Doyle (1987). Quality and quantity of DNA was measured according to Sambrook *et al*., 1989.

PCR amplification: The set of primers trnL-trnF was used to amplify the specific inter-genic region from the cpDNA of 43 individuals of *Salvia officinalis* (Table 2).
The PCR amplifications were carried out in Veriti 96-Well Thermal Cycles (Applied Biosystem) in a total volume of 25 µl containing Master mix (Cinnagen) and 30 ng genomic DNA, according to Ibrahim *et al*. 2012. The PCR program started with an initial phase of 3 minute at 94^{o}C, followed by 35 cycles of 30 s at 94^{o}C, 30 s at 54^{o}C, 1 min at 72^{o}C and 10 min final elongation at 72^{o}C (Wu Wei 2005).
The success of each PCR reaction was verified by electrophoresis of the reaction products on 1% agarose gels in 10X TBE buffer, stained with ethidium bromide (0.5µg/ml) and visualized under UV light.

Table 2. DNA sequence of the primer pairs used to amplify the specific inter-genic region from the cpDNA.

Primer pair	Sequence	Type	References
trnL-trnF	5'-CGAAATCGGTAGACGCTACG-3' 5'-ATTTGAACTGGTGACACGAG-3'	cpDNA	Taberlet *et al*., 1991

DNA digestion. After amplification, 15 µl of each PCR-cpDNA were separately digested (7 µl for each enzyme), with two restriction enzymes Alu1 and Taq1 (Promega). Total volume of the reaction was 25µl each tube containing 2.5µl buffer, 0.2µl from each enzyme (Alu1; Taq1) and 15.3 µl ddH_20 based on the manufacturer's protocol for restriction enzymes (Promega). The samples were incubated in 37^{0}C for 15 minutes and after that in 65^{0}C for 20 minutes. After that digestion products were separated with 1kb DNA marker by electrophoresis, in 2.5% agarose gel stained with Ethidium bromide in 10XTBE buffer for 2 hours in a voltage of 50V. Gels were visualized and photographed under UV-illuminator.

Data analysis.

The digested DNA fragments were scored by presence (1) or absence (0) for each genotype. The binary matrix was used to build the dendrograms of similarity among the genotypes

using the unweighted pair-group method with arithmetic average (UPGMA) cluster analysis of NTSYS 2.1 software.

RESULTS AND DISCUSSION

The set of primers trnF-trnL used in the present study successfully amplified the corresponding cpDNA regions in all *Salvia officinalis* genotypes analyzed (Figure1).

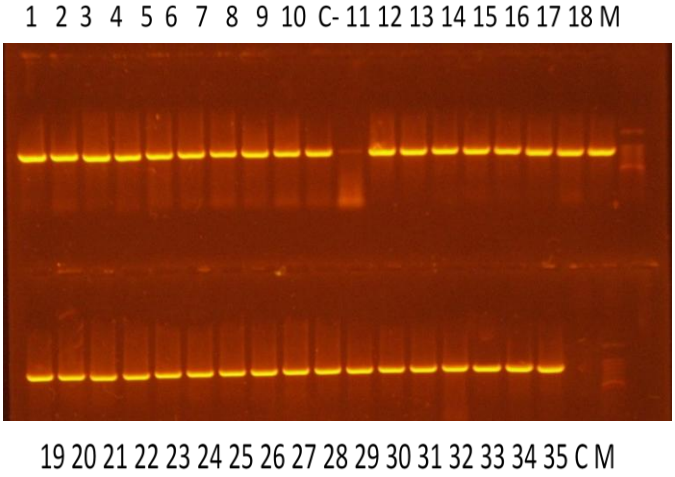


Figure 1. Amplified products of primer pairs trnL-trnF of genomic DNA from 35 out of 43 *Salvia* genotypes of Northern Albania (1-35 indicate the number described at Table 1/column 2 from left to right).

Restriction analysis of PCR products

Amplicons were digested in single reactions with restriction anzymes Taq1 and AluI. The first produced four major bands present at all genotypes, and two minor polymorphic bands (Figure 3A/3B).

(A)

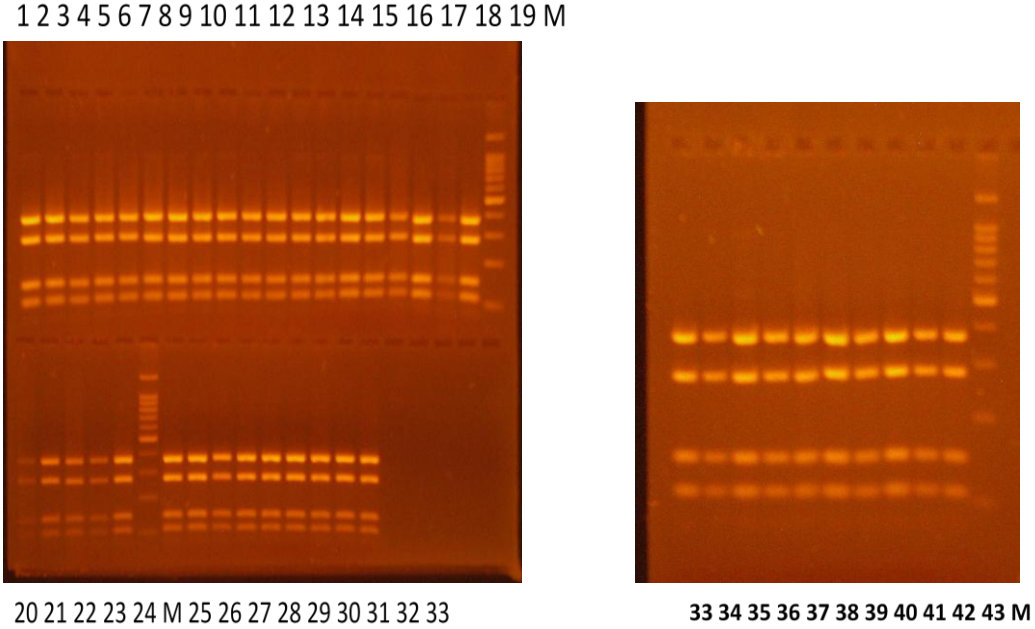


Figure 3. (A) Amplified and digested products of primer/enzyme combination trnL-trnF/ TaqI of genomic DNA from 43 Salvia accessions. 1-43 indicate the number in Table 1, M indicates GeneRulerTM 100bp DNA Ladder marker.

The second restriction enzyme produced four major bands, and three minor polymorphic bands (Figure 4).

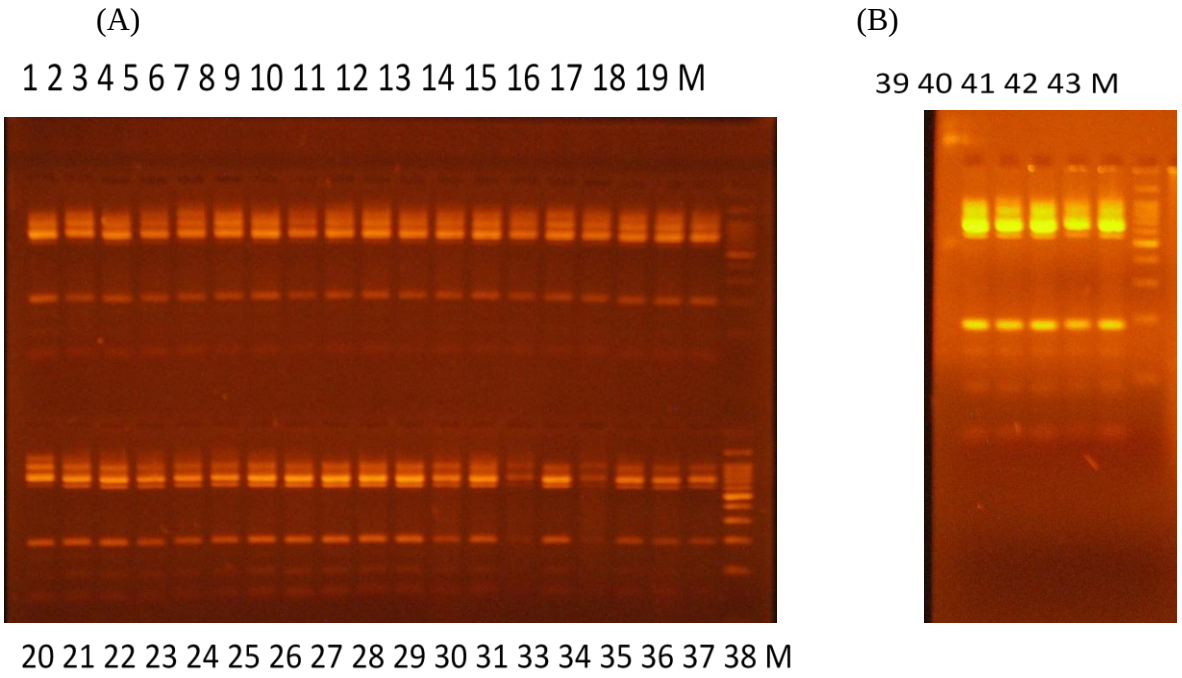
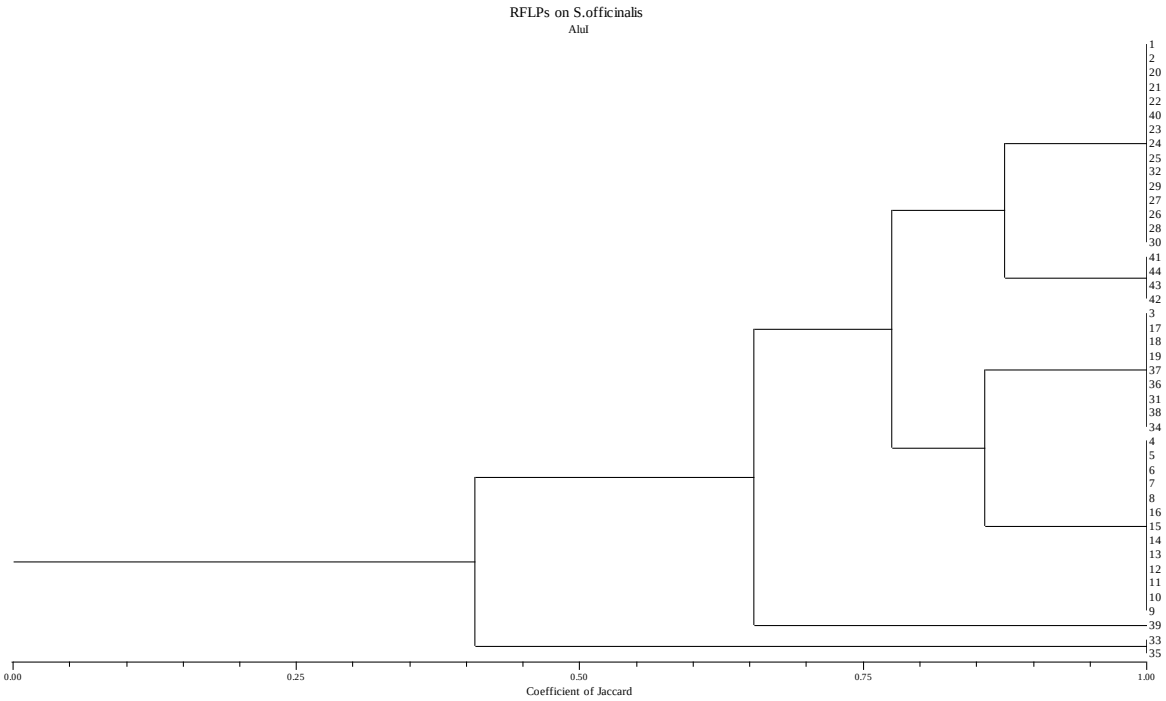


Figure 4. (B) Amplified and digested products of primer/enzyme combination trnL-trnF/ AluI of genomic DNA from 43 Salvia accessions. 1-43 indicate the number in Table 1, M indicates GeneRulerTM 100bp DNA Ladder marker.

The analysis of the RFLP-PCR data based on UPGMA cluster analysis produced the dendrograms of similarity displayed at Figures 5 and 6. Figure 5 makes clear than the AluI restriction of the amplicon from cpDNA can divide 43 Salvia genotypes into two main clusters which share 40% similarity, and distinguish genotypes 35 (Kruja6) and 33(Kruja3) of Kruja as quite distinct from the rest;



(A)

The second cluster is subdivided into two branches (which share 65% similarity) from which one with a single genotype (no 39 of Torovec), and the other subdivided in four clusters of genotypes. Considering the above the total number of genotypes is six out of 43.

(B)

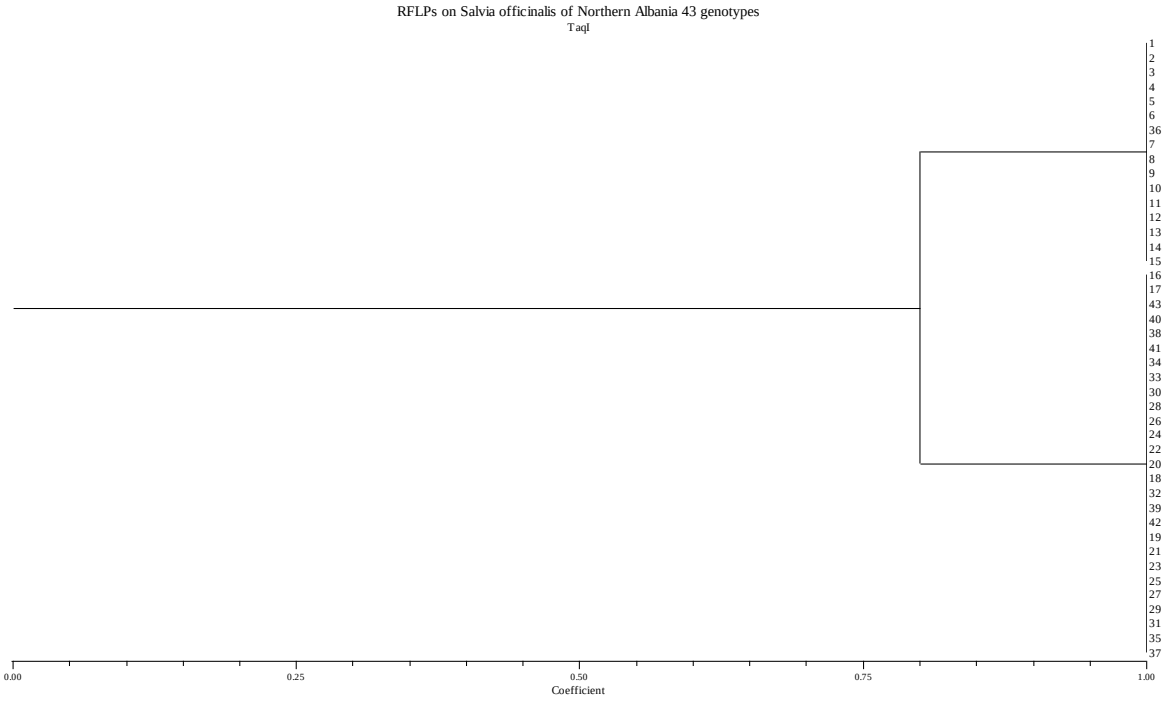


Figure 5 (A) Dendrogram of 43 Salvia accessions constructed from PCR-RFLP (restriction enzyme Alu1) marker-based genetic similarity. (B) Dendrogram of 43 Salvia accessions constructed from PCR-RFLP (restriction enzyme Taq1) marker-based genetic similarity.

Figure 5B groups the 43 genotypes in two clusters only, which share 80% similarity, being this way much more less informative than RFLP profiles produced by AluI (figure 5 A). Since the aim of the work was the use of cpDNA based PCR-RFLP profiles to discover possible genetic diversity among the main populations of sage of Northen Albania, the use of AluI profiles resulted much more informative than of TaqI. This result should be considered preliminary, and should be continued by implying other restriction enzymes.

CONCLUSIONS

- *Salvia officinalis* populations of Northern Albania display a low morphological diversity, thus the best way to analyze their genetic diversity, is the use of molecular tools.
- In order to define the most unique populations regarding the total genomic DNA, we used the RFLP-PCR on cpDNA templates.
- The set of primers trnF-trnL (Taberlet *et al*., 1991) amplified the target cpDNA region in all *Salvia officinalis* genotypes analyzed.
- The analysis of the RFLP-PCR data (AluI and TaqI), based on UPGMA cluster analysis produced dendrograms of similarity, which distinguished six main genotypes out of 43 analyzed.
- Since monoterpene coding genes are located in cpDNA, the results of this work will be used to continue the research on the identification of possible novel monoterpene coding genes from the chosen genotypes.

REFERENCES

1. Bacu A., Babani F., (2005). Molecular characterization of Salvia officinalis and Salvia triloba grown in Albania. AJNTS (Albanian Journal of Natural and Technical Sciences), 2005.Pg 65-71. ISSN: 2074-0867.
2. Bacu A., Loeser C., Marko O., Appenroth K., (2011). Amplified length polymorphisms (AFLP) group populations of Salvia officinalis of Albania in accordance to their geographical locations. International Journal of Ecosystem and Environment Research (IJEES). ISBN: 978-9928-4068-0-4. Vol I, Issue 1. 2011. Pg.172-176.
3. Badenes M. L. and Parfitt D. E., (1995). Phylogenetic relationships of the cultivated *Prunus* species from an analysis of chloroplast DNA variation. *Theor. Appl. Genet.* 90: 1035–1041.
4. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH (1985) Natural plant chemicals: sources of industrial and medicinal materials. Science 228:1154–1160
5. Bharat Singh • Ram A. Sharma (2015) Plant terpenes: defense responses, phylogenetic analysis, regulation and clinical applications. 3 Biotech 5:129–151 DOI 10.1007/s13205-014-0220-2.
6. Bohlmann J, Phillips M, Ramachandiran V, Katoh S, Croteau R (1999) cDNA cloning, characterization and functional expression of four new monoterpene synthase members of the Tpsd gene family from grand fir (Abies grandis). Arch Biochem Biophys 368:232–243
7. Boscherini G., Morgante M., Rossi P. *et al.*, (1994). Allozyme and chloroplast DNA variation in Italian and Greek populations of *Pinus leucodermis*. *Heredity* 73: 284–290.
8. Claßen-Bockhoff, R., P. WesteR, and E. Tweraser. 2003. The stamina lever arm mechanism in *Salvia*—a review. *Plant Biology* 5: 33–41.

9. Clebsch, B. (1997). A book of salvias. Timber Press, Portland, Oregon, USA
10. Clebsch, B. (2003). The new book of Salvias, sages for every garden. Timber Press, Portland, Oregon, U.S.A.
11. Demesure, B., Sodzi, N. and Petit, R. J. (1995). A set of universal primers for amplication of polymorphic noncoding regions of mitochondrial and chloroplast DNA in plants. _/ Mol. Ecol. 4: 129_/131.
12. Dhingdra, A. and Folta, M.K. (2005). ASAP: Amplification, sequencing and annotation of plastomes. BMC Genomics 6: 176 doi: 10.1186/1471-2164-6-176.
13. Doyle, J.J., and J.L. Doyle, (1987): A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. v. 9: 11-15.
14. Dumolin S., Demesure B. and Petit R. J., (1995). Inheritance of chloroplast and mitochondrial genomes in pedunculate oak investigated with an efficient PCR method. *Theor. Appl. Genet.* 91: 1253–1256.
15. Gielly L. and Taberlet P., (1994). The use of chloroplast DNA to resolve plant phylogenies: noncoding versus rbcL sequence. *Mol. Biol. Evol.* 11: 769–777.
16. Hayashi K, Kawaide H, Notomi M, Sakigi Y, Matsuo A, Nozaki H (2006) Identification and functional analysis of bifunctional entkaurene synthase from the moss Physcomitrella patens. FEBS Lett 580:6175–6181
17. Huang, J. C. and Sun, M. (2000). Genetic diversity and relationships of sweetpotato and its wild relatives in Ipomoea series Batatas (Convolvulaceae) as revealed by intersimple sequence repeat (ISSR) and restriction analysis of chloroplast DNA. _/ Theor. Appl. Genet. 100:1050_/1060.
18. Ibrahim, R.I.H.; Azuma, J.I., and Sakamoto, M. (2006). Complete nucteotide sequence of the cotton (*Gossypium barbadense* L.) chloroplast genome with a comparative analysis of sequence among 9 dicot plants. Gen. Genet. Syst. 81:311-321
19. Ibrahim, R.I.H.; Azuma , J.I., and Sakamoto, M. (2007). PCR_RFLP analysis of the whole chloroplast DNA from three cultivated species of cotton (*Gossypium* L.). Euphytica 156: 47-56
20. Ibrahim, R.I.H., Sakamoto, M., and Azuma J.I. (2012). PCR-RFLP and genetic diversity analysis of cp-DNA in some species of the genus *Salvia* L. Chromosome Botany 7: Pg 1-8
21. Keeling CI, Dullat HK, Ralph SG, Jancsik S, Bohlmann J (2010) Identification and function characterization of monofunctional ent-copalyl diphosphate and ent-kaurene synthases in white spruce (Picea glauca) reveal different patterns for diterpene synthase evolution for primary and secondary metabolism in gymnosperms. Plant Physiol 152:1197–1208
22. Keeling CI, Weisshaar S, Ralph SG, Jancsik S, Hamberger B, Dullat HK, Bohlmann J (2011) Transcriptome mining, functional characterization and phylogeny of a large terpene synthase family in spruce (Picea spp.). BMC Plant Biol 11:43
23. LI Q.Q.; Hui LI M.; Yuan J-Q.; Cui H.Zh.; Huang. Q. L.; Xiao P-G. (2013). Phylogenetic relationships of Salvia (Lamiaceae) in China: Evidence from DNA sequence datasets. Journal of Systematics and Evolution 51 (2): 184–195 (2013) doi: 10.1111/j.1759-6831.2012.00232.x
24. Martin D, Fa¨ldt J, Bohlmann J (2004) Functional characterization of nine Norway spruce terpene synthase genes and evolution of gymnosperm terpene synthases of the TPS-d sub-family. Plant Physiol 135:1908–1927
25. Palmer, J.D. (1987). Chloroplast DNA evolution and biosystematic uses of chloroplast DNA variation. Am.Nat. 130:6-29.

26. Parani, M., Rajesh, K., Lakshmi, M. et al. (2001). Species identification in seven small millet species using polymerase chain reaction restrition fragment length polymorphism of trnS-psbC gene region. _/ Genome 44:495_/499.
27. Parducci, L. and Szmidt, A. E. 1999. PCR-RFLP analysis of cpDNA in the genus Abies. _/ Theor. Appl. Genet. 98: 802_/808.
28. Rohlf, F. J. 1993. NTSYS-pc version 1.80. _/ Exeter Software, Setauket, NY.
29. Sambrook, J., Fritsch, E. F., & Maniatis, T. (2000). *Molecular Cloning: A Laboratory Manual.* 2nd ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, ISBN 0-87969-309-6.
30. Taberlet P., Gielly L., Pautou G. *et al.*, (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.* 17: 1105–1109.
31. Taberlet, P., Fumagalli, L., Wust-Saucy, A. G. et al. 1998. Comparative phylogeography and postglacial colonization routes in Europe. _/ Mol. Ecol. 7: 453_/464.
32. Tsumura, Y., Kawahara, T., Wickneswari, R. et al. 1996. Molecular phylogeny of Dipterocarpaceae in southeast Asia using RFLP of PCR-amplified chloroplast genes. _/ Theor. Appl. Genet. 93: 22_/29.
33. Turner G, Gershenzon J, Neilson EE, Froehlich JE, Croteau R (1999) Limonene synthase, the enzyme responsible for monoterpene biosynthesis in peppermint, localized to leucoplasts of oil gland secretory cells. Plant Physiol 120:879–886
34. Walker JB, Elisens WJ. 2001. A revision of Salvia section Heterosphace (Lamiaceae) in Western North America. Sida.19(3):571–589
35. Walker JB, Sytsma KJ, Treutlein J, Winks M. 2004. Salvia(Lamiaceae) is not monophyletic: Implications for the sys-tematic, radiations and ecological specializations of Salvia and tribe Mentheae. Am J Bot. 91(7):1115–1125
36. Wang G. Z., Matsuoka Y. and Tsunewaki K., (2000). Evolutionary features of chondriome divergence in*Triticum*(wheat) and *Aegilops* shown by RFLP analysis of mitochondrial DNAs. *Theor. Appl. Genet.* 100:221–231.
37. Wei W., YOULIANG ZH., LI CH., YUMING W., ZEHONG Y., RUIWU Y. (2005). PCR-RFLP analysis of cpDNA and mtDNA in the genus *Houttuynia* in some areas of China. Hereditas 142: pg: 24_/32
38. Williams DC, McGarvey DJ, Katahira EJ, Croteau R (1998) Truncation of limonene synthase preprotein provides a fully active 'pseudomature' from this monoterpene cyclase and reveals the amino-terminal arginine pair. Biochemistry 37:12213–12220
39. Vicario F., Vendramin G., Rossi P. *et al.*, (1995). Allozyme, chloroplast DNA and RAPD markers for determining genetic relationships between *Abies alba* and the relic population of *Abies nebrodensis*. *Theor. Appl. Genet.* 90:1012–1018.
40. Xu D. H., Abe A., Kanazawa A. *et al.*, (2001). Identification of sequence variations by PCR-RFLP and its application to the evaluation of cpDNA diversity in wild and cultivated soybeans. *Theor. Appl. Genet.* 102:683–688.
41. Zhang M, Liu J, Li K, Yu D (2013). Identification and Characterization of a Novel Monoterpene Synthase from Soybean Restricted to Neryl Diphosphate Precursor. PLoS ONE 8(10): e75972. doi:10.1371/journal.pone.0075972