







SEE-ERA.NET PLUS

ERA 91/01 "Preservation and establishment of true-to-type and virus free material of endangered grapevine cultivars in Croatia and Montenegro"

Project and workshop topics

Zagreb, September 27, 2012

Participants

- FAZ (HR) coordinator
- Ivan Pejić
- Maja Žulj Mihaljević
- Silvio Šimon
- Darko Preiner
- Edi Maletić

BOKU (A) – partner 1

- Astrid Forneck
- Ulrike Anhalt
- IRZ Geisenheim (D), part. 2
- Ernst Ruehl
 - Bettina Lindner

BTF (MNE) – partner 3

- Vesna Maraš
- Milena Mugoša (Tomić)
- Sanja ŠućurMiroslav Čizmović

Key information

- Coordinator: FAZ (HR)
- Partners: BOKU (A), IRZ Geisenheim (D), BTF (MNE)
- Title: ERA 91/01 "Preservation and establishment of true-to-type and virus free material of endangered grapevine cultivars in Croatia and Montenegro"
- Duration: 2 years
- Funds requested: 145.000,00 EUR
- Funds aproved: 122.400,00 EUR
- Budget share: FAZ (39%)

BTF (29%)

BOKU (16%)

IRZ Geisenheim (16%)

Project concept

- Croatia & Montenegro are neighboring countries that base their development on strong tourism sharing similar potentials and problems > main targets of the project
- 2. HR & MNE have rich grapevine germplasm that might be very relevant for **global biodiversity preservation** purposes, and
- This germplasm might contribute development of local products (wines) of benefit for tourism development in HR & MNE
- 4. Project sustainability envisioned through safe accession duplicates, education & training

Project idea

Croatia Austria Montenegro Germany Field expeditions in HR & MNE to collect and conserve rare native grapevine varieties: - Conservation is international obligation (Kartagena protocol) Native grape varieties - source for unique local wines supporting the fast growing tourism in both countries Primary description and quality assessment: Standard description methods in situ Bunch morphology and must analyses, microwinifications DNA fingerprinting and clonal variation analysis: - SSR markers & training for MNE (**Zagreb**) -AFLP, REMAP, SNP & training for HR (Vienna) **Propagation (Geisenheim):** MNE & HR - ELISA testing - National ex situ collections Grafting Certification - Mother blocks for nurseries - Dissemination to winemakers **EU Vitis database** - Training for end users EU ex situ collections

Work packages

WP-1 Field expedition, cultivar identification and evaluation

- Field expedition on the territory of Croatia and Montenegro cultivar and Vitis vinifera no name genotypes (NN) identification and evaluation by basic ampelographic methods
- Grape and tissue sampling for evaluation of grape quality, molecular marker analyzes, virus testing and grafting purpose.
- Cultivar identification by SSR markers conducted by coordinator's (FAZ).

WP-2 Intravarietal studies and confirmation of SSR data (by other marker systems)

 Molecular analysis for inter- and intra-varietal variability via S-SAP conducted by Partner 1 (BOKU, Austria).

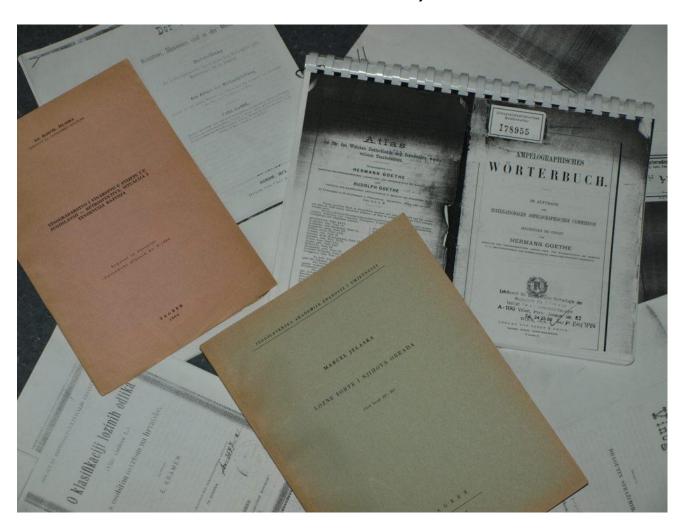
WP-3 Virus testing, propagation of virus-free material and its maintaining

- Laboratory and nursery research on virus infections of grape cultivar populations, as well as their ecotype (clonal) differences conducted by Partner 2 (IRZ Geisenheim, Germany).
- Propagation for long term conservation of selected and analyzed material.

Aim 1: Make a thorough survey through available literature and available databases already dealing with national and international grapevine conservation efforts

Methodology: All available books, S&T papers related to grapevine cultivars, their history and agronomic properties and conservation efforts will be systematically screened through classical library and advanced edatabases. This will encompass diploma and PhD thesis and interviews with local producers.

D01 – FAZ: Bibliography (searching for and reading available books, S&T papers related to native varieties)



D01 – BTF: Bibliography (searching for and reading available books, S&T papers related to

native varieties)

Heirap Circhanol Serobut

Beja 17. Kranoba

Факсимил Зайисника (йрва сйрана)

Rovinski (1994) navodi "da se u Crmnici vinogradi mogu nači samo u Godinju i Boljevićima". Da li je Rovinski mislio na veće površine vinograda sa organizovanim zasadima ili su ratovi, zapuštenost i bolesti u tom periodu toliko devastirali vinograde da ili je bilo moguće nači samo u ovim selima (prim.aut.). On dalje nastavlja da "kada su vinogradi izloženiji suncu i na nižem mjestu grožde je slade i vino bolje. Smatra se da je vino najbolje u Sotonićima, naročito lokalitet Mačuge, pa Godinje, Boljevići i u nižim djelovima Limljana i Boljevića. Ali je u visočijim selima Podgor, Gornje Brčele, Bukovik, Gluhi Do-bolja rakija".

M. Plamenac. (1891). u "Grlici" ističe da se "grožde po boji dijeli u Crmnici, na tri sorte: crno, ride i bijelo. Crno grožde, kome je zrno okruglo, na kratkoj peteljci, zove se kratošija. Grozdovi kratošije obično su nabijeni. Ima, pak, jedna vrsta kratošije, kojoj grozdovi nijesu zbijeni, nego zrna poreda, i to se zove reavica. Crno grožde, kome su jagode ovalne, zove se vranac. Ako su zrna vranca krupnija nego u običnog, takav zove se krstač. Ride grožde u koga su zrna okrugla, zove se šijerovina, ako li je zrno sitnije a ovalne forme, zove se lisičina (lisica). Bijelo grožde naziva se samo jednim imenom-bijelo grožde, premda ima i bijelog kome su jagode malo krupnije ili sitnije, a tako isto više bijelo ili nažuto. Samo ima malo loze bijele kojijema grožde ima jaki miris i zove se muškaćelica, muškat. Ove loze je vrlo malo u Crmnici." U daljem tekstu autor opisuje čauš i razakliju. "Čauš je donesen iz Carigrada, krupnih ovalnih zrna, naročito nježno i slatko grožde. Rozaglija je sorta koja može biti sviju tri boja, ovalnih je hobica, tvrde opne (komina). Ova sorta se koristi samo za jelo, a ne obrazuje se kao ostale sorte već raste kao odrina (za lozu napravljena specijalna rešetka-odar) a ako se loza penje uz drvo, naziva se podrevina".

24

пословник

ВИНОГРАДАРСКЕ ЗАДРУГЕ

На основу чл. 33 тач. 1 правила падзории одбор прописује овај пословник.

Задругари.

Uman 1.

Ко хове да ступи у задругу, мора за полиссе управном одбору писмену малбу у којој ће поред молбе за пријем, најавати: колико има вемљанита пасађеног америчком лозом и колико уписује удела.

Кад управии одбор прими молитела за задругара оп hе се преко једног од својих чланова претходно уверити, да ли вови задругар заиста има околиво земдвита засађенот пиновох дозом колико је пријамио, колико на истом земланиту има чокота дозе и према томе допети оддуку, колико је исти задругар дужав уписати удела.

После оне коначие одлуке о пријему новоги задругара у задругарство, задругар је дужан да поднесе писмену најаву, коју мора оперити управии одбор.

Опако оперену изјаву — с једним преписом управиц одбор подноси суду ради увођења у списац задругира.

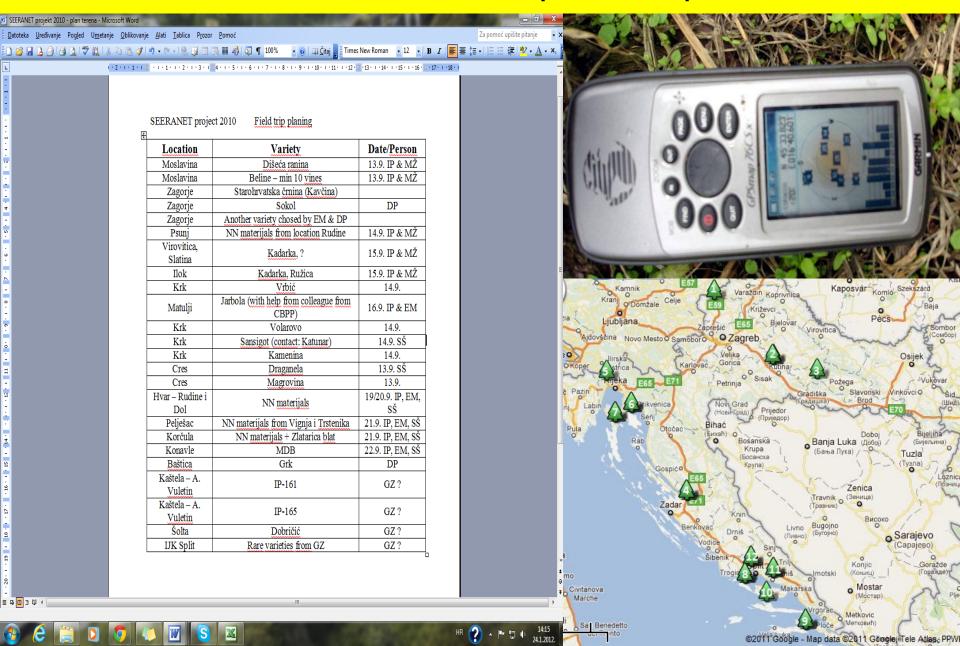
Сваки задругар, кад поставе, добива од задруге један примерак њених правила, на којима ће стајати

Aim 2: Perform surveys (field expeditions) to identify and collect <u>rare and neglected</u> indigenous *Vitis vinifera* undetermined genotypes, not previously evaluated and <u>not present</u> in the existing national *Vitis ex situ* collections

Methodology:

- search for old grape native varieties not previously genotyped by molecular markers.
- existing ex situ collections to be sampled as well in order to check and establish the new and fully compatible SSR genotype database
- emphasis to be given to extremely rare genotypes previously not collected and/or evaluated
- A special effort to find and sample genetic variation (mutant ecotypes)
 within the perspective variety populations as well as individual vines with
 absent symptoms of economically relevant virus diseases.

D02 – FAZ: Field expedition plan



D02 – FAZ: Field expedition plan



D03 - FAZ: Ampelographic description and evaluation of sampled genotypes Microsoft Excel - Ampelografske analize ERA 91-01 - 10 - B I U | 臺 | 臺 | 臺 | 臺 | ◎ % 00 % % | 章 | 章 | □ - 🔈 - A - 😭 M -Sampled **Photos** Ampelographic descriptors (OIV) genotypes ERA91/01 leaf MAGROVINA DRAGANELA SANSIGOT DIŠEĆA RANINA STARA BELINA AROMATIČNA **BELINA JARBOLA REBULA** SOKOL Glavinuša, okatac DOBRIČIĆ Zlatarica blatska b 17 18 20 21 22 23 24 25 26 H ← → H \ List1 / List2 / List3 \ AMPELOGRAPHIC DESCRIPTORS

D03 – BTF: Ampelographic description and evaluation of sampled genotypes

shoot tip

SEE-ERA NET PLUS (ERA 91/01)

"Passervation and establishment of true-to-type and virus fice material of endangered grape vire cultivars in Croatia and Montenegro"

Name of variety or synonym: ICRATOSUA Code of variety (number of sample): MINE 02 Fumber and code of the taken samiles from this location: 10 Date of sampling: 11.09.2010. year Vineyards and place: Danilo vgad (Marlo vina) Flame of vineyard: Jashmik

GPS position and the vine's position in vineyard:
MMR 02-1-M 42' 31.743'; EO 19'00 597
MMR 02-2-M 42' 31.744'; EO 19'00 597
MMR 02-3-M 42' 31.744'; EO 19'00 609
MMR 02-4-M 42' 31.741'; EO 19'00 609
MMR 02-4-M 42' 31.741'; EO 19'00 609
MMR 02-4-M 42' 31.743'; EO 19'00 610'
MMR 02-4-M 42' 31.743'; EO 19'00 627
MMR 02-4-M 42' 31.733'; EO 19'00 627
MMR 02-8-M 42' 31.733'; EO 19'00 624'
MMR 02-8-M 42' 31.733'; EO 19'00 614'
MMR 02-8-M 42' 31.733'; EO 19'00 614'
MMR 02-9-M 42' 31.733'; EO 19' 00 614'



Data about vineyard's owner: Name and surname: Spasoje Vijouis

Address: Pasici bb. SI 410 Danile ugrad

Phone number +382 (9.949.300)

DESCRIPTION OF VARIETY				
Training system, vigorous and age of the vine	without defined training system, slightly vigorous, over 30 years			
Rating of vine's health	poor health, times dy ingout, tirible symptoms of powday milde w (
Ripening date	the end of the September			
Description of cluster	medium large, hosse, conital			
Description of berry	globose, small dark blue to pumple color, colorbes junice			
Description of leaf	large matus kaf, five leber, bas face of kaf and large back			
Description of the young				

SHE-HRA NET PLUS (HRA 91.01)
"Preservation and establishment of true-to-ype and wire fine material of endangered graps wire cultivars in Croatia and Montree gro"

Name of variety or synonym: CUBRICA Code of variety (number of sample): IDME 07 Fumber and code of the taken samles from this location: 10 Date of sampling: 12 09: 2010 . year Vineyards and place: Podgorisa's vineyards, Doljani Fame of vineyard: Raciza

GPS position and the wire's position in vineys rd:
MNH 071-14 14738078; EO 19 18377
MNH 077-14 14738079; EO 19 18377
MNH 077-14 14738087; EO 19 18377
MNH 077-14 14738078; EO 19 18377
MNH 077-14 14738078; EO 19 18377
MNH 077-9 M 14738078; EO 19 18377
MNH 077-9 M 14738078; EO 19 18377
MNH 077-10-14 1738078; EO 19 18377



Data about vineyard's owner: Name and surname: Sasa Vijosevis

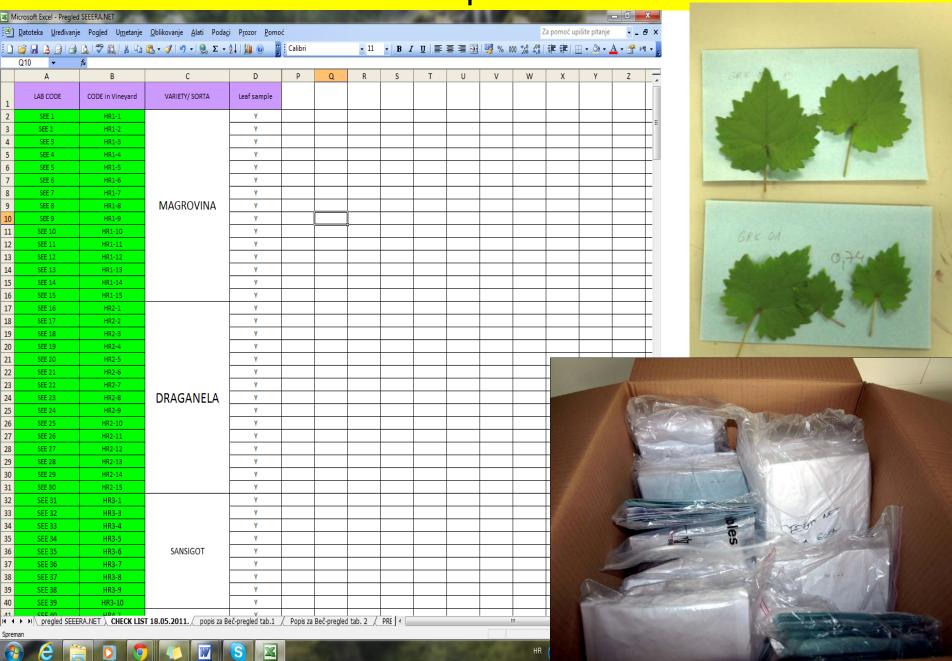
Address: Doljani bb. 20 000 Pod gorica.

Phone number: +382 (7 245 301

DESCRIPTION OF VARIETY				
Training system, vigorous and age of the vine	modified pergola, medium vigorous, over 10 years			
Rating of vine's health	relatively good health, visible symptoms vine's mites (continued on leaf			
Ripening date	mildle of the September			
Description of cluster	medium large, medium loose, sylindric conical			
Description of berry	globose to slightly ellipsoid, small, dark blue color			
Description of leaf	large matus haf, three lebes, slightly proleted face of haf			
Description of the young				



D04 – FAZ: Leaf samples for DNA extraction



D04 – BTF: Leaf samples for DNA extraction



Kontant worka Hirosla Cirmovid RAČUN BR Biotelinière famillet Ul Mihaila colière Br. 1. odiel en vineyadanstro i vinerate 0: pronocat see era NeT- PLUS DATUM: EXPRESS REPRESENTED BY KINGSCLIFFE DISTRIBUTION MONTENEGRO D.O.O. 27,04,11 Ul. Marka Miljanova 52, 81000 Podgorica Tel.020/633-971 P.I.B.: 02303337 PDV: 30/31-00730-4 25 5022 5182 DOC Nesuclinais RAČUN IZDAO: PRI PLAĆANJU OBAVEZNO NAVEDITE BROJ RAČUNA SEE-ERA, NET - PLUS -> VINOUA LOZA

Legend of sent samples (leaves - Vitis vinifera)

Code	Locality	Owner	Number of samples
MNE 1	Račica,Doljani- Podgorica	Dragiša Vujošević	10
MNE 2	Markovina — Danilovgrad	Spasoje Vujović	10
MNE 7	Račica,Doljani- Podgorica	Saša Vujošević	10
MNE 12	Boljevići,Crmnica Bar	Jovan P. Plamenac	5
MNE 13	Beri- Podgorica	Ljubo Perović	1
MNE 15	Beri- Podgorica	Ljubo Perović	5
MNE 18	Beri- Podgorica	Veselin Perović	1
MNE 17	Godinje,Crmnica Bar	Krsto Leković	6
MNE 20	Otočići,Crmnica Bar	Božo Vujačić	3
MNE 21	Donji Medun, Podgorica	Mihailo Laković	5
Total nu	56		

April, 2011

Aim 3: Determine neglected indigenous varieties with distinguished enological potential (based on quality of grape) and collect their cuttings for fast clonal selection;

Methodology:

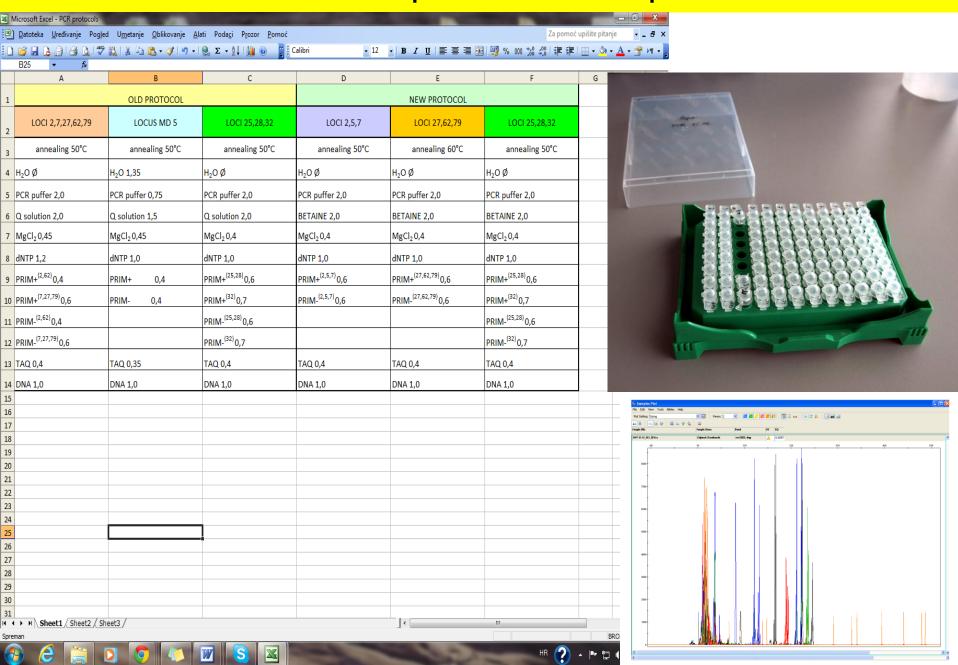
- During the project year 1 and 2, up to 10 (?) neglected varieties suitable for quality wine production in each WBC country will be selected in farmers' vineyards (in situ) based on the sensory and analytical evaluation (quality of grapes estimated by sugar and acid content, primary aromas, color intensity, etc.).
- All grapes from 20 30 vines per variety will be selected and harvested from farmers' vineyards and transported to experimental wine cellar.
- Same vines will be used for WP2 and WP3

 Aim 4: Perform positive genotype identification and assessment of its genetic variability using both standard ampelographic and modern molecular tools;

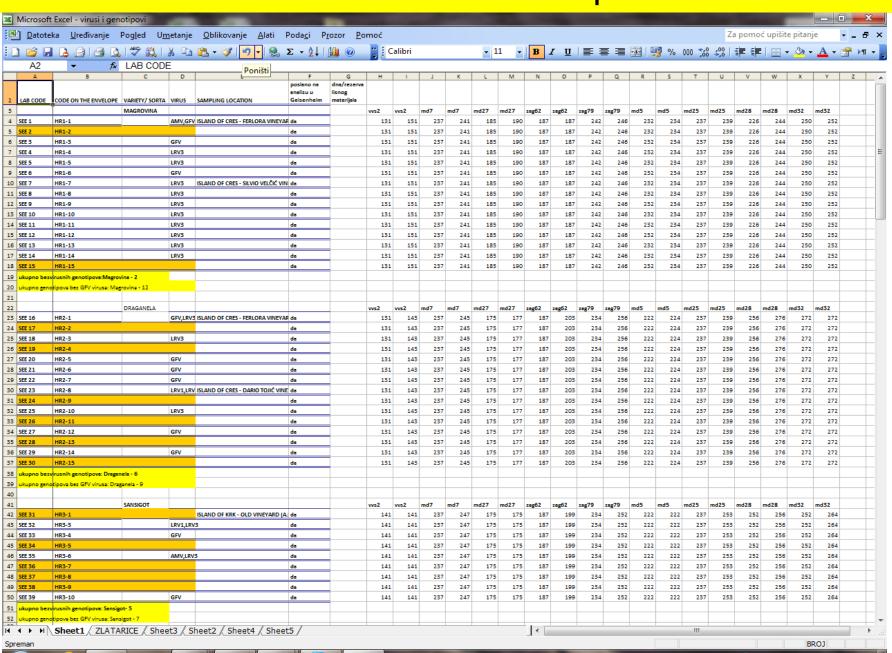
Methodology:

- SSR analysis of all collected samples, both from HR & MNE to be analyzed with 9 SSR markers;
- Combine the results of SSR analysis and ampelographic descriptions, as well as enological estimates to make final report on genotype description and its value.

D08 – FAZ: Optimized SSR protocol



D09 & D10 - FAZ: SSR profiles



 Aim 5: To check the inter- and intravarietal variation in order to estimate the potential for clonal selection and propose the strategy for biodiversity preservation;

Methodology:

 Multiple samples (vines) per variety population analyzed by S-SAP markers in order to estimate the level of intravarietal variation and search for dominant ecotype.

D11- BOKU: S-SAP protocol

Universität für Bodenkultur Wien

University of Natural Resources and Applied Life Sciences, Vienna





Department of Crop Sciences, Division of Viticulture and Pomology Konrad Lorenz-Strasse 24, 3430 Tulln, Austria

Tulln, 01/25/2012

ERA 91/01 (HRV & MNE Endangered Grapes) Progress of Project at BOKU Vienna

Introduction:

A modified S-SAP (sequence-specific amplified polymorphism) method by Wegscheider et al. 2009 with universal primers for retrotransposons was used to study the diversity of the grape from Croatia and Montenegro. Included in the study were 214 clones from Croatia and Montenegro.

Method:

Transposon display after the protocol of Wegscheider et al. 2009.

DNA (13.5 µL) was digested with Msel (Fermentas, St. Leon-Rot, Germany) in a total volume of 25 µL. The digestion was incubated for 2 hr at 65 ℃. Restricted DNA was further purified using the E.Z.N.A MicroElute DNA Clean-Up Kit (Omega Bio-Tek, USA). After purification, template DNA (25 µL) was prepared by adding 5 µL of a ligation mix (50 pmol Msel adapter, 100 mM ATP, 10x T4 ligase buffer, and 1 U T4 Ligase (Fermentas, St. Leon-Rot, Germany), and was incubated overnight at room temperature (20 ℃). T4 ligase was inactivated by heating up to 65 ℃ for 10 min. In the preamplification step, the primer M(0), homologous to the adapter sequence, was combined with one of six labeled (IRD700 and IRD800) universal retrotransposon primers: F0100, F0103, F0104, F0105, F0113, and F0117 (Table 1). The PCR reaction mixture contained 2.25 μL template DNA, 1.5 μM M(0), 1.5 μM transposon primer, 1x PCR buffer, 3 mM MgCl2, 0.2 mM dNTPs, and 1 U Taq DNA polymerase recombinant (Fermentas, St. Leon-Rot, Germany) in a final volume of 15 µL. The unselective PCR was conducted using the following program: 94 °C · 60 s+ 26 x (94 °C · 30 s, 56 °C · 60 s, 72 °C · 60 s) + 72 °C · 6 min. The selective amplification was carried out in a total volume of 10 µL containing 1 µL preamplified DNA (diluted 1:10), 0.5 µM selective Msel primer (M22, M23, M24, M25, M27) (Table 1), 0.5 µM transposon primer, 1x PCR buffer, 2.5 mM MgCl2, 0.2 mM dNTPs, and 0.75 U Taq DNA polymerase recombinant (Fermentas, St. Leon-Rot, Germany) using the following cycle profile: 94 °C ⋅ 60 s+ 12 x (94 °C ⋅ 30 s, 65 °C ⋅ 30 s, 72 °C ⋅ 60 s) [annealing temperature was reduced by 0.7 °C in each of the 12 cycles] + 26 x (94 °C ⋅ 30 s, 56 °C ⋅ 30 s, 72 °C · 60 s) + 72 °C · 6 min.

Bands were detected in a 6% polyacrylamide gel and visualized by the automated LI-COR NEN 4300 DNA annalyzer (LI-COR Biosciences, Bad Homburg, Germany).

Results so far:

Protocol was established for the analysis of the clone DNAs from Croatia and Montenegro and primers were tested. After the primer test four primer combinations F0105a-M27, F0104a-M27, F0103a-M27, and F0100a-F27 were chosen (Table 1). Each primer combination approximately produced around 70 marker bands (poly- and monomorphic bands). The laboratory work on the LI-COR NEN 4300 DNA analyzer (LI-COR Biosciences, Bad Homburg, Germany) could be successfully completed and all clones were analyzed. The data analysis and statistics of the inter- and intra- variation is ongoing but will be completed by the end of January 2012.

Table 1:Msel primers and universal retrotransposon-based primers (Wegscheider et al. 2009) used in the S-SAP analyses

Primer code	DNA sequence
M(0)	5' GATGAGTCCTGAGTAA 3'
M22	5' GATGAGTCCTGAGTAACAA 3'
M23	5' GATGAGTCCTGAGTAACTT 3'
M24	5' GATGAGTCCTGAGTAACAC 3'
M25	5' GATGAGTCCTGAGTAACAT 3'
M27	5' GATGAGTCCTGAGTAACTG 3'
F0100a	5' TAGGTCGGAACAGGCTCTGATACCA 3'
F0103a	5' ACCGAGCAACTTGAGCTCTGATACCA 3'
F0104a	5' CTAGGGTCAAGGGGGCTCTGATACCA 3'
F0105a	5' GGGAAATGGTCCGCTCTGATACCA 3'
F0113a	5' AGTTCATCGTAGGTGGGCGCCA 3'
F0117a	5' ATCCCCAGCGGAGTCGCCA 3'

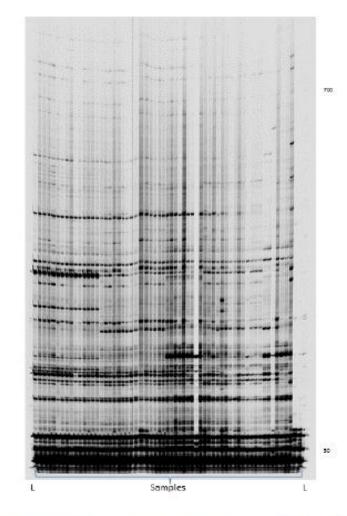


Figure 1: Figure displays the primer amplification F0103-M27 in an 6% Acrylamid gel detected by the LI-COR NEN 4300 DNA analyzer (LI-COR Biosciences, Bad Homburg, Germany).

References:

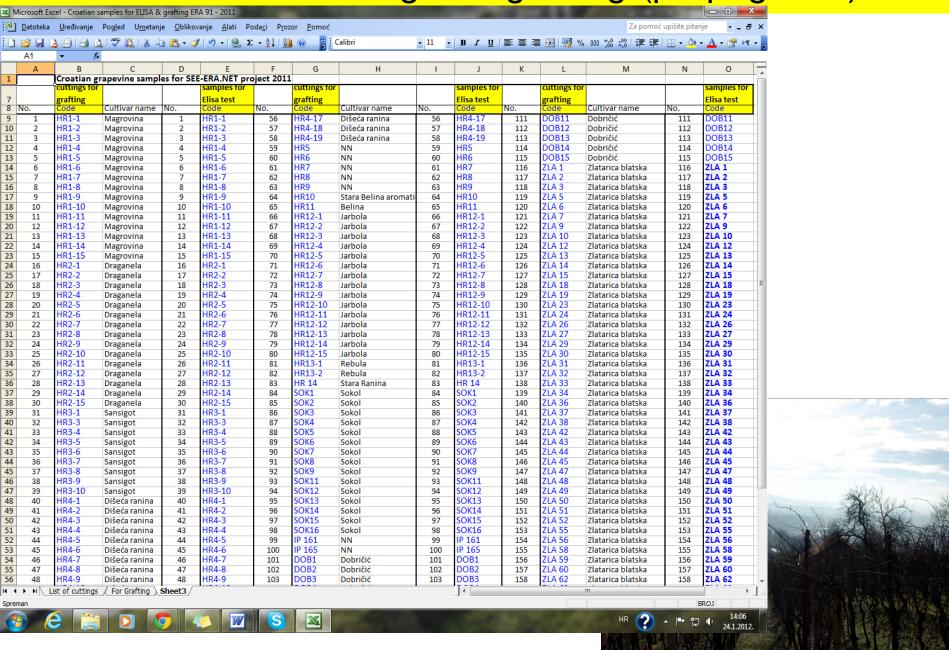
Wegscheider E,Benjak A,Forneck A. (2009) Clonal Variation in Pinot noir Revealed by S-SAP Involving Universal Retrotransposon-Based Sequences Am. J. Enol. Vitic. 60(1):104-109

 Aim 6: Assess presence of plant viruses within collected material and do sanitary selection using accurate laboratory tests;

Methodology:

According to the results of ampelographic, SSR and S-SAP analyzes (determination of true-to-type genotype) the cuttings (buds) for propagation have been selected and all verified samples were shipped to Partner 2 to perform ELISA and other required (PCR) tests.

D06 – FAZ: Cuttings for grafting (propagation)

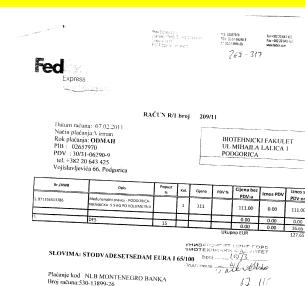


D06 – BTF: Cuttings for grafting (propagation)

Legend of sent samples (cuttings - Vitis vinifera),

Code	Locality	Owner	Number of cattings
MNE 1	Račica,Doljani- Podgorica	Dragiša Vujošević	50
MNE 2	Markovina — Danilovgrad	Spasoje Vujović	49
MNE 7	Račica,Doljani- Podgorica	Saša Vujošević	50
MNE 12	Boljevići,Crmnica Bar	Jovan P. Plamenac	21
MNE 13	Beri- Podgorica	Ljubo Perović	4
MNE 15	Beri- Podgorica	Ljubo Perović	19
MNE 16	Beri- Podgorica	Veselin Perović	5
MNE 17	Godinje,Crmnica Bar	Krsto Leković	25
MNE 20	Otočići,Crmnica Bar	Božo Vuj <i>a</i> čić	13
MNE 21	Donji Medun, Podgorica	Mihailo Laković	23
Total nun	259		

January, 2011



FAKTURISÃO Danijela Smolović

NAPOMENA: Oslobođeno plaćanja PDV-a prema članu 21.stav 4. b Pravilnika Klmmell y

DIREKTOR

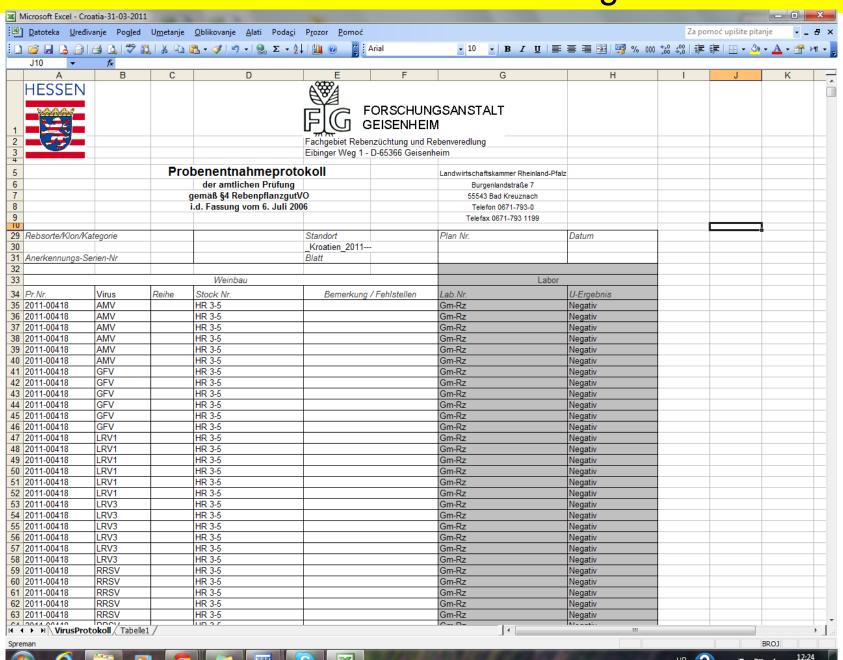
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 Aim 7: Ensure proper maintaining of material for future use in research and production

Methodology:

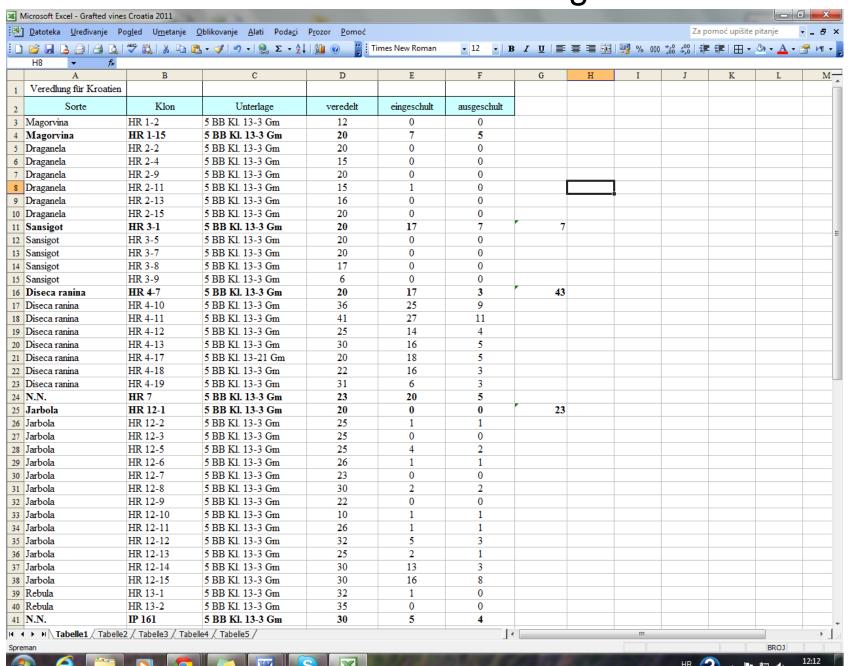
• It is expected that up to 15 different cultivars/genotypes will be necessary to propagate with 3 – 4 clones per cultivar in average leads to between 45 and 60 stock vines to be thoroughly tested and propagated. Those ones to be approved as virus-tested will be propagated into 15-20 grafts.

D12 – FAG: Virus testing

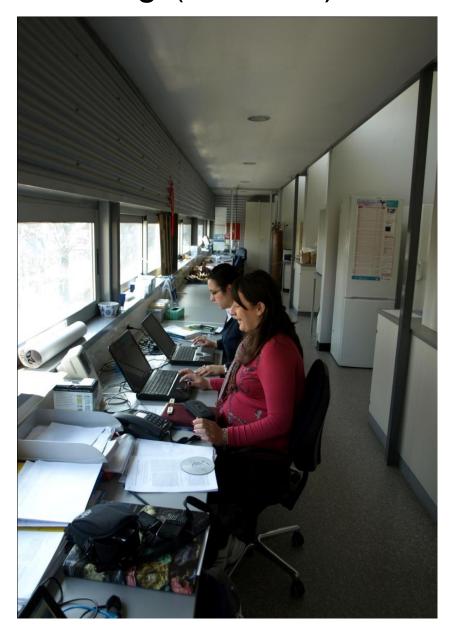


24.1.2012.

D13 – FAG: Grafting



Milena Mugoša has been on FAZ for one month training (lab work)





Maja Žulj Mihaljević has been on BOKU for one month training (lab work)



Project at the end: results' dissemination

- Professional and scientific papers (data analysis)
- Dissemination through scientific meetings (two papers accepted)
- This workshop to make familiar professional comunity and all relevant stakeholders with project results
- Thank you for coming and your interest in our work!

Workshop: Advanced management with autochthonous grapevine varieties

Topics:

- Field identification, collection and evaluation of grapevine autochthonous cultivars (D. Periner & M. Mugoša)
- Genetic identification of cultivars by SSR markers and its practical applications (M. Žulj Mihaljević)
- Clonal variation consequences and methods of detection (U. Anhalt)
- Cultivars' propagation (virus testing, grafting & certification scheme) - E. Rühl
- Autochthonous cultivars' quality assessment (E. Maletić and V. Maraš)
- Discussion on strategy of autochthonous cultivars' revitalization including administrative steps (moderators: I. Pejić and E. Rühl)