54 Croatian
14 International Symposium on Agriculture
2019

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

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Europska unija
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EUROPSKI STRUKTURNI I INVESTICIJSKI FONDOVI

Operativni program
KONKURENTNOST
I KOHEZIJA

Republika Hrvatska

Vodice, 2019

# CENTERS OF EXCELLENCE

## 2014/2015

## - 13 Centers of Excellence have been established

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

- 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

## Host Institution

**FAZ**
University of Zagreb
Faculty of Agriculture

## + 5 Member Institutions

**IPTPO**
Institute for Agriculture
and Tourism Poreč

**PMF**
University of Zagreb
Faculty of Science

**PIO**
Agricultural Institute
Osijek

**IJK**
Institute for Adriatic
Crops Split

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

CoE CroP-BioDiv

**Research team (2018):**

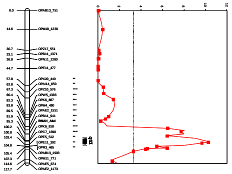
| | |
|---|---|
| 30 | Senior researchers |
| 13 | PhD students |
| 3 | Admin/Tech |

# OBJECTIVES

Conservation of PGR

Phenotyping

Genotyping



Bioinformatics

Dissemination

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

# 1 CONSERVATION OF PGR

**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 stong link between germplasm collections, scientific research and breeding programmes

# 2 PHENOTYPING

**Objective:**

Apply novel phyenotypic tools for the analysis of plant traits

**Background:**

- reseach infrastructure: many small reseach 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

# 3 GENOTYPING

**Objective:**

Introduce novel genotyping methods

**Background:**

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

**Tasks:**

1. Otpimization of genotyping protocols
2. Evaluate and compare new genotyping methods according to the specific needs

# 4 BIOINFORMATICS

**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 disconected 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 entrepeneurs 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
Alliums


Common bean


Dalmatian pyrethrum
Dalmatian sage

# 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

# e.g. WHEAT

'Klara' x 'Bezostaja 1'
Recombinant inbred lines (RILs)

Diversity Arrays Technology
IT'S A BULLSEYE WITH DArT

| Genome | No. of markers | % markers | Length (cM) |
|---|---|---|---|
| A | 458 | 42.13 | 2,215 |
| B | 522 | 48.02 | 1,993 |
| D | 107 | 9.84 | 451 |

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:**

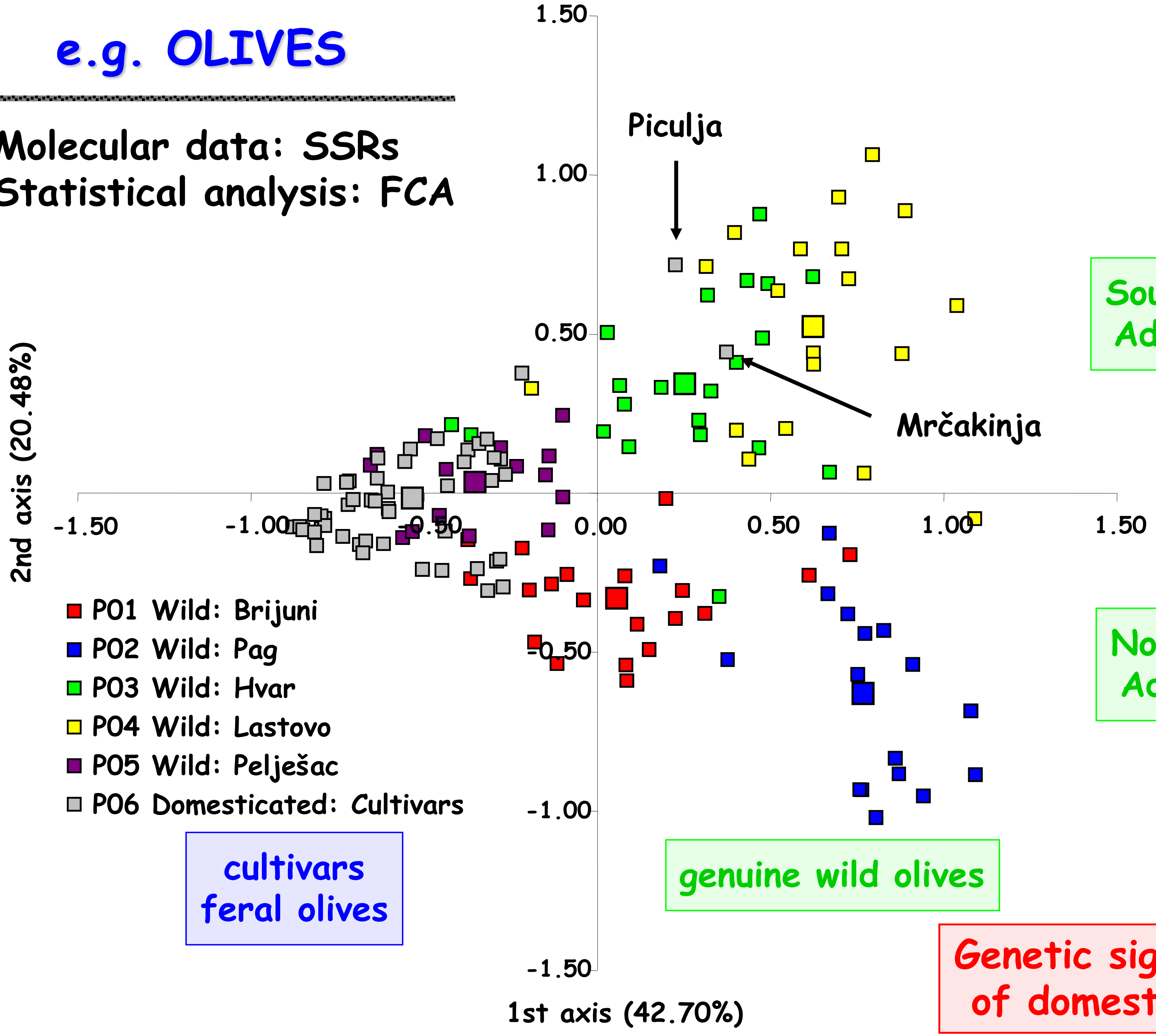
- numerous traditional cultivars
- nursery production

**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**



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 resouces

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

# e.g. COMMON BEAN

- 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


'Kukuruzar'


'Tetovac'


'Biser'

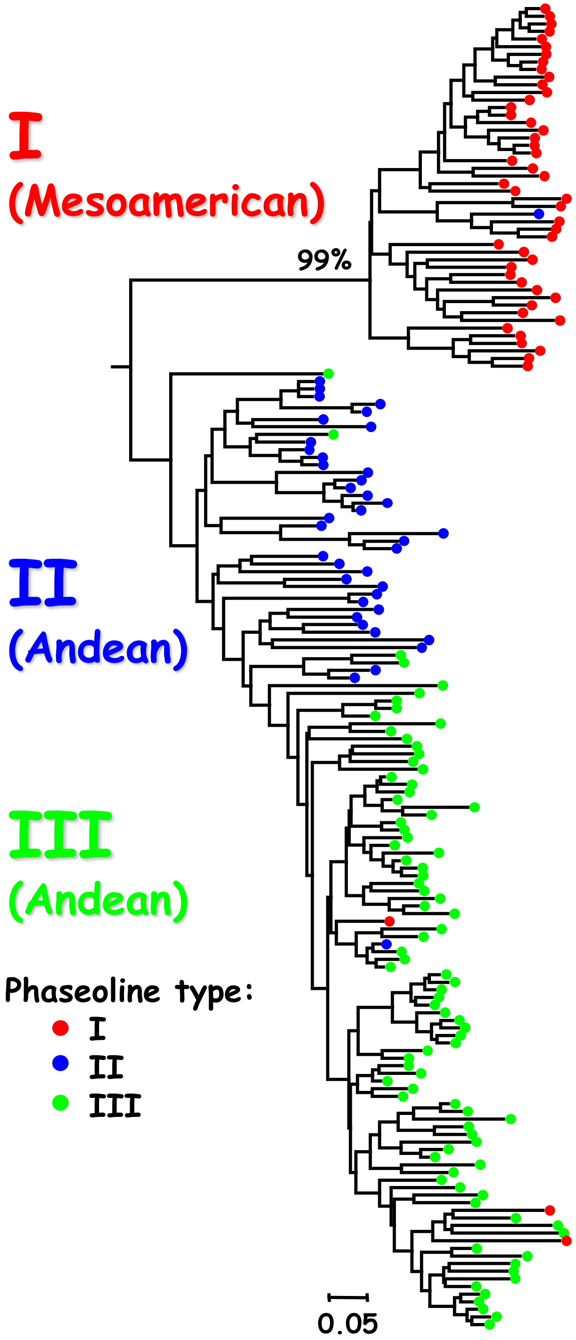

'Trešnjevac'


'Puter'


'Dan i noć'


'Zelenčec'

# PHASEOLINE TYPE ANALYSIS



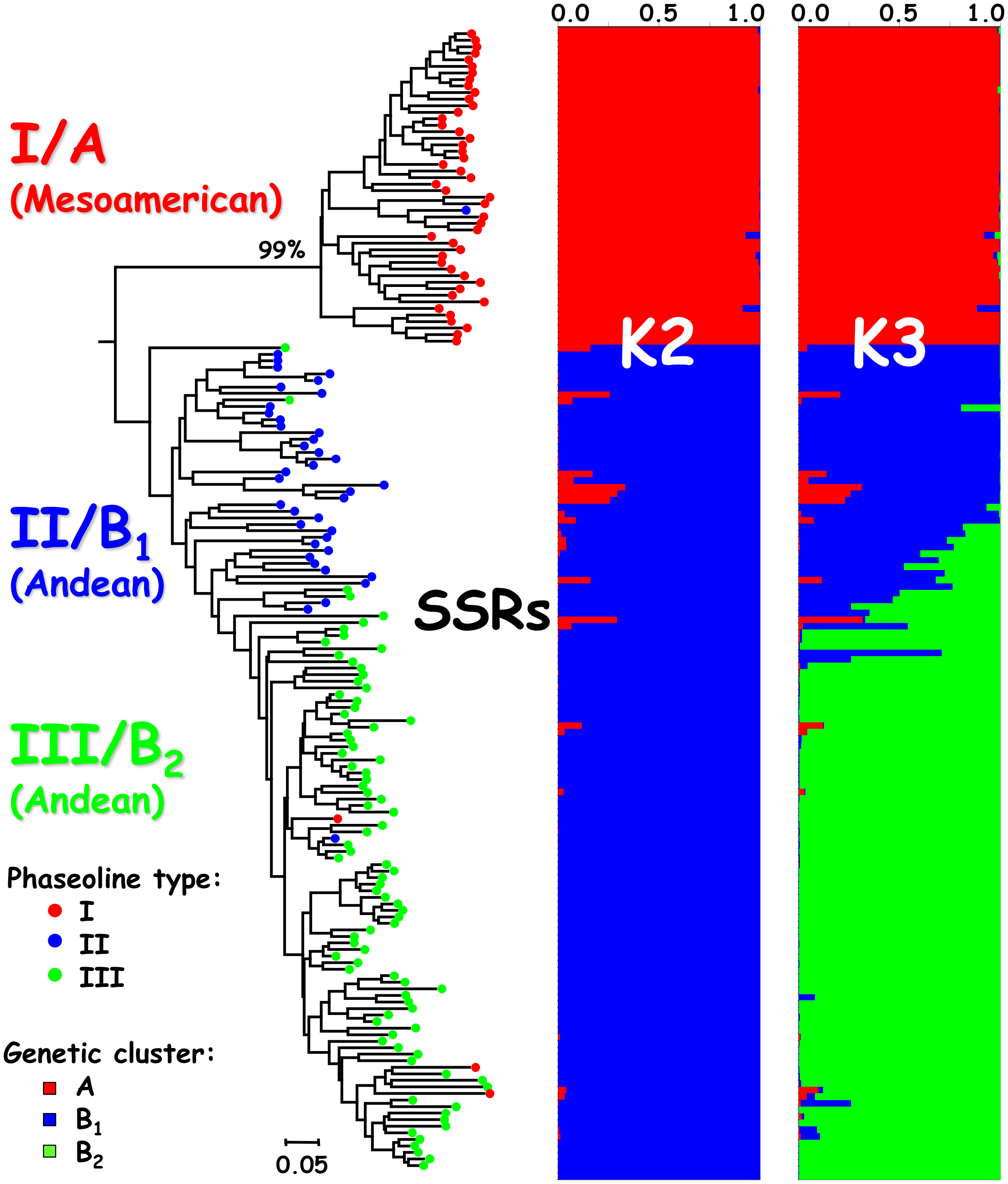
(1) Phaseoline type analysis

- 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)

# 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
  - $B_1$ - type II Andean
  - $B_2$ - type III Andean

# SNPs

Diversity Arrays Technology
IT'S A BULLSEYE WITH DArT

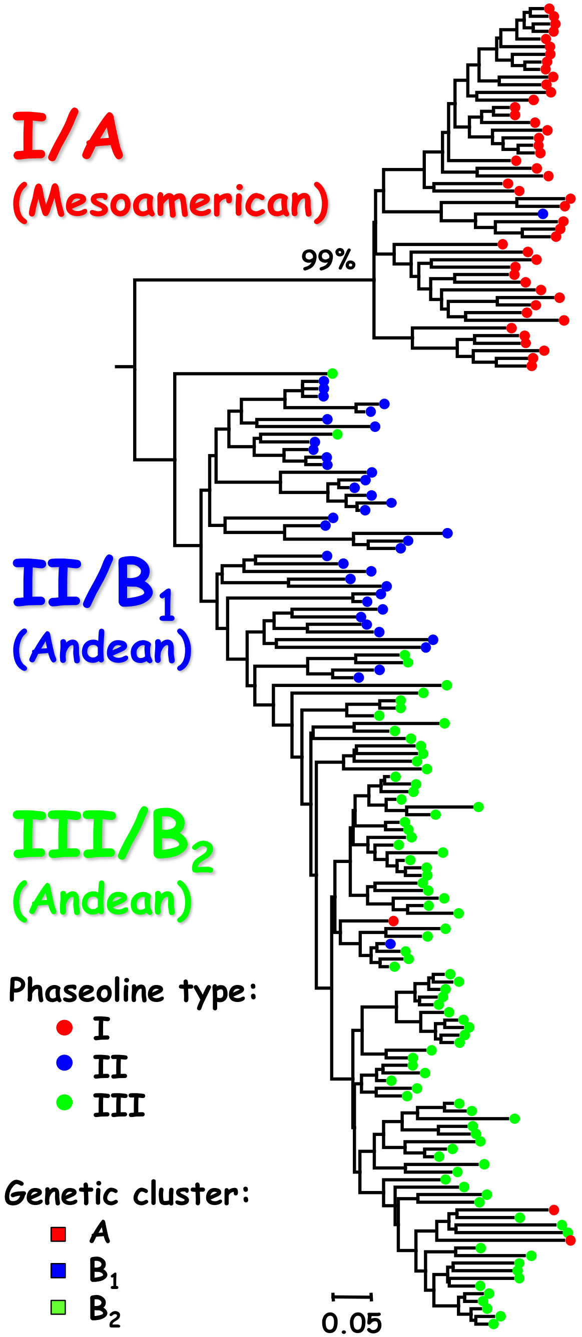
- Diversity Arrays Technology (DArT) based on genome complexity reduction and SNP detection through hybridization of PCR fragments

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

| | Valdisser et al. (2017) | This study |
|---|---|---|
| No. of accessions | 188* | 174 |
| No. of polymorphic markers | 5,961 | 6,599 |
| SNP/Mbp | 11.58 | 12.85 |
| Observed heterozygosity ($H_O$) | 0.037 | 0.009 |
| Expected heterozygosity ($H_E$) | 0.443 | 0.373 |

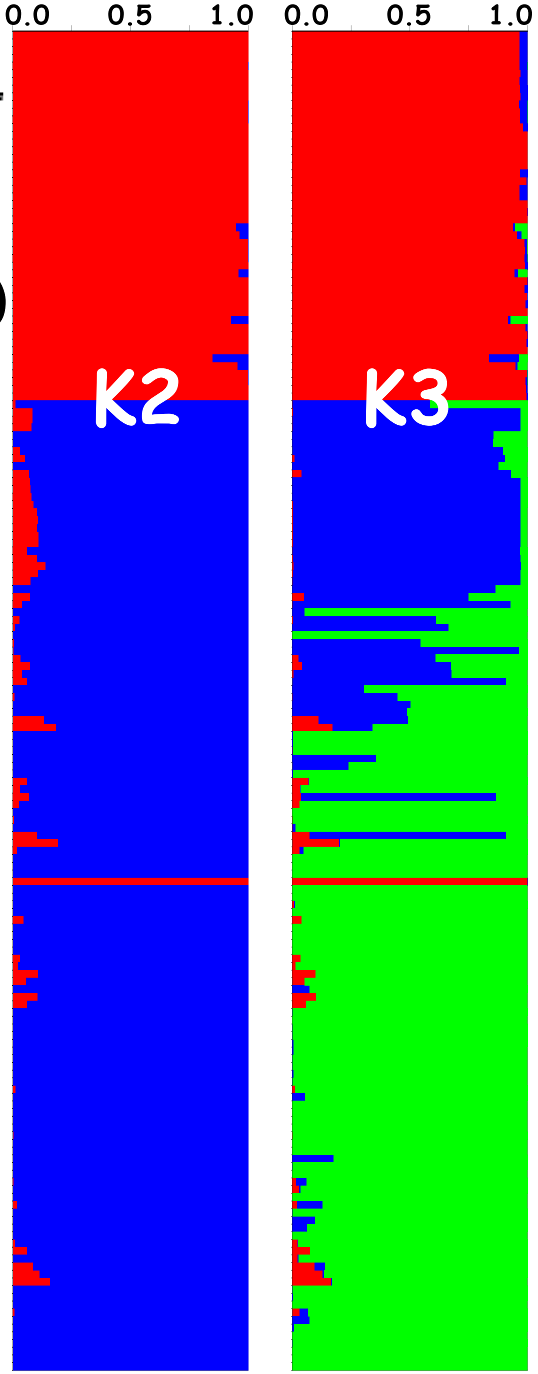
*91 landraces + 97 cultivars from all over the world

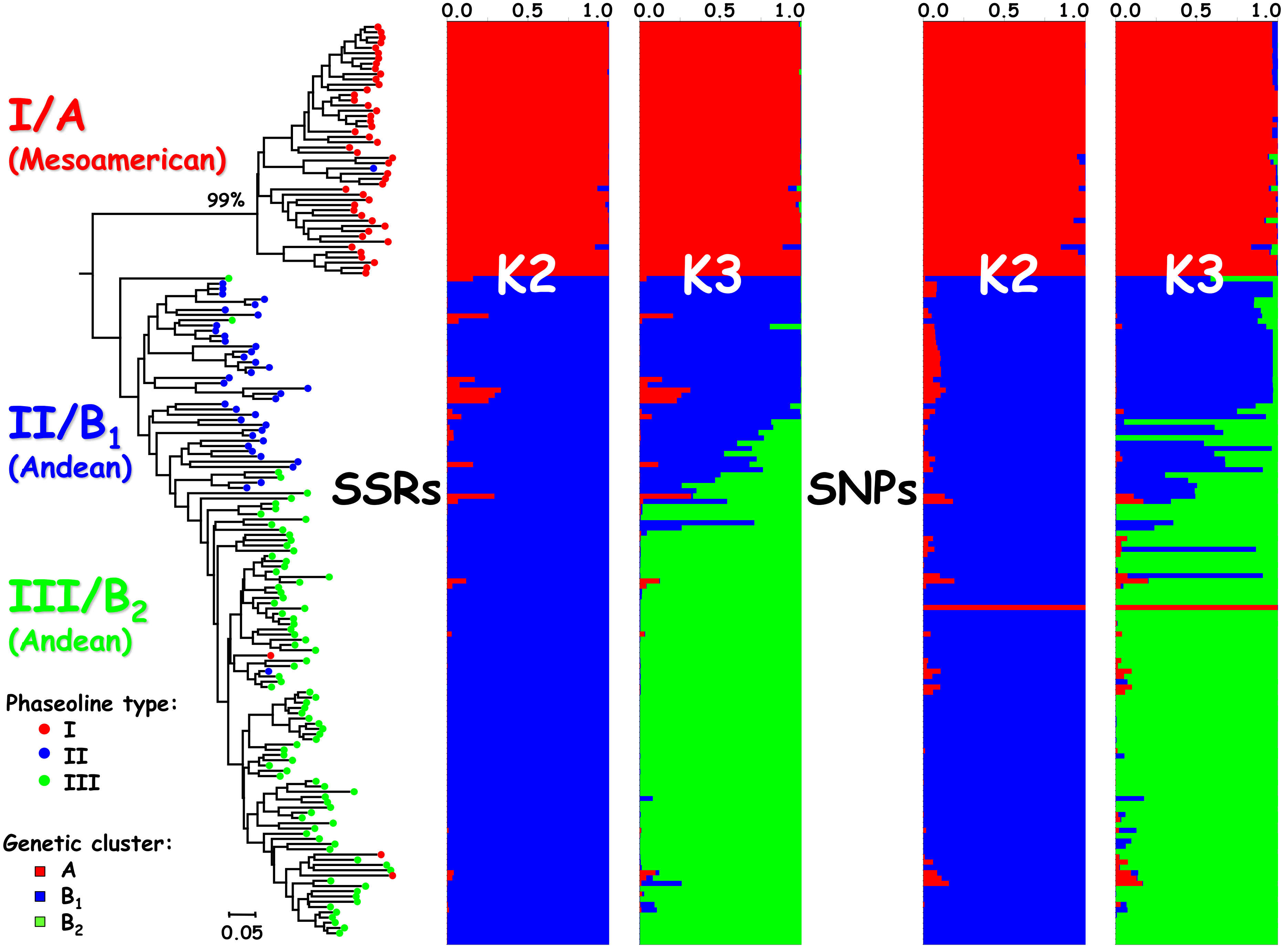
# SNPs



## (4) SNPs

- 923 markers
  (linkage equilibirum; LE)
- Bayesian model-
  based clustering
  method for inferring
  population structure
- same approach as for
  SSRs
- congruent results

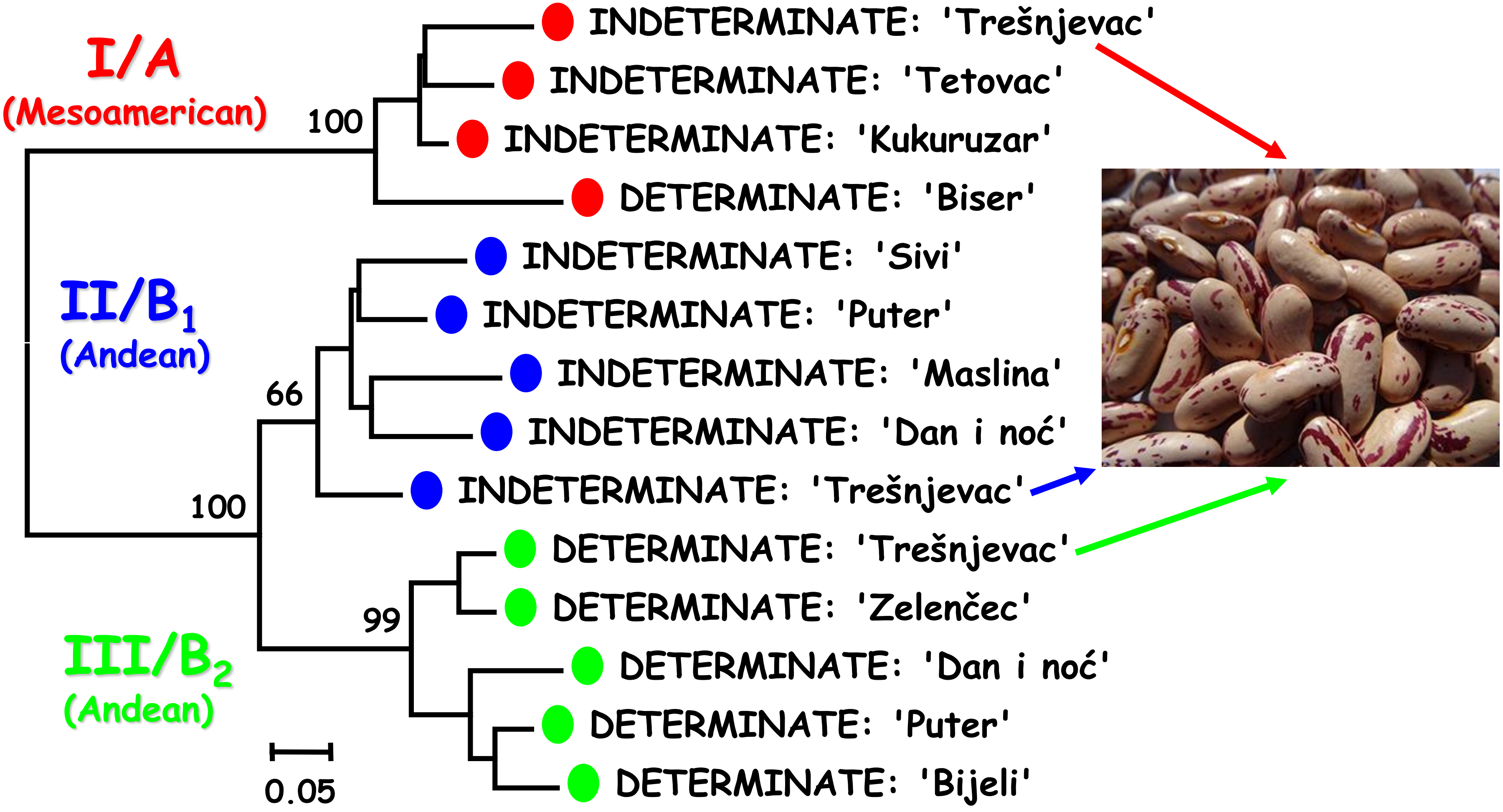



I/A
(Mesoamerican)
99%
II/B1
(Andean)
III/B2
(Andean)
Phaseoline type:
● I
● II
● III
Genetic cluster:
🟥 A
🟦 B1
🟩 B2
0.05
SSRs
SNPs
K2
K3
K2
K3
0.0
0.5
1.0
0.0
0.5
1.0
0.0
0.5
1.0
0.0
0.5
1.0

# RELATIONSHIPS AMONG MORPHO-GENETIC GROUPS

**Microsatellite markers (SSRs)**

- genetic distance: Cavalli-Sforza chord distances
- tree: Neighbor-joining method



# 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)

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