

COST-STSM-FP1202-17507

PROJECT REPORT

**"RESEARCH OF GENETIC VARIABILITY OF PEDUNCULATE OAK IN THE
BOSNIAN PROVENANCE TEST"**

SARAJEVO, JUNE, 2014

COST STSM Reference Number: COST-STSM-FP1202-17507

Period: 2014-05-01 00:00:00 to 2014-05-31 00:00:00

COST Action: FP1202

STSM type: Regular (from Bosnia and Herzegovina to Germany)

STSM Applicant: Mirzeta Memisevic Hodzic, JP "Bosanskohercegovačke sume", 71000 Sarajevo (BA), mirzeta.memisevic.hodzic@gmail.com

STSM Topic: Research of genetic variability of pedunculate oak in the Bosnian provenance test

Host: Dr Monika Konnert, Bayerisches Amt für Forstliche Saat-und Pflanzenzucht, 83317 Teisendorf (DE), Monika.Konnert@asp.bayern.de

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"Research of genetic variability of pedunculate oak in the Bosnian provenance test"

1. Purpose of the STSM

In order to complete my Ph.D. thesis "Morphological phenological and genetic variability of pedunculate oak (*Quercus robur* L.) in the Bosnian provenance test" at Faculty of Forestry, University of Sarajevo (Address: Zagrebačka 20, 71000 Sarajevo, phone: +387 33812490/491, Fax: + 387 33812488, E-mail: info@sfsa.unsa.ba) I worked on isozyme analysis of pedunculate oak at Bayerisches Amt für Forstliche Saat-und Pflanzenzucht (83317 Teisendorf, Forstamtsplatz 1, Tel: +49 (0) 8666 9883-0 Fax: +49 (0) 8666 9883-30, Email: poststelle@asp.bayern.de).

I used ASP's laboratories and facilities extract isoenzymes from fifty samples per population for twenty populations for subsequent electrophoretic analysis.

Aim of research

Pedunculate oak (*Quercus robur* L.) used to represent an important part of economic forests of Bosnia and Herzegovina. In nature, pedunculate oak has a large distribution area Bosnia and Herzegovina's territory is the central southern part of the natural distribution of oak, and so has a specific genetic structure in relation to the central and northern part of the area. So this species with its distribution in Bosnia and Herzegovina builds a specific link of southern and eastern provenances of the Balkan Peninsula with those from Central Europe, and plays a very important role in the movement of genes from north to south and west to east and vice versa.

Natural populations of pedunculate oak in Bosnia and Herzegovina, represent specific communities in the disappearance. They differ from those of its optimum in central Europe and are very important for maintaining the diversity of pedunculate oak in Europe. The effectiveness of conservation measures and reintroduction depends on the degree of knowledge of its diversity in Bosnia and Herzegovina. One of the ways how to get valid results is setting up provenance trials. This experiment will represent an *ex situ* conservation measure, and the results obtained from the the experiment will provide a large amount of data required for reintroduction and conservation measures *in situ*.

The aim of this study was to answer the question: what is the genetic structure of the remaining pedunculate oak populations in Bosnia and Herzegovina through research of provenance, and to ensure predisposition for future production of forest reproductive material (seeds and plants), experimental zoning of pedunculate oak in Bosnia and Herzegovina and preserving *in situ* and *ex situ*.

Future goal of setting up the experiment is also the determination of the main features of Bosnian pedunculate oak, compared to those from Central and Eastern Europe, but through a long-term comparative study.

At this early stage of research of variability we will concentrate on one small segment, on results of early juvenile stages of plant development. Early tests can provide basic guidelines for the selection of individual provenances of pedunculate oak, and are essential for the production of seedlings in the nursery and planting in the field.

2. Description of the work carried out during the STSM

Materials

In February 2014 I collected samples for isozyme analysis: one twig (only living plant parts with buds) in winter dormancy stage from 50 individuals per population/provenance were collected from twenty provenance from the provenance experiment set up in 2009.

Table 1: List of Populations/Provenances for isoenzyme analysis

No	Country	Provenance	Locality	Population ID	North latitude	East longitude	Altitude (m)
1	B&H	Bos. Brod	Zborišta	BH2	45° 05' 27"	18° 00' 38"	84
2	B&H	Bos. Gradiška	Lipnica	BH5	45° 06' 64"	17° 18' 63"	91
3	B&H	Bijeljina	Patkovača	BH6	44° 43' 50"	19° 13' 30"	93
4	B&H	Srebrenik - Hrgov	Dubrave	BH9	44° 49' 06"	18° 34' 11"	133
5	B&H	Bos. Dubica	Knežica	BH13	45° 06' 24"	16° 40' 32"	145
6	B&H	Jelah	-	BH17	44° 39' 09"	17° 56' 46"	181
7	B&H	Živinice	D. Dubrave	BH19	44° 27' 58"	18° 41' 09"	216
8	B&H	Žepče	Žepački lug	BH20	44° 25' 35"	18° 03' 10"	224
9	B&H	Kotor Varoš	-	BH23	44° 39' 07"	17° 21' 35"	252
10	B&H	Ključ	Velečevo	BH24	44° 30' 56"	16° 48' 42"	260
11	B&H	Mutnica	Cazin	BH25	44° 58' 55"	15° 50' 54"	270
12	B&H	Kačuni	Nezirovići	BH28	44° 03' 59"	17° 56' 13"	443
13	B&H	Drvar	Unac	BH29	44° 23' 39"	16° 21' 54"	462
14	B&H	Stojčevac	Iliđa	BH33	43° 48' 40"	18° 17' 25"	506
15	B&H	Bugojno	Kopčić	BH35	44° 06' 00"	17° 26' 31"	537
16	B&H	Olovo	-	BH36	44° 07' 44"	18° 36' 11"	542
17	B&H	Miljevina	-	BH38	43° 31' 06"	18° 38' 56"	627
18	B&H	Bos. Grahovo	Crni lug	BH41	44° 01' 05"	16° 38' 24"	703
19	B&H	Mrkonjić Grad	Čadavica	BH42	44° 27' 04"	16° 58' 42"	753
20	B&H	Sokolac	Lug	BH44	43° 55' 17"	18° 48' 53"	866

Samples were kept at -20°C until beginning on May, when I brought them to Bayerisches Amt für Forstliche Saat-und Pflanzenzucht, where I did analysis using isoenzymes as biochemical markers.

The following five steps are conducted during isoenzyme analysis:

1. Extraction of the enzymes from the plant material,
2. Application of the extracts on a gel (starch, PA)
3. Separation of the enzymes in an electric field (electrophoresis)
4. Staining of the gel (enzyme-specific)
5. Evaluation of the banding pattern (Zymogramme)

During my stay at ASP I finished the first step and started with steps 2-4 for trial purpose. The extraction of the enzymes from buds were performed with extraction buffers pH 7,2 and small amount of Polyclar. (100 ml Homogenatpufer Tris/HCl 0,1 M pH 7,2 contains: PVP (3%) 3 g; β -Mercaptoethanol (0,07 mM) 30 μ l.)

For hand grinding of buds I used a small mortar and pestle. I used 3-4 buds per sample, 50 samples per population. After homogenization, crude extracts were absorbed onto small paper cuttings for application onto the gels - paper wicks, 3x8 mm.

Plates with extracted enzymes are stored on -60°C until July 2014 for completing the work at ASP.

3. Future collaboration with the host institution

In July 2014 a second visit at ASP Teisendorf will take place, in order to finish the work done so far and to start writing a publication. Furthermore a continuous collaboration between University of Sarajevo and ASP Teisendorf was established in the field of genetic investigations.

4. Confirmation by the host institution of the successful execution of the STSM

In attachment.

Acknowledgments

First I would like to thank Dr Monika Konnert for giving me this opportunity to work at Bayerisches Amt für Forstliche Saat-und Pflanzenzucht and for her support during obtaining and transkription of the results. Also, I want to thank the collaboration of the colleagues at the laboratory.

Mr Sc Mirzeta Memisevic Hodzic

June 2nd, 2014