AcademicPres

Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not. Bot. Hort. Agrobot. Cluj 38 (3) 2010, 302-307

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Chloroplast DNA Diversity of Oak Species in Eastern Romania

Ioan Calin MOLDOVAN[1)], Nicolae SOFLETEA[1)], Alexandru Lucian CURTU[1)], Ioan Vasile ABRUDAN[1)], Dragos POSTOLACHE[2)], Flaviu POPESCU[2)]

[1)] *University of Transilvania, Brasov, Faculty of Silviculture and Forest Engineering, 1 Sirul Beethoven Str., Brasov, Romania; calin.moldovan@unitbv.ro, lucian.curtu@unitbv.ro, nic.sofletea@unitbv.ro, abrudan@unitbv.ro*

[2)] *ICAS, Arboretum Simeria, 1 Biscaria Str., Simeria, Romania; dragospostolache@yahoo.com, flaviu.popescu@icashd.ro*

Abstract

The chloroplast DNA of 34 sessile oak (*Quercus petraea*) and 27 pedunculate oak (*Q. robur*) populations covering the entire natural distribution of the two oak species in Eastern Romania was investigated using four large regions of the chloroplast genome by PCR and RFLP technique. A total of seven chloroplast DNA haplotypes *sensu lato* have been observed by analysing 305 mature trees. However, due to the high resolution of the electrophoresis method a total of 22 chloroplast variants could have been detected, with new mutations and fragment combinations in two of the amplified regions: psbC/trnD and trnT/trnF. All of the haplotypes belong to the phylogenetic lineages A and E, which originate from the Balkan Peninsula. Most of genetic diversity is distributed among populations (G_{ST}=0.779). The chloroplast DNA haplotypes are shared by the two oak species. Different dispersal abilities may explain the higher value of genetic differentiation among populations in sessile oak than in pedunculate oak.

Keywords: chloroplast DNA, genetic diversity, oak, *Quercus robur*, *Q. petraea*, spatial distribution, Eastern Romania

Introduction

The study of chloroplast DNA (cpDNA), which is maternally inherited in most of the angiosperms including oaks, allows the identification of glacial refugia and postglacial recolonisation routes (Dumolin-Lapegue *et al.,* 1997; Mátyás and Sperisen, 2001). Several studies, based on a series of cpDNA markers, reported patterns of geographic variation across the European continent (Petit *et al.,* 2002 a, b). However, only a few studies have analysed the distribution of chloroplast genetic diversity at finer scales within regions (Slade *et al.,* 2007).

The pedunculate oak (*Quercus robur*) and the sessile oak (*Q. petraea*) are among the most important broadleaved forest tree species in Europe. In Romania, pedunculate oak and sessile oak occupy approximately 2%, respectively 10% of the forest area, having high ecological and economic value (Sofletea and Curtu, 2007). The distribution ranges of the two species overlap and stretch in Romania on both sides of the Carpathian Mountains, occupying sites in the hilly and plain region (Sanda *et al.,* 2004; Sofletea and Curtu, 2007). Sessile oak occurs mainly at the top of the hills, with rare inclusions in the lower plains and mountain areas (Florescu and Nicolescu, 1996). In Romania, the studies on cpDNA were focused on a small number of populations (Bordács *et al.,* 2002; Petit *et al.,* 2002 b) or on the distribution of chloroplast DNA variation among five oak species within a single forest (Curtu *et al.,* 2007). A recent study presented new data on cpDNA variation in Central, Western and Southern Romania (Popescu and Postolache, 2009). However, cpDNA diversity studies among oak populations in Eastern Romania are scanty.

The aim of this study was to characterize the chloroplast genetic diversity in Eastern Romania, in Moldova region in particular, by analysing numerous populations of the two oak species, *Q. robur* and *Q. petraea*.

Materials and methods

Plant material

Dormant buds or leaf samples from 35 populations of sessile oak and 26 of pedunculate (Tab. 1) oak were collected. All of the sampled populations are naturally regenerated forests, with an age of over 100 years. Plant material has been collected from 5 trees by taking into account the minimum distance of 100 meters between sampled individuals. The populations were selected to evenly cover the natural distribution of the two taxa in Eastern Romania. The samples were collected in 2007 and after collection, the samples were kept at low temperature (-60°C) in a freezer.

DNA extraction and PCR-RFLP methods

The DNA isolation of the plant material was done using Dneasy Plant Mini Kit (Qiagen, Hilden, Germany) or ZR Plant/Seed DNA Kit (Zymo Research, U.S.A.) following manufacturer specification with small modifica-

tions (Toader *et al.,* 2009). Four regions of the cpDNA, *trnD-trnT* (DT), *psaA-trnS* (AS), *trnC-trnD* (CD), and *trnT-trnF* (TF) were amplified and analyzed to estimate genetic variability of sessile oak and pedunculate oak in Eastern Romania.

The PCR programs were as follows: 4 min denaturation at 94°C followed by 30 cycles of three steps [50 s denaturation at 94°C, 50 s (1 min for AS) annealing at 58°C (for AS and CD and TF) or 56°C (for DT) and 2 min (for DT and TF) or 4 min (for AS and CD) elongation at 72°C]. The final extension was held at 72°C for 10 min. Two amplified DNA fragments (AS and TF) were digested with restriction enzyme *Hinf I* (Demesure *et al.,* 1995; Taberlet *et al.,* 1991) and the next two amplified DNA

Tab. 1. Location of the sampled populations and sample size per species and population

No.	County	Forest Districts	Species	No. of samples	Lat	Long
1/2	Bacau	Bacau	*Q.petrea/Q.robur*	5/5	46.26	26.56
3/4	Bacau	Fântânele	*Q.petrea/Q.robur*	5/5	46.40	26.47
5	Bacau	Livezi	*Q.robur*	5	46.23	26.44
6	Bacau	Moinesti	*Q.petrea*	5	46.32	26.35
7	Bacau	Oituz	*Q.petrea*	5	46.13	26.35
8	Bacau	Sascut	*Q.petrea*	5	46.15	27.08
9	Bacau	Tg. Ocna	*Q.petrea*	5	46.17	26.29
10	Bacau	Traian	*Q.robur*	5	46.39	27.02
11/12	Bacau	Zeletin	*Q.petrea/Q.robur*	5/5	46.26	27.19
13	Botosani	Botosani	*Q.robur*	5	47.49	26.40
14	Botosani	Darabani	*Q.robur*	5	48.04	26.47
15/16	Botosani	Dorohoi	*Q.petrea/Q.robur*	5/5	47.59	26.21
17	Botosani	M. Eminescu	*Q.petrea*	5	47.42	26.34
18	Botosani	Trusesti	*Q.robur*	5	47.43	27.05
19/20	Galati	Grivita	*Q.petrea/Q.robur*	5/5	46.04	27.44
21/22	Iasi	Ciurea	*Q.petrea/Q.robur*	5/5	47.09	27.34
23	Iasi	Dobrovat	*Q.robur*	5	46.58	27.49
24/25	Iasi	Hârlau	*Q.petrea/Q.robur*	5/5	47.25	27.06
26	Iasi	Iasi	*Q.petrea*	5	47.21	27.28
27/28	Iasi	Pascani	*Q.petrea/Q.robur*	5/5	47.16	26.42
29	Iasi	Padureni	*Q.robur*	5	46.58	27.28
30	Iasi	Podu Iloaiei	*Q.petrea*	5	47.11	27.08
31	Iasi	Raducaneni	*Q.petrea*	5	47.00	27.59
32	Neamt	Brates	*Q.petrea*	5	46.46	26.11
33/34	Neamt	Gârcina	*Q.petrea/Q.robur*	5/5	46.59	26.31
35	Neamt	Manastirea Neamț	*Q.petrea*	5	47.15	26.19
36/37	Neamt	Roman	*Q.petrea/Q.robur*	5/5	46.60	26.50
38	Neamt	Vaduri	*Q.petrea*	5	46.57	26.15
39/40	Suceava	Adâncata	*Q.petrea/Q.robur*	5/5	47.44	26.11
41	Suceava	Falticeni	*Q.robur*	5	47.30	26.15
42	Suceava	Gura Humorului	*Q.robur*	5	47.35	25.52
43/44	Suceava	Malini	*Q.petrea/Q.robur*	5/5	47.25	25.56
45	Suceava	Solca	*Q.robur*	5	47.42	25.53
46	Vaslui	Bacesti	*Q.robur*	5	46.49	27.17
47	Vaslui	Bârlad	*Q.petrea*	5	46.23	27.35
48/49	Vaslui	Brodoc	*Q.petrea/Q.robur*	5/5	46.44	27.41
50	Vaslui	Epureni	*Q.petrea*	5	46.17	27.57
51	Vaslui	Husi	*Q.petrea*	5	46.38	28.04
52	Vaslui	Vaslui	*Q.robur*	5	46.33	27.41
53/54	Vrancea	Adjud	*Q.petrea/Q.robur*	5/5	46.06	27.13
55	Vrancea	Dumitresti	*Q.petrea*	5	45.36	26.48
56	Vrancea	Focsani	*Q.petrea*	5	45.45	27.07
57/58	Vrancea	Gugesti	*Q.petrea/Q.robur*	5/5	45.35	27.10
59	Vrancea	Panciu	*Q.petrea*	5	45.57	27.04
60	Vrancea	Soveja	*Q.petrea*	5	46.02	26.41
61	Vrancea	Vidra	*Q.petrea*	5	45.56	26.53

fragments (CD and DT) were digested with *Taq I* enzyme (Demesure *et al.,* 1995). The resulting fragments from digestion were separated on a polyacrylamide gel with a concentration of 8% and with 0.75 mm thickness. The voltage was increased gradually in electrophoresis chamber. Initial voltage was maintained for 40 min at 100 V, then 10 min at 300 V and at 500 V differentiated, depending on the size of fragments resulting from digestion (3h 20 min for AS and CD, 2h and 40 min for DT and 1 h and 40 min for TF). The staining of the gels was done with Syber Gold and the visualization was done with a ultraviolet light system (GelDoc-It, UVP Imaging System). The encoding fragments and determination of the *sensu lato* haplotypes was done in accordance with the European nomenclature (Petit *et al*., 2002 a).

Data analysis

The frequency and the distribution of haplotypes were mapped using ARCWIEW 9.2 software. HAPLODIV and HAPLONST softwares (available at http://www.pierroton.inra.fr/genetics/labo/Software) were used to calculate genetic diversity parameters. Based on haplotypes frequency, the following genetic parameters were calculated: within-population genetic diversity h_S, total genetic diversity h_T, coefficient of genetic differentiation G_{ST}, by ignoring genetic distances between haplotypes, and N_{ST}, taking into account the genetic distances between haplotypes (Pons and Petit, 1996).

Results and discussion

By combining the observed electrophoretic band patterns for each of the four amplified chloroplast DNA region, a total of 22 chloroplast variants were identified in Eastern Romania (Fig. 1). These chloroplast variants can be grouped to seven *sensu lato* European chloroplast DNA haplotypes: 4, 5, 6, 15, 16, 17 and 30 (Petit *et al.,* 2002 b). Most of the 22 chloroplast variants identified in the present study were not previously reported. The source of the large number of polymorphisms identified is due to the identification of a new band, noted 1', and which is located below the band 1, in the CD region of the chloroplast genome. In the TF region a new combination of bands (2020203) was also observed (Tab. 2). The observed *sensu lato* chloroplast haplotypes belong to the phylo-geographical lineages A (haplotypes 4, 5, 6 and 30) and E (haplotypes 15, 16 and 17), having their origin in the Southern Balkans.

Haplotype 4 was identified with a higher frequency in the southern part of Moldova, with its frequency decreasing towards the north. The same haplotype was previously identified in Southern and in Eastern Romania (Bordács *et. al.,* 2002). The most probable origin of the haplotype 4 is in Eastern Balkans, in a glacial refugium along the Bulgarian cost of the Black See (Bordács *et al.,* 2002). Haplotype 5 was identified in Eastern Romania with a high frequency in the northern part of Moldova. It is was also found outside the Carpathian basin and becomes dominant in the western part of Romania (Banat) and in Transylvania (Popescu and Postolache, 2009). The high frequency of haplotype 5 in Northern Moldova may be explained by oak migration from Transylvania to Moldova through mountain passes, when the climate was warmer, or by a route along the Sub-Carpathians. One of the possible postglacial colonisation routes, from Transylvania to Moldova, is Oituz pass, in Eastern Charpathians, because haplotype 5 was identified in the sampled populations from the surrounding areas. This hypothesis is supported by the occurrence of highly elevated sessile oak populations in the Carpathian Mountains (Fekete and Blattny, 1913). Another less likely hypothesis is that haplotype 5 was spread by humans into Moldova.

Haplotype 6 was found only in two oak populations in Northern Moldova, together with the haplotype 5. This haplotypes may originate from a secondary glacial refugium in the Carpathian Basin (Bordács *et al.,* 2002). Oak populations with haplotypes 15 and 16 were localized mainly in the Central part of Moldova. However,

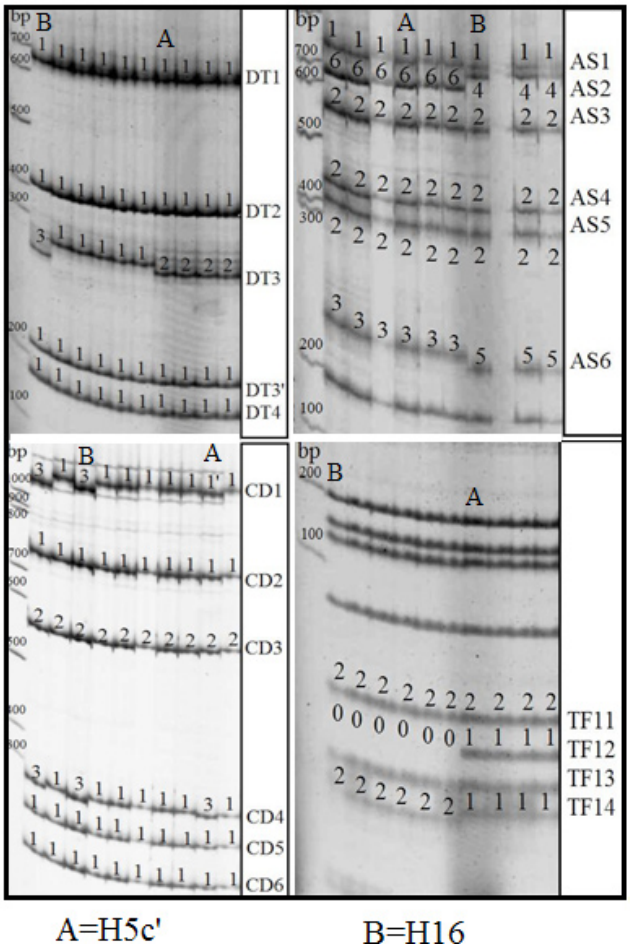

Fig. 1. Band patterns on polyacrylamide gels for each amplified region of the chloroplast genome. H5c'-haplotype 5c', H16-haplotype 16

Tab. 2. Coding of chloroplast DNA haplotypes observed in Eastern Romania

Chloroplast haplotype	Band combination for each haplotype			
	DT	AS	CD	TF
4a	11111	162223	11231	2020212
4a'	11111	162223	1'1231	2020212
4a"	11111	162223	1'1231	2020211
4b	11111	162223	11231	2020202
5a	11211	162223	11231	2020212
5a'	11211	162223	1'1231	2020212
5b	11211	162223	11231	2020213
5b'	11211	162223	1'1231	2020213
5c	11211	162223	11231	2020211
5c'	11211	162223	1'1231	2020211
5d	11211	162223	11231	2020202
6	21211	162223	11131	2020212
6'	21211	162223	1'1131	2020212
15	11311	142225	11211	2020202
15'	11311	142225	1'1211	2020202
16	11311	142225	31231	2020202
16a	11311	142225	31231	2020203
17a	11311	142223	11231	2020212
17a'	11311	142223	1'1231	2020212
17e	11311	142223	11231	2020213
30'	11211	262223	1'1231	2020212
30a	11111	262223	1'1231	2020212

they are also present in Southern Romania (Bordács *et. al.,* 2002). Haplotype 17 has a very low frequency (only 2.3%) is the studied region. For the first time the haplotype 30 was identified in sessile oak populations. Up to now it was observed only in pedunculate oak populations (Popescu and Postolache, 2009).

Tab. 3. Relative frequency of haplotypes by species and geographical regions

Species/ regions	Chloroplast DNA Haplotype frequency							Total
	4	5	6	15	16	17	30	
Q. robur	0.20	0.31	0.06	0.23	0.18	0.01	0.01	1
Q. petraea	0.19	0.32	0.01	0.16	0.26	0.03	0.05	1
Total	0.20	0.31	0.03	0.19	0.22	0.02	0.03	1
Southern Moldova	0.37	0.16	0.02	0.13	0.28	0.01	0.05	1
Northern Moldova	0.01	0.49	0.03	0.26	0.17	0.03	0.01	1

The geographical distribution and relative frequency of chloroplast DNA haplotypes for each population is shown in Fig. 2. Pedunculate oak and sessile oak populations belonging to the same forest district (locality) were merged in the spatial distribution of the haplotypes. The two oak species share all of the 7 haplotypes (Tab. 3). The most common chloroplast haplotype in both taxa is haplotype 5, followed by haplotypes 4, 15 and 16. These haplotypes are also the most common haplotypes in Romania (Popescu and Postolache, 2009). Haplotype 5 has the highest frequency (31%) both in Moldova and in the whole country. This haplotype 5 is dominant in Northern Moldova with a frequency of 49%. In contrast, Southern Moldova is dominated by haplotype 4 with a frequency of 37%. A high frequency in Southern Moldova has also haplotype 16 (28%).

In the eastern part of Romania a clear geographical structure in the spatial distribution of the haplotypes can be distinguished (Fig. 2). Northern Moldova is dominated by haplotype 5 and southern Moldova by haplotype 4. This is supported by higher N_{ST} values compared to the G_{ST} values, corresponding to a phylogeographic distribution. The oak postglacial colonization was not always evenly. It is well known that acorns can be transported over longer distance by various vectors (e.g. birds) (Petit *et. al.,* 1997), allowing the occupation of certain territories only by one or few of the haplotypes.

The presence of a relatively high number of chloroplast haplotypes in a small region and their mixing pattern in Eastern Romania make very difficult the control of seeds transfer based on cpDNA data only. Therefore, it is recommended that acorns from Moldavian seed stands should be used only within the forest districts. In this way, the bastardization of valuable oak populations is avoided.

Although the sessile oak in Eastern Romania is close to the eastern boundary of its distribution range, the same haplotypes observed in pedunculate oak, were found also in sessile oak populations. The genetic differentiation between the populations of the same species was higher in sessile oak (G_{ST}=0.802) than in pedunculate oak (G_{ST}=0.754) (Tab. 4), which is consistent with other studies in Europe (Bordács *et al.,* 2002; Petit *et al.,* 2003). Moreover, the geographic structure is lower in pedunculate oak compared with sessile oak, because the N_{ST} value is higher than the G_{ST} value in pedunculate oak. This structure may be explained by differences in the dissemi-

Tab. 4. Genetic diversity and differentation between species and regions

Species/regions	Number of population	Number of haplotypes	h_S (standard error)	h_T (standard error)	G_{ST} (standard error)	N_{ST} (standard error)
Q. robur	26	7	0.196 (0.0586)	0.800 (0.0252)	0.754 (0.0736)	0.791 (0.0674)
Q. petraea	35	7	0.156 (0.0445)	0.787 (0.0248)	0.802 (0.0561)	0.806 (0.0592)
All populations	61	7	0.174 (0.0357)	0.785 (0.0173)	0.779 (0.0451)	0.797 (0.0444)
Southern Moldova	32	7	0.231 (0.0574)	0.764 (0.0358)	0.697 (0.0713)	0.712 (0.0703)
Northern Moldova	29	7	0.110 (0.0379)	0.682 (0.0546)	0.838 (0.0528)	0.888 (0.0465)

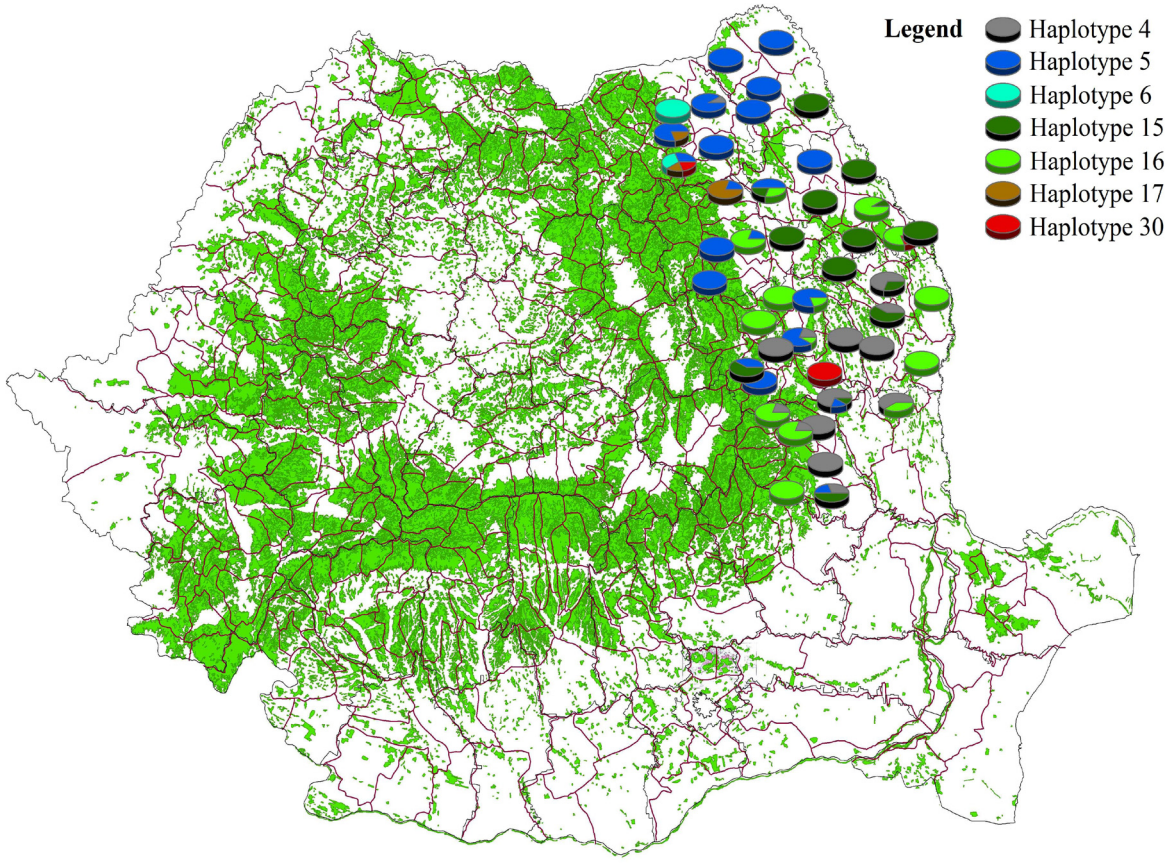

Fig. 2. Distribution of oak chloroplast haplotypes in Eastern Romania

nation of the two oak species. It seems that *Q. robur* acorns can be better dispersed over long distances than the *Q. petraea* acorns (Petit *et al.,* 2003). The total genetic diversity throughout Moldova was higher in pedunculate oak than in sessile oak (Tab. 4). A more extensive seed movement in pedunculate oak may explain this result.

The total genetic diversity was higher for the southern part of the study region (h_T=0.764) in comparison with the northern part (h_T=0.682). The decrease of genetic variation from south toward the north, along the recolonization routes, was reported in other studies (Farcas *et al.,* 2006).

Conclusions

A large number of chloroplast DNA variants have been observed on a relatively small area in Eastern Romania (Moldova). The most common DNA chloroplast haplotypes were haplotypes 5, 16, 4, and 15, which belong to two lineages originating from the Balkans. No species-specific haplotype has been found. Haplotype 5 is very common in Northern Moldova, while haplotypes 4 has a very high frequency in the south of the study region. Haplotype 5, which is very frequent in oak populations from the nearby Transylvania, might have passed the Carpathian Mountains, rather than coming from the south of Romania. The natural occurrence of haplotypes 6 in Northern Moldavia is questionable. Artificial seed transfer may explain its presence in only two isolated populations in the northern part of Moldova. The comparison of these chloroplast DNA data with those obtained from fossil pollen studies may better explain the current spatial distribution of chloroplast genetic diversity in Eastern Romania. Moreover, the fine scale distribution of the chloroplast haplotypes in Eastern Romania, together with data from other parts of the country, may help in future the control of the movement of forest reproductive material in oak species.

Acknowledgments

This paper is supported by ANCS-CNMP through the Partnership OAKGIS no. 51-029/2007 and the Sectoral Operational Programme Human Resources Development (SOP HRD), financed by the European Social Fund and by the Romanian Government under the contract number POSDRU/6/1.5/S/6

References

Bordács, S., F. Popescu, D. Slade, U. M. Csaikl, I. Lesur, A. Borovics, P. Kezdy, A. O. Konig, D. Gomory, S. Brewer, K. Burg and R. J. Petit (2002). Chloroplast DNA variation of white oaks in northern Balkans and in the Carpathian Basin. Forest Ecology and Management 156:211-222.

Curtu, A. L., O. Gailing, L. Leinemann and R. Finkeldey

(2007). Genetic variation and differentiation within an natural community of five oak species (*Quercus* spp.). Plant Biology 9:116-126.

Demesure, B., N. Sodzi and R. J. Petit (1995). A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Molecular Ecology 4:129-131.

Dumolin-Lapègue, S., B. Demesure, V. Le Corre and R. J. Petit (1997). Phylogeographic structure of white oaks throughout the European continent. Genetics 146:1475-1487.

Farcas, S., F. Popescu and I. Tantau (2006). Spacial and temporal distribution of pedunculate oak, common ash and hornbeam during late glacial and postglacial period in Romania. Ed. Presa Universitara Clujeana (in Romanian).

Fekete, L. and T. Blattny (1913). The distribution of forest trees and shrubs in Hungary, Vols. I-II (in Hungarian). Selmecbánya, Joerges A. Ny

Florescu, I. L. and N. V. Nicolescu (1996). Forestry, Vol. I Forest research. Ed. Lux Libris, Brasov (in Romanian).

Mátyás, G. and C. Sperisen (2001). Chloroplast DNA polymorphism provide evidence for glacial re-colonisation of oaks (*Quercus ssp.*) across the Swiss Alps. Theor. Appl. Genet. 102:12-20

Petit, R. J., C. Bodénès, A. Ducousso, G. Roussel and A. Kremer (2003): Hybridization as a mechanism of invasion in oaks. New Phytologist 161:151-164.

Petit, R. J., E. Pineau, B. Demesure, R. Bacilieri, A. Ducousso and A. Kremer (1997). Chloroplast DNA footprints of postglacial recolonization by oaks. Proc. Natl. Acad. Sci. USA. 94:9996-10001.

Petit, R. J., S. Brewer, S. Bordács, K. Burg, R. Cheddadi, E. Coart, J. Cottrell, U. M. Csaikl, B. C. Van Dam, J. D. Deans, S. Espinel, S. Fineschi, R. Finkeldey, I. Glaz, P. G. Goicoechea, J. S. Jensen, A. O. König, A. J. Lowe, S. F. Madsen, G. Mátyás, R. C. Munro, F. Popescu, D. Slade, H. Tabbener, S. G. M. De Vries, B. Ziegenhagen, J. L. de Beaulieu and A. Kremer (2002b). Identification of refugia and post-glacial colonisation routes of European white oaks based on chloroplast DNA and fossil pollen evidence. Forest Ecology and Management 156:49-74.

Petit, R. J., U. M. Csaikl, S. Bordács, K. Burg, E. Coart, J. Cottrell, B. van Dam., J. D. Deans, S. Dumolin-Lapegue, S. Fineschi, R. Finkeldey, A. Gillies, I. Glaz, P. G. Goicoechea, J. S. Jensen, A. O. König, A. J. Lowe, S. F. Madsen, G. Mátyás, R. C. Munro, M. Olalde, M. H. Pemonge, F. Popescu, D. Slade, H. Tabbener, D. Taurchini, S. G. M. de Vries, B. Ziegenhagen and A. Kremer (2002a). Chloroplast DNA variation in European white oaks Phylogeography and patterns of diversity based on data from over 2600 populations. Forest Ecology and Management 156:89-102.

Pons, O. and R. J. Petit (1996). Measuring and testing genetic differentiation with ordered versus unordered alleles. Genetics 144:1237-1245.

Popescu, F. and D. Postolache (2009). Genetic variability of the oak stands in Romania, like a result of the interaction between the postglacier evolution of the vegetation and the anthropic influences. Revista padurilor 5:49-54 (in Romanian).

Sanda, V., N. Babaras and S. Stefanut (2004). Atlas Florae Romaniae III Genul *Quercus*. Ed. Ion Borcea, Bacau (in Romanian).

Slade, D., Ž. Škvorc, D. Ballian, J. Gračan and D. Papeš (2007). The chloroplast DNA polymorphism of white oaks of Section *Quercus* in the Central Balkans. Silvae Genetica 57(4-5):227-234.

Sofletea, N. and A. L. Curtu (2007). Dendrolgy. Ed. Universitatii Transilvania, Brasov (in Romanian).

Taberlet, P., L. Gielly, G. Pautou and J. Bouvet (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology. 17:1105-1109.

Toader, V. A., I. C. Moldovan, N. Sofletea, I. V. Abrudan and A. L. Curtu (2009). DNA isolation and amplification in oak species *(Quercus spp.)*. Buletinul Universitatii Transilvania Brasov 2(51):45-50.

www.qiagen.com. DNeasy Plant Handbook: Protocol: Purification of Total DNA from Plant Tissue (Mini Protocol).

www.zymoresearch.com. ZR Plant/Seed DNA Kit: Protocol for isolation of DNA.