ORIGIN AND GENETIC DIVERSITY OF CROATIAN COMMON BEAN LANDRACES
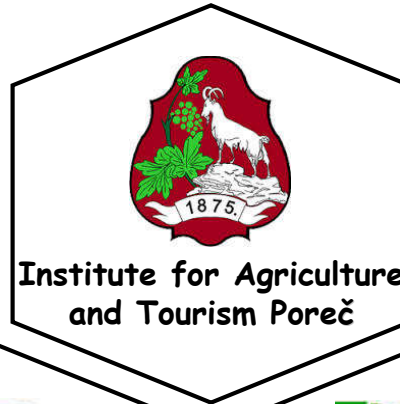
Zlatko Šatović

University of Zagreb, Faculty of Agriculture, Zagreb, Croatia

Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Zagreb, Croatia
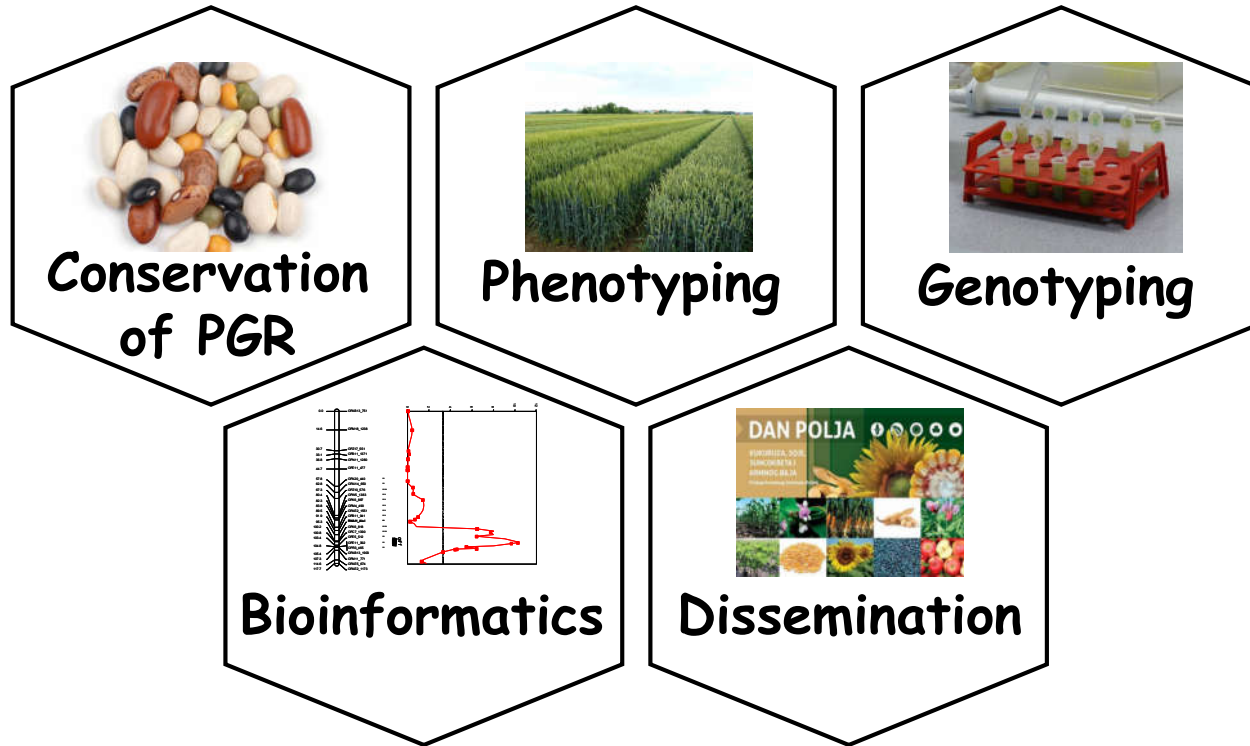
E-mail: zsatovic@agr.hr

Belgrade, 2018

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

University of Osijek, Faculty of Agriculture

CoE CroP-BioDiv

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

MODEL PLANT SPECIES

Major Crops

Maize

Wheat

Soybean

Traditional Crops

Grapevine

Olives

Promising Crops

Brassicas
Alliums

Common bean

Dalmatian pyrethrum
Dalmatian sage

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PLAN

(1) Introduction: Common Bean Domestication

(2) Materials and Methods

(3) Origin and genetic diversity

(4) Further research

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○ Origin of wild common bean: Mesoamerica

→ Divergence: Andean wild bean diverged from Mesoamerican prior to domestication (~100,000 BP)

DOMESTICATION

Domestication: Independently domesticated in Mesoamerica and the Andes (~8,000 BP)

🔴 Mesoamerican centre: indeterminate (maize-bean-squash multicrop system)

🔵 Andean centre: determinate types (no suitable crop: root crops, quinoa)

DIVERSIFICATION

Domestication:
🔴 Mesoamerican centre: indeterminate
🔵 Andean centre: determinate

Diversification: Adaptation to new environments and local preferences
🟢 Andean centre: indeterminate (after maize introduction: ~7,000-6,000 BP)
🟠 Mesoamerican centre: determinate (determinacy has been selected independently in both centres)

INTRODUCTION TO EUROPE
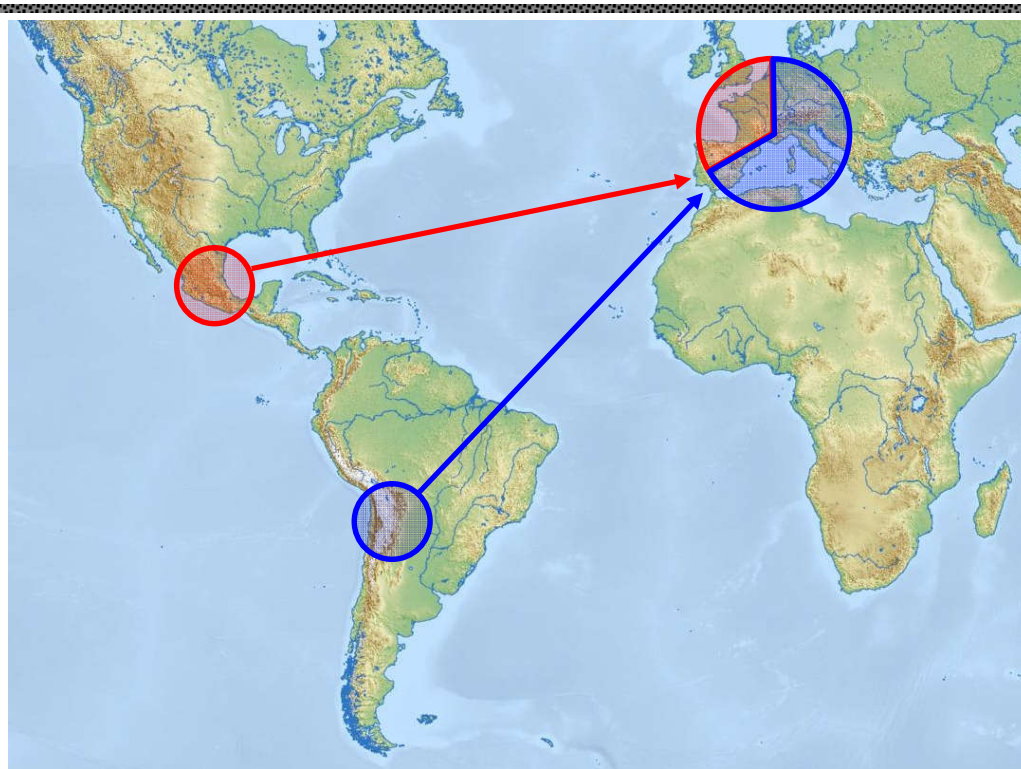
⇒ **Introduction to Europe:**
   16th century
   Protugal and Spain
   several introductions

Origin (phaseoline type):
   Mesoamerican 33%
   Andean 67%
Hybrids:
   up to 33%

- Balkans:

(A) The proportion of landraces of the Mesoamerican origin tends to increase in eastern and south-eastern Europe (Albania, Bulgaria, Macedonia, Greece)

(B) The proportions found in accessions from Bosnia and Herzegovina, Croatia, Serbia and Slovenia were very similar to those found in the Iberian Peninsula and Italy indicating that common bean was introduced mainly from the Mediterranean Basin

PLAN

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- 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'

(1) Phaseoline type analysis
      - a DNA marker for phaseolin-type diversity
(2) Microsatellite markers (SSRs)
      - 26 markers / 135 alleles
(3) DArTseq SNP genotyping (SNPs)
      - 6,599 polymorphic SNP markers

PLAN

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PHASEOLINE TYPE ANALYSIS

I
(Mesoamerican)

99%

II
(Andean)

III
(Andean)

Phaseoline type:
- I
- II
- III

0.05
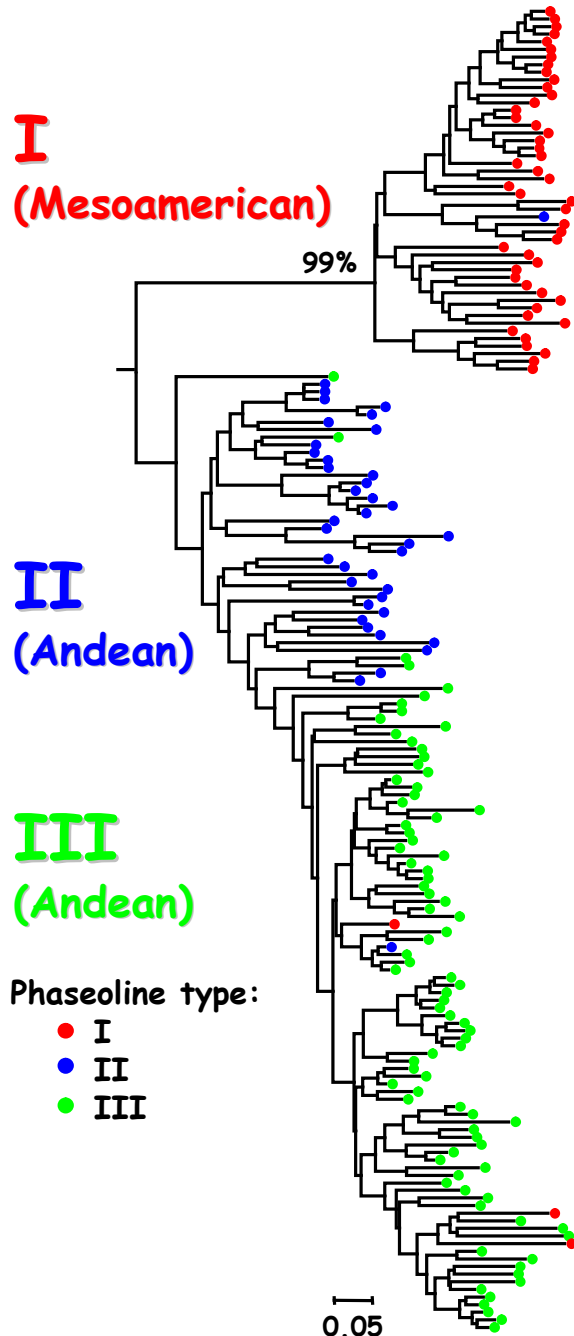
(1) Phaseoline type analysis
- three types:
>   1. type I - Mesoamerican ("S")
>   2. type II - Andean ("H" or "C")
>   3. type III - Andean ("T")

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

SSRs

I/A
(Mesoamerican)

99%

II/B₁
(Andean)

III/B₂
(Andean)

Phaseoline type:
- ● I
- ● II
- ● III

Genetic cluster:
- ■ A
- ■ B₁
- ■ B₂

SSRs

K2

K3

0.05
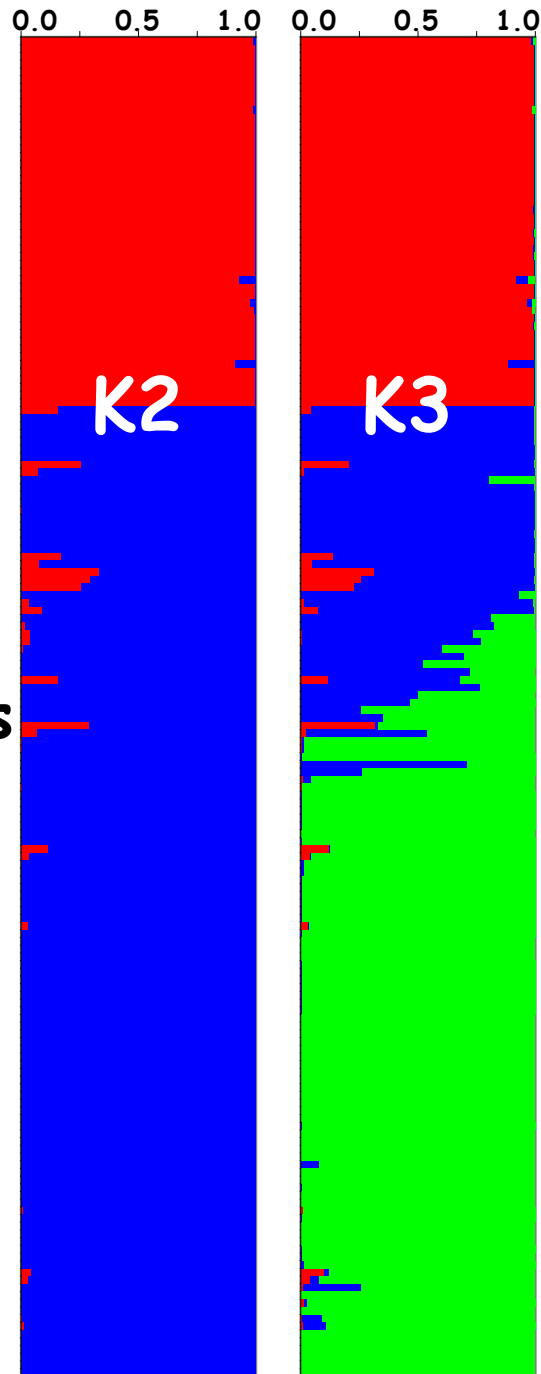
(3) **Microsatellites**
- Bayesian model-based clustering method for inferring population structure
- proportions of membership (Q) of each accession in each genetic cluster assuming two (K2) or three clusters (K3)
- clusters / phaseoline:
  A – type I Mesoamerican
  B₁ – type II Andean
  B₂ – type III Andean

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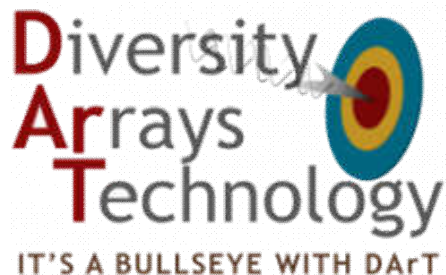
– Diversity Arrays Technology (DArT) based on genome complexity reduction and SNP detection through hybridization of PCR fragments

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

	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

I/A
(Mesoamerican)

99%

II/B$_1$
(Andean)

III/B$_2$
(Andean)

Phaseoline type:
● I
● II
● III

Genetic cluster:
■ A
■ B$_1$
■ B$_2$

0.05

(4) SNPs
- 923 markers
  (linkage equilibirum; LE)
- Bayesian model-
  based clustering
  method for inferring
  population structure
- same approach as for
  SSRs
- congruent results
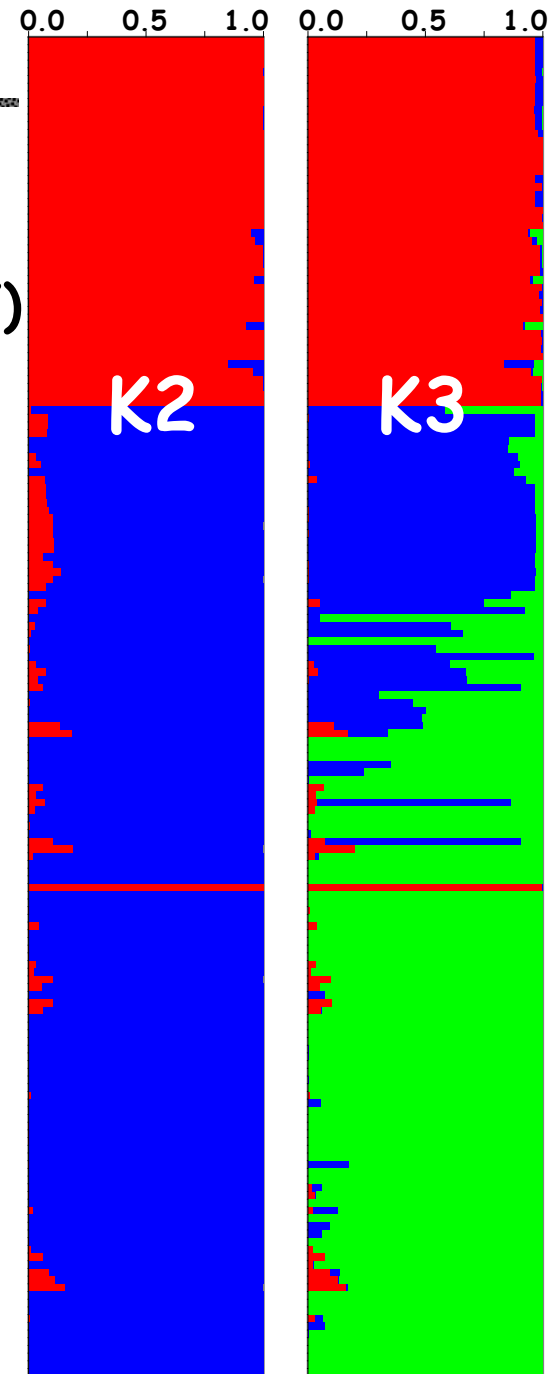
0.0 0.5 1.0   0.0 0.5 1.0

K2   **K3**

I/A
(Mesoamerican)

99%

II/B₁
(Andean)

III/B₂
(Andean)

SSRs

SNPs

K2

K3

K2

K3

Phaseoline type:
● I
● II
● III

Genetic cluster:
■ A
■ B₁
■ B₂

0.05

0.0   0.5   1.0

I/A
(Mesoamerican)

99%

II/B$_1$
(Andean)

SSRs

III/B$_2$
(Andean)

Phaseoline type:
- I
- II
- III

Genetic cluster:
- A
- B$_1$
- B$_2$

0.05
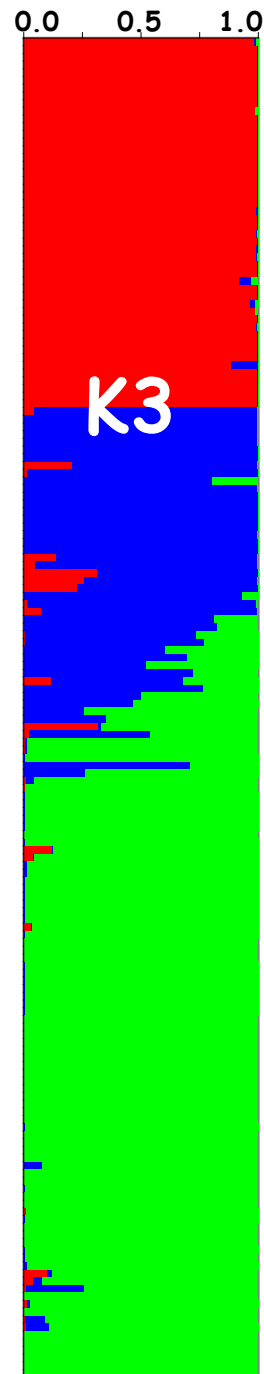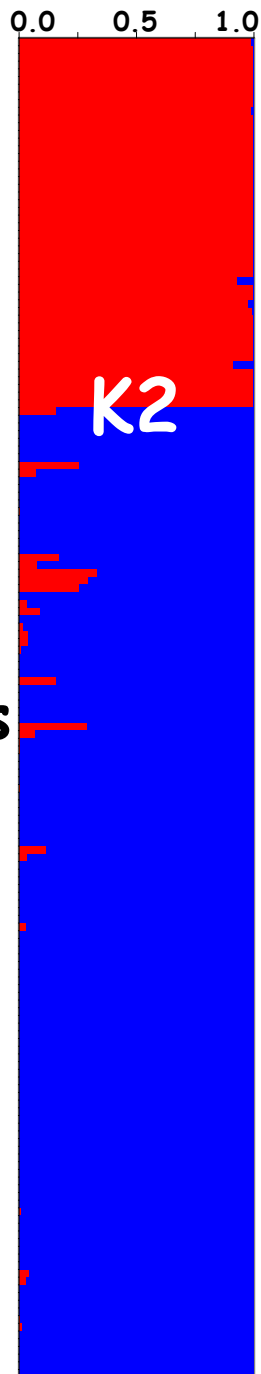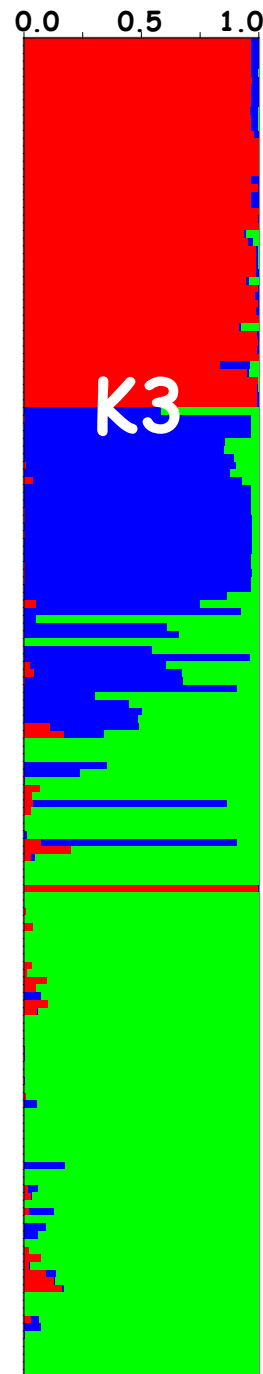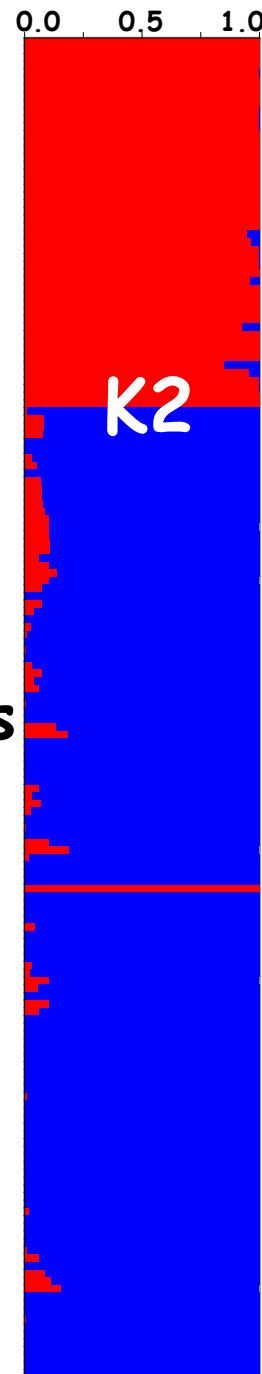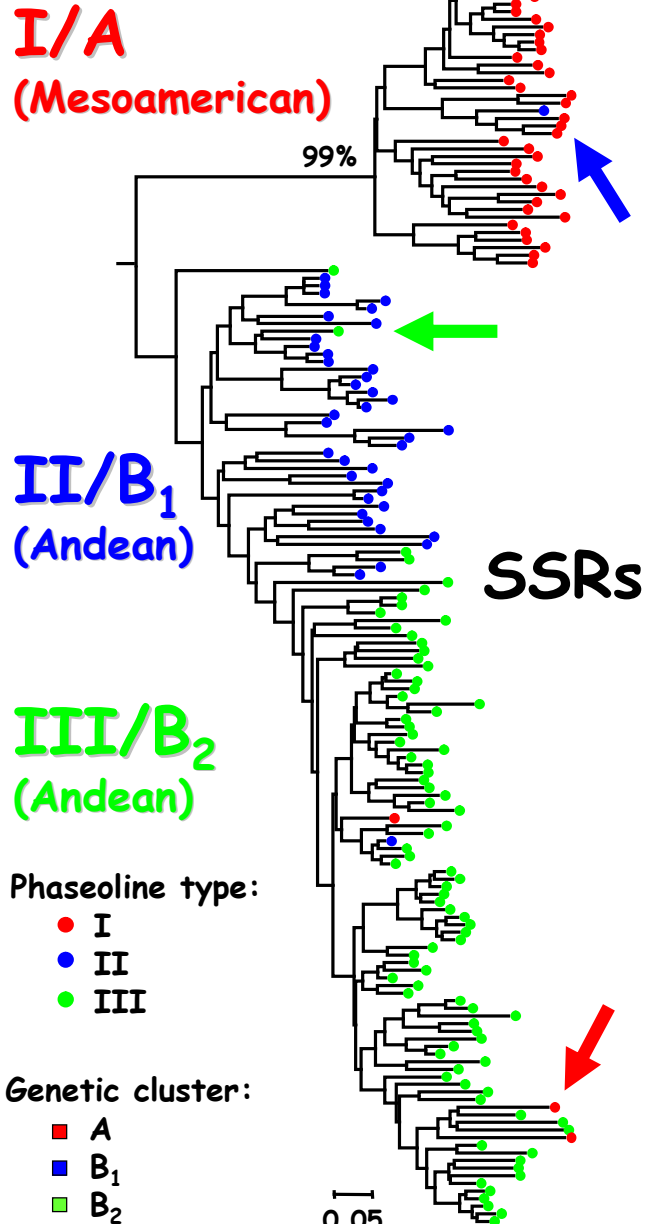
(1) True-types
- phaseolin type matches the cluster membership (SSRs and SNPs)
- percentage of cluster membership (Q) higher than 75%

(2) Offtypes
- putative hybrids
(2.1) Non-corresponding
  no correspondence between phaseolin types and clusters

I/A
(Mesoamerican)

99%

II/B$_1$
(Andean)

III/B$_2$
(Andean)

Phaseoline type:
● I
● II
● III

Genetic cluster:
■ A
■ B$_1$
■ B$_2$

0.05

SSRs

0.0   0.5   1.0   0.0   0.5   1.0
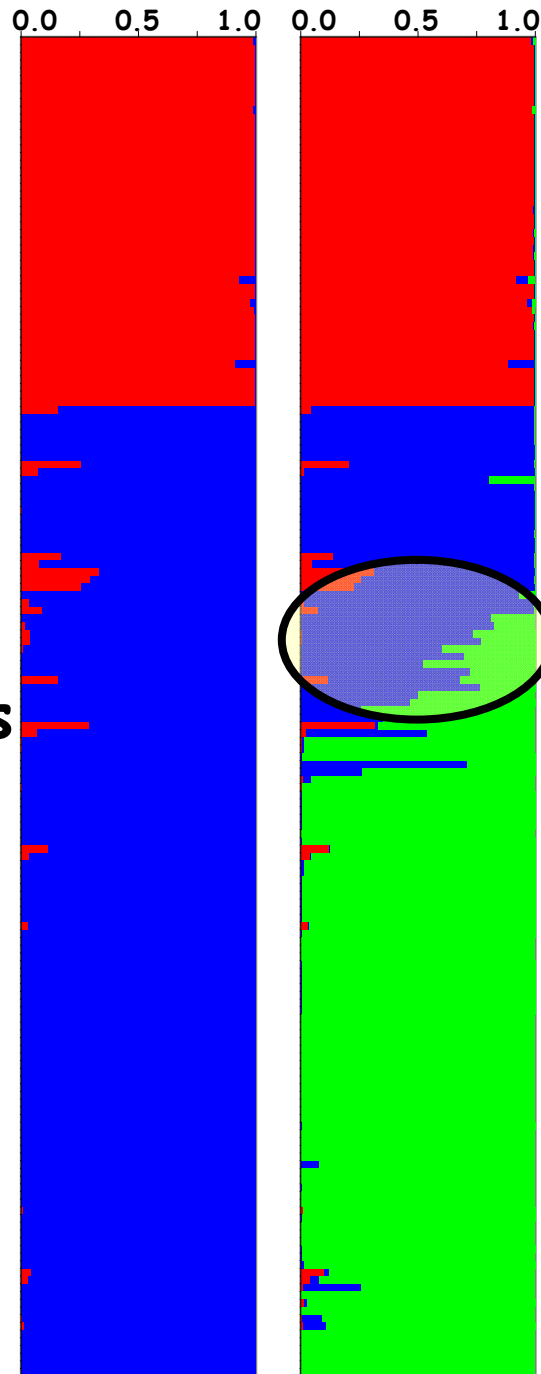
(2.2) Mixed origin
- percentage of
  cluster membership
  (Q) lower than 75%
  based on SSRs or
  SNPs

I/A
(Mesoamerican)

99%

II/B$_1$
(Andean)

SSRs

III/B$_2$
(Andean)

SNPs

Phaseoline type:
- I
- II
- III

Genetic cluster:
- A
- B$_1$
- B$_2$

0.05

ORIGIN

CROATIA

Offtypes 22%
I/A 25%
III/B$_2$ 39%
II/B$_1$ 14%
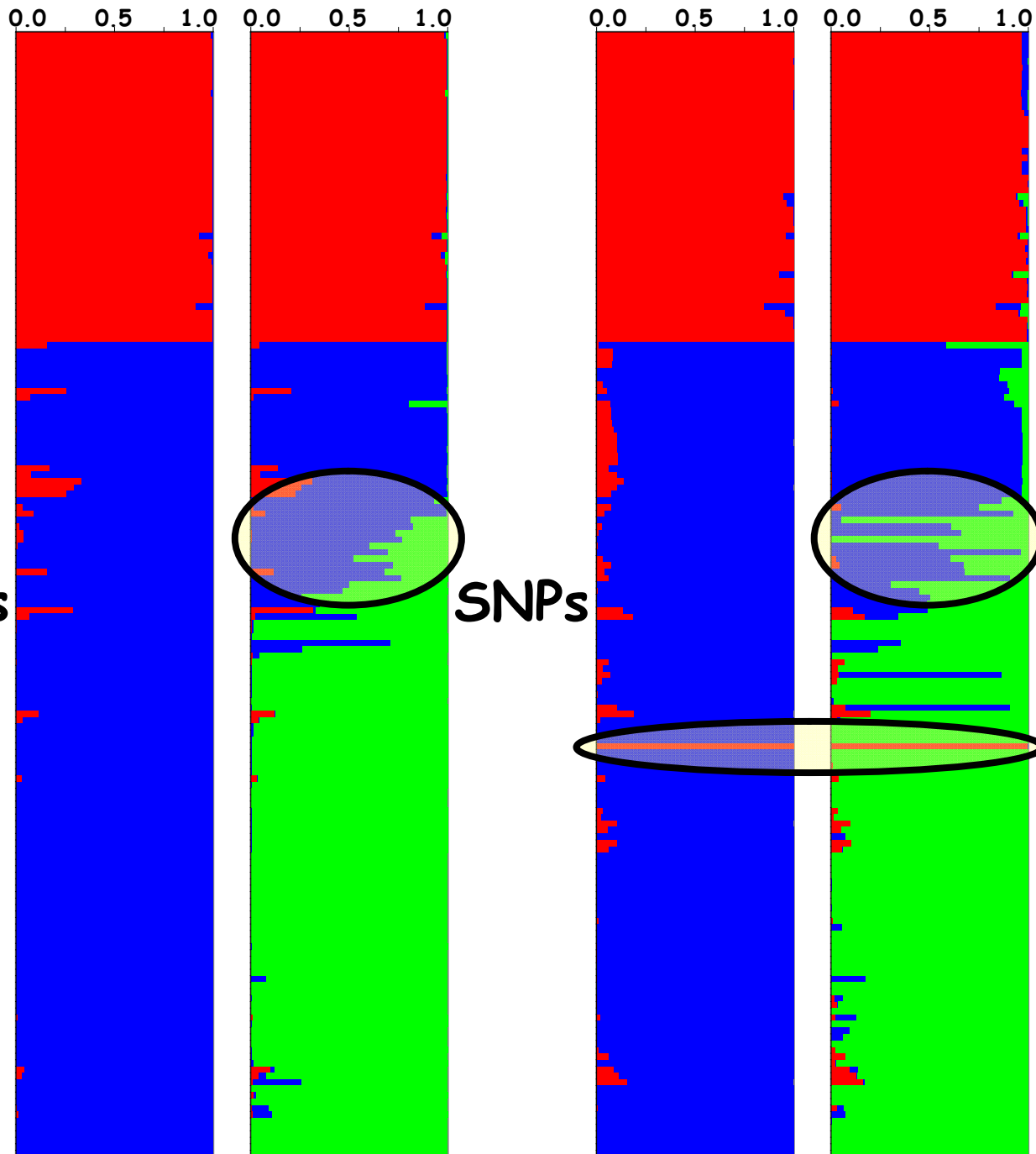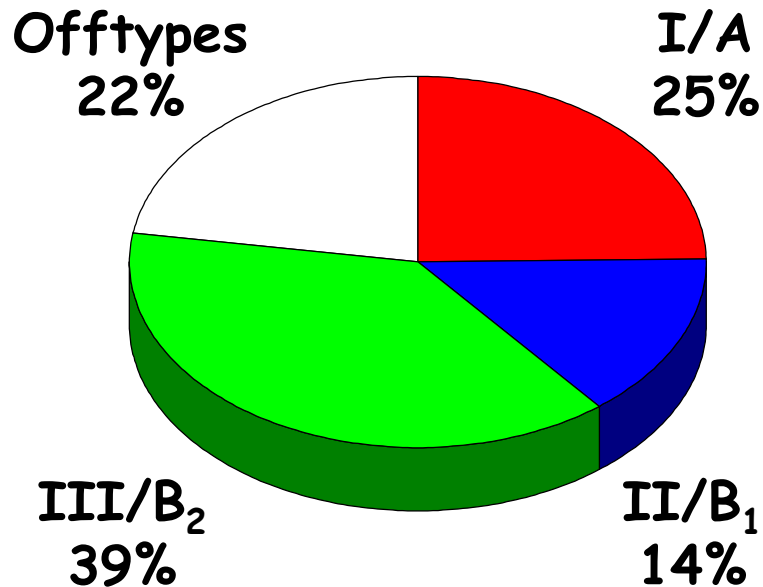
174 accessions
(1 plant/accession)

PORTUGAL

Offtypes 32%
I/A 15%
III/B$_2$ 21%
II/B$_1$ 32%
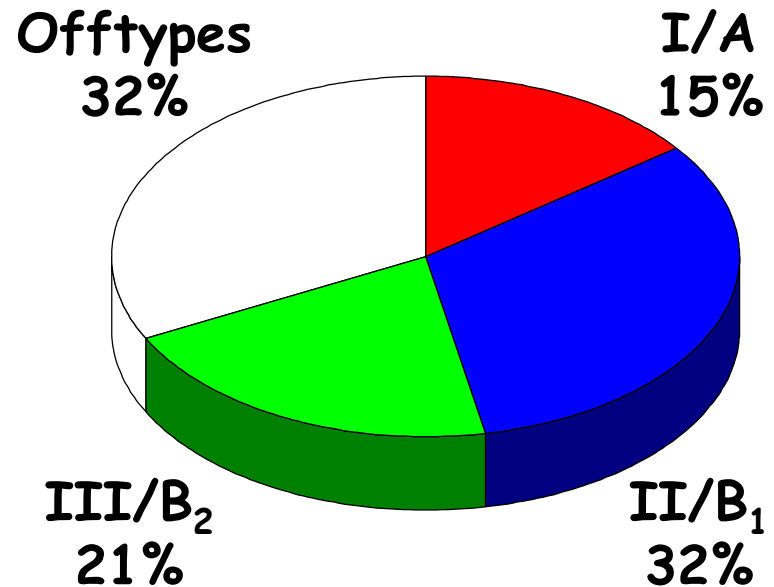
175 accessions
(10 plants/accession)

Leitão et al. (2017)

GENETIC DIVERSITY

(1) SSRs

    26 markers

    $H_O = 0.000$ **(autogamy)**

    II/B1: highest H_E
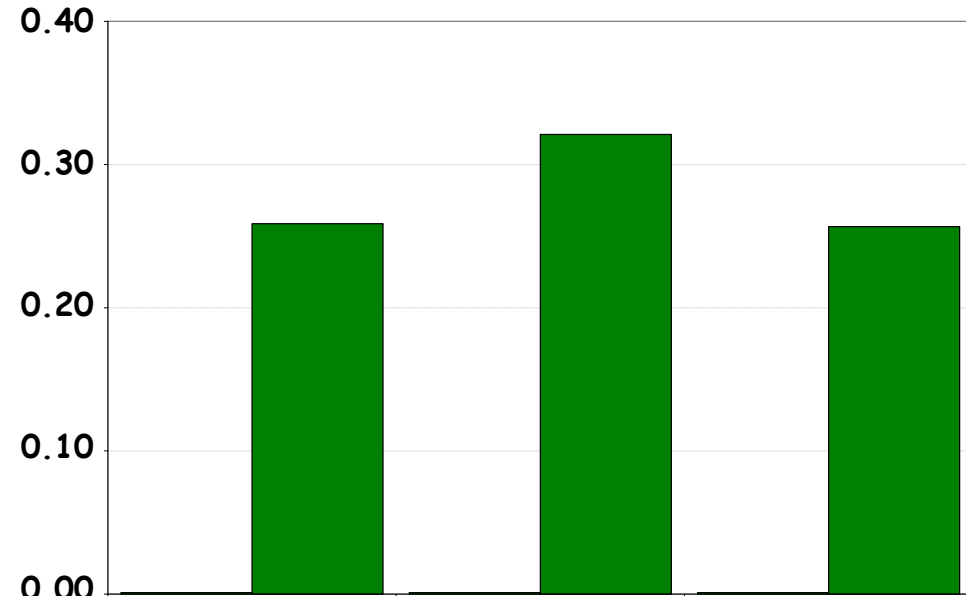
(2) SNPs

    6,599 markers

    $H_O > 0.000$

    I/A: highest H_E
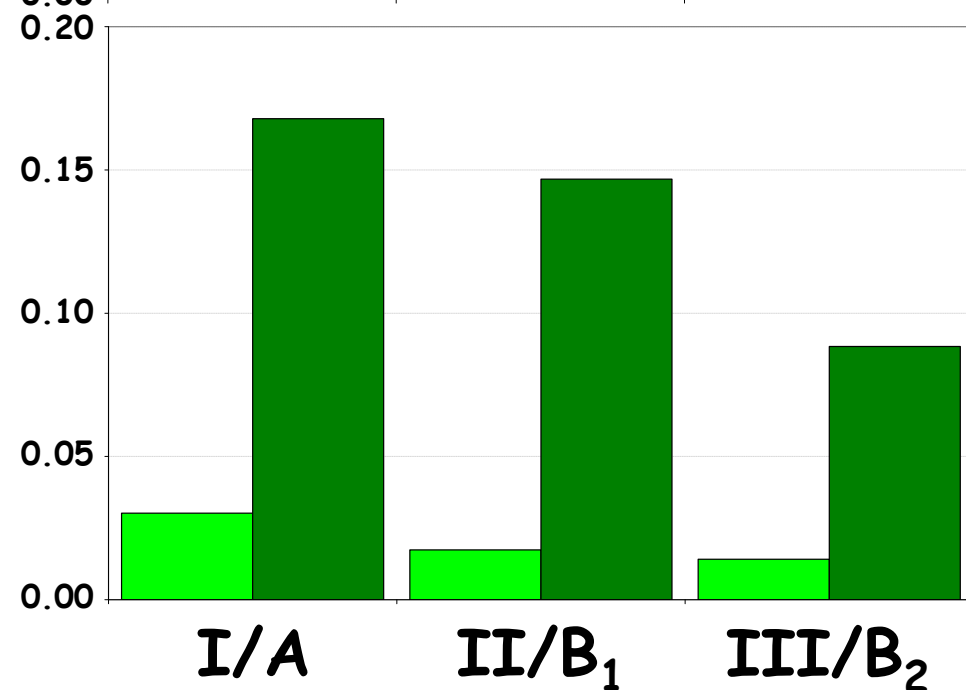
☐ Observed heterozygosity (H_O)

■ Expected heterozygosity (H_E)
    = gene diversity

MORPHO-GENETIC GROUPS

- from 10 morphotypes to 16 morpho-genetic groups

I/A (Mesoamerican)	II/B1 (Andean)	III/B2 (Andean)
'Trešnjevac' INDETERMINATE	'Trešnjevac' INDETERMINATE	'Trešnjevac' DETERMINATE
'Kukuruzar' INDETERMINATE	'Puter' INDETERMINATE	'Puter' DETERMINATE
'Tetovac' INDETERMINATE	'Dan i noć' INDETERMINATE	'Dan i noć' DETERMINATE
'Biser' DETERMINATE	'Sivi' INDETERMINATE	'Zelenčec' DETERMINATE

MORPHO-GENETIC GROUPS

I/A (Mesoamerican)	II/B1 (Andean)	III/B2 (Andean)
'Trešnjevac' INDETERMINATE	'Trešnjevac' INDETERMINATE	'Trešnjevac' DETERMINATE
'Kukuruzar' INDETERMINATE	'Puter' INDETERMINATE	'Puter' DETERMINATE
'Tetovac' INDETERMINATE	'Dan i noć' INDETERMINATE	'Dan i noć' DETERMINATE
'Biser' DETERMINATE	'Sivi' INDETERMINATE	'Zelenčec' DETERMINATE

MORPHO-GENETIC GROUPS

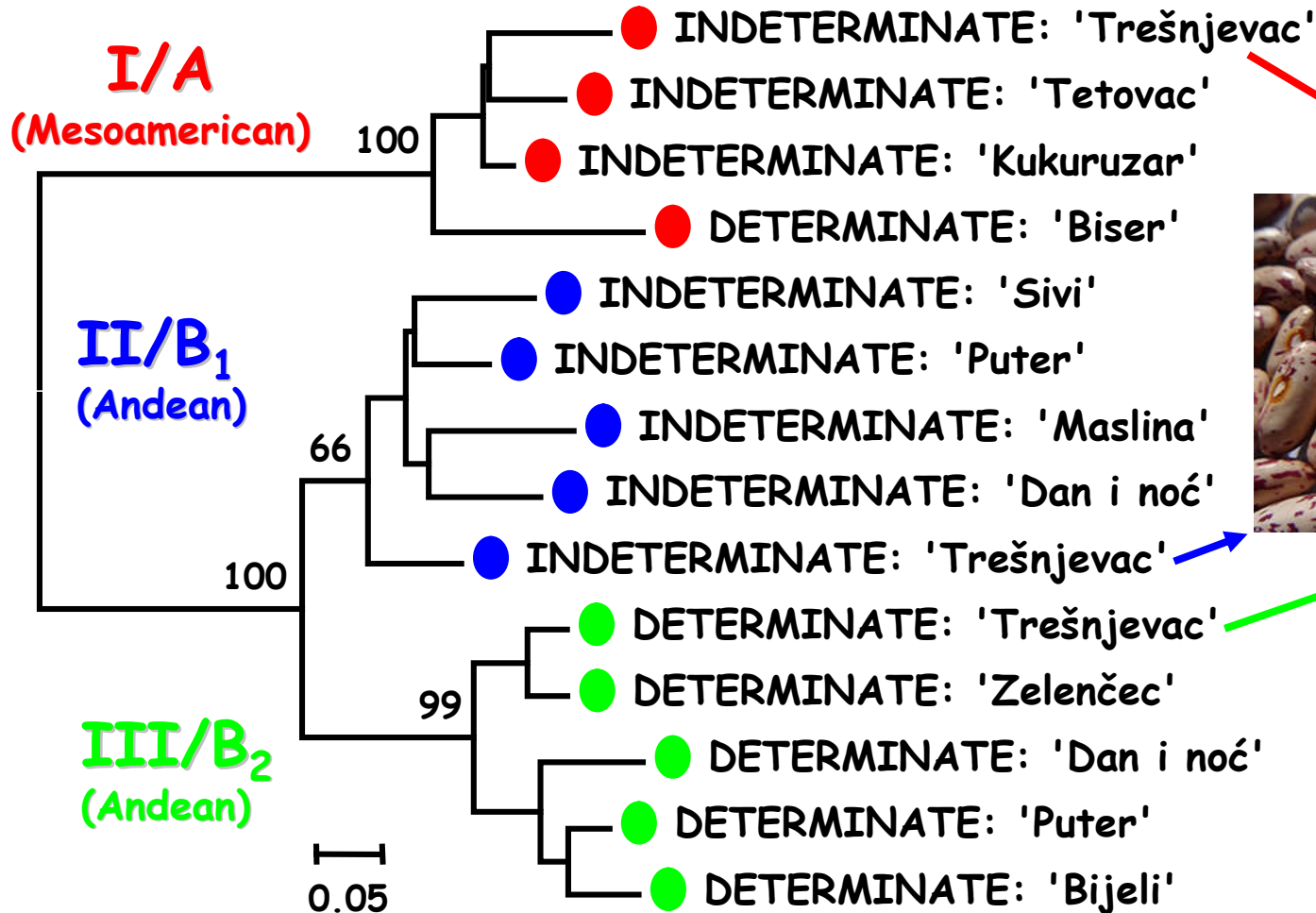| I/A (Mesoamerican)	II/B1 (Andean)	III/B2 (Andean)
'Trešnjevac' INDETERMINATE	'Trešnjevac' INDETERMINATE	'Trešnjevac' DETERMINATE
'Kukuruzar' INDETERMINATE	'Puter' INDETERMINATE	'Puter' DETERMINATE
'Tetovac' INDETERMINATE	'Dan i noć' INDETERMINATE	'Dan i noć' DETERMINATE
'Biser' DETERMINATE	'Sivi' INDETERMINATE	'Zelenčec' DETERMINATE

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- 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)

ACKNOWLEDGEMENTS

http://biodiv.iptpo.hr