Centre of Excellence
for Biodiversity and Molecular Plant Breeding
(CoE CroP-BioDiv): Objectives and Activities

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# CENTERS OF EXCELLENCE

## 2014/2015

- 13 Centers of Excellence have been established

<table>
<thead>
<tr>
<th>No.</th>
<th>Centre</th>
<th>Field</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>CoE for Advanced Materials and Sensing Devices</td>
<td>Natural Sciences</td>
</tr>
<tr>
<td>2</td>
<td>CoE for Science and Technology</td>
<td>Natural Sciences</td>
</tr>
<tr>
<td>3</td>
<td>CoE for Quantum and Complex Systems and Representation of Lie Algebras</td>
<td>Natural Sciences</td>
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<tr>
<td>4</td>
<td>CoE for Reproductive and Regenerative Medicine</td>
<td>Biomedicine</td>
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<tr>
<td>5</td>
<td>CoE for Research in Viral Immunology and the Development of New Vaccines</td>
<td>Biomedicine</td>
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<tr>
<td>6</td>
<td>CoE for Basic, Clinical and Translational Neuroscience</td>
<td>Biomedicine</td>
</tr>
<tr>
<td>7</td>
<td>CoE for Biodiversity and Molecular Plant Breeding</td>
<td>Biotechnical Sciences</td>
</tr>
<tr>
<td>8</td>
<td>CoE for Marine Bioprospecting</td>
<td>Biotechnical Sciences</td>
</tr>
<tr>
<td>9</td>
<td>CoE for Personalised Health Care</td>
<td>Inter-disciplinary</td>
</tr>
<tr>
<td>10</td>
<td>CoE for Data Science and Cooperative Systems</td>
<td>Technical Sciences</td>
</tr>
<tr>
<td>11</td>
<td>CoE for Integrative Bioethics</td>
<td>Humanities</td>
</tr>
<tr>
<td>12</td>
<td>CoE for Croatian Glagolitism</td>
<td>Humanities</td>
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<tr>
<td>13</td>
<td>CoE for School Effectiveness and Management</td>
<td>Humanities</td>
</tr>
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</table>

- established for a period of five years
- status of the CoE may be prolonged for the next five years
THE PROJECT

2017

- call for projects
  financed by Ministry of Regional Development and EU Funds
  (European Structural and Investment Funds;
  Operational Programme Competitiveness and Cohesion 2014/20)
- for 10 CoE from a STEM field
- overall budget: ~50,000,000.00 EUR
- project
  Biodiversity and Molecular Plant Breeding
  Coordinated by: CoE CroP-BioDiv
  Start of project: 01/01/2018
  Duration: 5 years
  Budget: ~5,000,000.00 EUR
CoE CroP-BioDiv

+ 5 Member Institutions

Host Institution

University of Zagreb
Faculty of Agriculture

Institute for Agriculture and Tourism Poreč

University of Zagreb
Faculty of Science

Agricultural Institute Osijek

Institute for Adriatic Crops Split

J.J. Strossmayer University of Osijek
Faculty of Agrobiotechnical Sciences Osijek

Research team (2018):
30 Senior researchers
13 PhD students
3 Admin/Tech
OBJECTIVES

Conservation of Plant Genetic Resources >> increase the benefits
Phenotyping >> high-throughput phenotyping (HTP) platforms
Genotyping >> next-generation sequencing (NGS) techniques
Bioinformatics >> genome-wide association studies (GWAS)
Dissemination >> workshops, summer schools
Objective:
Increase of benefits arising out of use of plant genetic resources for food and agriculture

Background:
National Programme for Conservation and Sustainable Use of PGRFA
- numerous collections / limited budget

Tasks:
1. Further characterization and evaluation of accessions on agronomic, biochemical and genetic levels
2. Create a strong link between germplasm collections, scientific research and breeding programmes
2 PHENOTYPING

Objective:
Apply novel phenotypic tools for the analysis of plant traits

Background:
- research infrastructure: many small research groups, underutilized equipment, obsolete technology
- new solutions: high-throughput phenotyping (HTP)

Tasks:
1. Optimization of phenotyping protocols
2. Establish links with key institutions possessing infrastructure for high-throughput phenotyping
Objective:
Introduce novel genotyping methods

Background:
- research infrastructure: many small research groups, underutilized equipment, obsolete technology
- new solutions: next-generation sequencing techniques

Tasks:
1. Optimization of genotyping protocols
2. Evaluate and compare new genotyping methods according to the specific needs
Objective:
Implement novel statistical methodology

Background:
- hundreds of PCs; underutilized facilities at the University Computing Centre (SRCE) in Zagreb
- new solutions: genome-wide association studies (GWAS)

Tasks:
1. Relocate the data management and analysis processes from servers and PCs to computer clusters
2. Develop pipelines for big data bioinformatics
5 DISSEMINATION

Objective:
Communicate the activities of the CoE

Background:
- small and disconnected research groups
- ineffective transfer of knowledge
- general public: misconceptions about plant breeding

Tasks:
1. Academia: scientific papers, workshops, summer schools, scientific conference
2. Farmers and entrepreneurs in agriculture: joint activities, round tables
3. General public: popular articles, internet, radio, TV
MODEL PLANT SPECIES

Major Crops
- Maize
- Wheat
- Soybean

Traditional Crops
- Grape vine
- Olives

Promising Crops
- Brassicas
- Common bean
- Alliums
- Dalmatian pyrethrum
- Dalmatian sage
- Common bean
1 MAJOR CROPS

- maiz, wheat, soybean

Background:
- plant breeding programmes based on classical breeding methods

Goal:
- introduction of molecular breeding methods
- next-generation sequencing; GWAS
- traits: physiological traits
  quality traits
  resistance traits
'Klara' x 'Bezostaja 1'
Recombinant inbred lines (RILs)

<table>
<thead>
<tr>
<th>Genome</th>
<th>No. of markers</th>
<th>% markers</th>
<th>Length (cM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>458</td>
<td>42.13</td>
<td>2,215</td>
</tr>
<tr>
<td>B</td>
<td>522</td>
<td>48.02</td>
<td>1,993</td>
</tr>
<tr>
<td>D</td>
<td>107</td>
<td>9.84</td>
<td>451</td>
</tr>
</tbody>
</table>

No. of SNPs: 1,087
No. of linkage groups: 26
Total map length: 4,660 cM
Intermarker distance: 5.24 cM
2 TRADITIONAL CROPS

- grape vine, olives

Background:
- numerous traditional cultivars
- nursery production

Goal:
- detailed characterization of traditional cultivars (homonymy, synonymy, intracultivar diversity)
- introduction of molecular markers techniques in nursery production
- olives: phenotyping/genotyping of wild olive genetic resources
e.g. OLIVES

Molecular data: SSRs
Statistical analysis: FCA

2nd axis (20.48%)

1st axis (42.70%)

P01 Wild: Brijuni
P02 Wild: Pag
P03 Wild: Hvar
P04 Wild: Lastovo
P05 Wild: Pelješac
P06 Domesticated: Cultivars

Piculja
Mrčakinja

Southern Adriatic
Northern Adriatic

cultivars
feral olives

genuine wild olives

Genetic signatures of domestication
3 PROMISING CROPS

- Brassicas/Alliums, common bean, Dalmatian pyrethrum/sage

Background:
- no breeding programme
- richness of plant genetic resources

Brassicas: traditional cultivars (collard greens: B. oleracea var. acephala)
  wild relatives (B. incana, B. botterii, B. cazzae, B. mollis)

Alliums: traditional cultivars (onion, garlic, shallot...)

Common bean: traditional cultivars

Dalmatian pyrethrum/sage: wild populations

Goal:

Brassicas/Alliums: biochemical/genetic analyses; phylogeny

Common bean: QTL for bioactive nutrient content

Dalmatian pyrethrum: population genetics; pyrethrins

Dalmatian sage: population genetics; chemotypes
- the majority of the common bean production is based on local landraces
- grown by small-scale farmers in low input production systems
- no plant breeding programme

- landraces known by their traditional names
different seed coat patterns and colours
also found in neighbouring countries
(central Europe, western Balkans)
174 accessions >> 10 morphotypes

- aim: origin and genetic diversity
- goal: association mapping study
(1) Phaseoline type analysis
   - three types:
     1. type I - Mesoamerican ("S")
     2. type II - Andean ("H" or "C")
     3. type III - Andean ("T")

(2) Microsatellite markers (SSRs)
   - genetic distance: $D_{psa}$
   - tree: Neighbor-joining method
   - two well-supported clades
     (Mesoamerican vs Andean)
   - subclade
     (Andean type III)
Phaseoline type:
- I
- II
- III

Genetic cluster:
- A
- B₁
- B₂

SSRs

(3) Microsatellites
- Bayesian model-based clustering method for inferring population structure

- proportions of membership (Q) of each accession in each genetic cluster assuming two (K2) or three clusters (K3)

- clusters / phaseoline:
  - A - type I
    - Mesoamerican
  - B₁ - type II
    - Andean
  - B₂ - type III
    - Andean
**SNPs**

- Diversity Arrays Technology (DArT) based on genome complexity reduction and SNP detection through hybridization of PCR fragments

Schmutz et al. (2014) >> a reference genome for common bean
Valdisser et al. (2017) >> characterization of common bean core collection

<table>
<thead>
<tr>
<th></th>
<th>Valdisser et al. (2017)</th>
<th>This study</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of accessions</td>
<td>188*</td>
<td>174</td>
</tr>
<tr>
<td>No. of polymorphic markers</td>
<td>5,961</td>
<td>6,599</td>
</tr>
<tr>
<td>SNP/Mbp</td>
<td>11.58</td>
<td>12.85</td>
</tr>
<tr>
<td>Observed heterozygosity ($H_O$)</td>
<td>0.037</td>
<td>0.009</td>
</tr>
<tr>
<td>Expected heterozygosity ($H_E$)</td>
<td>0.443</td>
<td>0.373</td>
</tr>
</tbody>
</table>

*91 landraces + 97 cultivars from all over the world
(4) SNPs
- 923 markers
  (linkage equilibrium; LE)
- Bayesian model-based clustering method for inferring population structure
- same approach as for SSRs
- congruent results
Phaseoline type:
- I
- II
- III

Genetic cluster:
- A
- B₁
- B₂

I/A (Mesoamerican)
II/B₁ (Andean)
III/B₂ (Andean)
RELATIONSHIPS AMONG MORPHO-GENETIC GROUPS

Microsatellite markers (SSRs)
- genetic distance: Cavalli-Sforza chord distances
- tree: Neighbor-joining method

I/A
(Mesoamerican)

II/B₁
(Andean)

III/B₂
(Andean)

- INDETERMINATE: 'Trešnjevac'
- INDETERMINATE: 'Tetovac'
- INDETERMINATE: 'Kukuruzar'
- DETERMINATE: 'Biser'
- INDETERMINATE: 'Sivi'
- INDETERMINATE: 'Puter'
- INDETERMINATE: 'Maslina'
- INDETERMINATE: 'Dan i noć'
- DETERMINATE: 'Trešnjevac'
- DETERMINATE: 'Zelenčec'
- DETERMINATE: 'Dan i noć'
- DETERMINATE: 'Puter'
- DETERMINATE: 'Bijeli'

- 0.05
- 100
- 66
- 100
- 99
ASSOCIATION MAPPING

- goal: Identification of SNP markers linked to the quantitative trait loci (QTL) related to bioactive nutrient contents in common bean

1. PGR: Croatian common bean landraces
2. Phenotyping: The assessment of seed mineral diversity (Mg, Ca, Fe, Zn, K, P) and phytic acid content
3. Genotyping:
   - SSRs - genetic diversity and structure
   - SNPs - DArTseq high-density SNP genotyping
3. Bioinformatics:
   Genome-Wide Association Study (GWAS)
Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv)

Please visit: http://biodiv.iptpo.hr