Institute of Molecular Genetics and Genetic Engineering
Belgrade
Serbia

University of Belgrade

- **31 faculty**
- **90 000 students**
- **400 000 graduates**

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IMGGE, Serbia, Belgrade

Institute of Molecular Genetics and Genetic Engineering (IMGGE - formerly Centre for Genetic Engineering) was founded in 1986. The Institute is associated to International Center for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy, as an affiliated centre and it is involved in all activities within this international organization.

In the field of fundamental research, Institute's projects are mostly focused on research related to analysis of genome organization and regulation of gene expression in different organisms. Furthermore, IMGGE is also oriented to biotechnological and commercial applications in human and veterinary medicine, agriculture, food production, and pharmaceutical industry.

The Institute consists of six laboratories:

- Laboratory for Molecular Hematology
- Laboratory for Molecular Biology
- Laboratory for Human Molecular Genetics
- Laboratory for Microbial Molecular Genetics and Ecology
- Laboratory for Molecular Genetics of Industrial Microorganisms
- *Laboratory for Laboratory Plant Molecular Biology*

12 members
9 PhD
1 associate professor (Faculty of Biology)
2 principal research fellow
1 senior research associate
2 research associates

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Current project

- **The role of transcription factors and small RNAs in abiotic stress response in plants and genetic diversity of plant species important for agriculture and biotechnology**
- **Bilateral projects:**
  - **France**
  - **Slovenia**
  - **Portugal**
- **SEE ERA NET**

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Sustainable preservation of indigenous South Eastern European legumes and their traditional food and feed products SEELEGUMES

WP2 - Describing SEE legumes will be focused on a conventional and molecular characterization of the collected species and an analysis of their nutritional and other values

Proteomic analysis-2D electrophoresis; heat stable proteins, phosphoproteom

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WP2 - Describing SEE legumes

- Testing of drought tolerance of *Pisum* sp. *(Pisum sativum*, *P.arvense*, *P.fulvum*, *P.abyssinicum)* and *Lathyrus (L.sativus* and *L.cicera)* in greenhouse

- Growing of winter-season legumes in greenhouse and on the field-intercropping

MILESTONES:

- **delay of 1st instalement**
- **problem with IEF apparatus**

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WP2 - Describing SEE legumes

- Clonning end expression analysis of DREB2A transcription factor from *Pisum sativum* (Model Legume Congress Sainte Maxime 2011)

- Clonning of DREB1A transcription factor from *Pisum sativum*

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WP2 - Describing SEE legumes

- Molecular evaluation of ancient legume seeds
- Protocol on ancient DNA extraction form charred legume seeds
- Press-releases in national and local newspapers and television and radio media on the extraction of aDNA from charred pea and bitter vetch seeds

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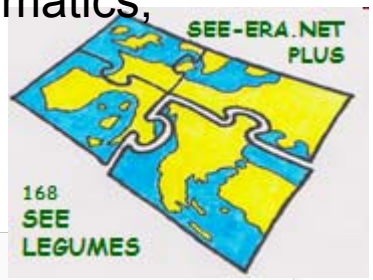
WP2 - Describing SEE legumes

Protocol on ancient DNA extraction form charred legume seeds

- Aleksandar Medović (Museum of Vojvodina, Novi Sad, Serbia) – archaeobotany, crop history
- Petr Smýkal (Palacky University, Olomouc, Czech Republic) – crop evolution, molecular taxonomy
- Bojan Zlatković (University of Niš, Faculty of Sciences and Mathematics, Department of Biology and Ecology, Niš, Serbia) – plant taxonomy, ecogeography, in situ conservation



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WP2 - Describing SEE legumes

Protocol on ancient DNA extraction form charred legume seeds

Protocol according to **Dr Robin Allaby, The University of Warwick, School of Life Science**
Petr Smýkal - PCR and sequencing of PCR products

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- Jovanović Ž., Stanisavljević N., Nikolić A., Medović A., Mikić A., Radović S., Đorđević V. (2011): Pisum & Ervilia Tetovac – made in Early Iron Age Leskovac. Part two. Extraction of the ancient DNA from charred seeds from the site of Hissar in South Serbia. Field and Vegetable Crops Research, 48, 227-232.

- Medović A., Mikić A., Ćupina B., Jovanović Ž., Radović S., Nikolić A., Stanisavljević N. (2011): Pisum & Ervilia Tetovac – made in Early Iron Age Leskovac. Part one. Two charred pulse crop storages of the fortified hill fort settlement Hissar in Leskovac, South Serbia. Field and Vegetable Crops Research, 48, 219-226.

- Medović A., Jovanović Ž., Stanisavljević N., Radović S., Mikić A., Đorđević V., Ćupina B. (2010): An archaeobotanical and molecular fairy tale about the early Iron Age Balkan princess and the charred pea. Pisum Genetics, 42, 35-38.

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Upon analysis of chloroplast DNA -derived and phylogenetically informative markers, namely trnSG, matK, rbcLA, trn FL we came into the following conclusons:

- we deal truly with **authentic ancient DNA** - based on mutations detected by sequences, which arose in process of aging, eg. upon chemical modification of bases (this is very important for any further work)
- we are **NOT able (yet) to amplify nuclear encoded genes (appart from ribosomal ITS spacer**) - likely due to extensive DNA fragmentation (but beside perhaps flower colour we have at the moment little to analyze - but this might change soon- after for example Dpo gene cloning (in Spain) which govern pod dehiscence - one of the few legume domestication traits
- we are **not able to amplify mitochondrial fragment** of cox1 gene (again likely due to low copy number and extensive fragmentation) but this is little iformative for pea, having only 2-3 SNPs

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BUT – we get nicely cpDNA and comparative analysis of above mentioned **chloroplast genes** (present in thousands of copies/ cell eg. trnSG, matK, rbcLA, trn FL) positioned **that ancient sample to one of the groups having part of Pisum elatius, but also cultivated/ or pre-domestification peas (eg. Pisum sativum/ Pisum humile)**

- at the moment upon analysis of recent **Serbian "wild pea"** which Bojan Zlatkovic found - these **two samples are related**.

These ancient peas could be actually both already domesticated/cultivated peas (*Pisum sativum*), as well as semi/wild peas of *Pisum elatius*.
Currently existing (semi)wild pea in that region might be direct descendant of the archeological sample.
Further analysis is clearly needed clarify the status and origin.

DEFINITELY THIS IS FIRST SUCH ANALYSIS OF ANCIENT LEGUME !!!!! SEEDS

WP2 - Describing SEE legumes

- 2D electrophoresis, phosphoprotein/HS fractions
- Western blot with anti dehydrin antibody and small HSP
- Oxidative fingerprinting

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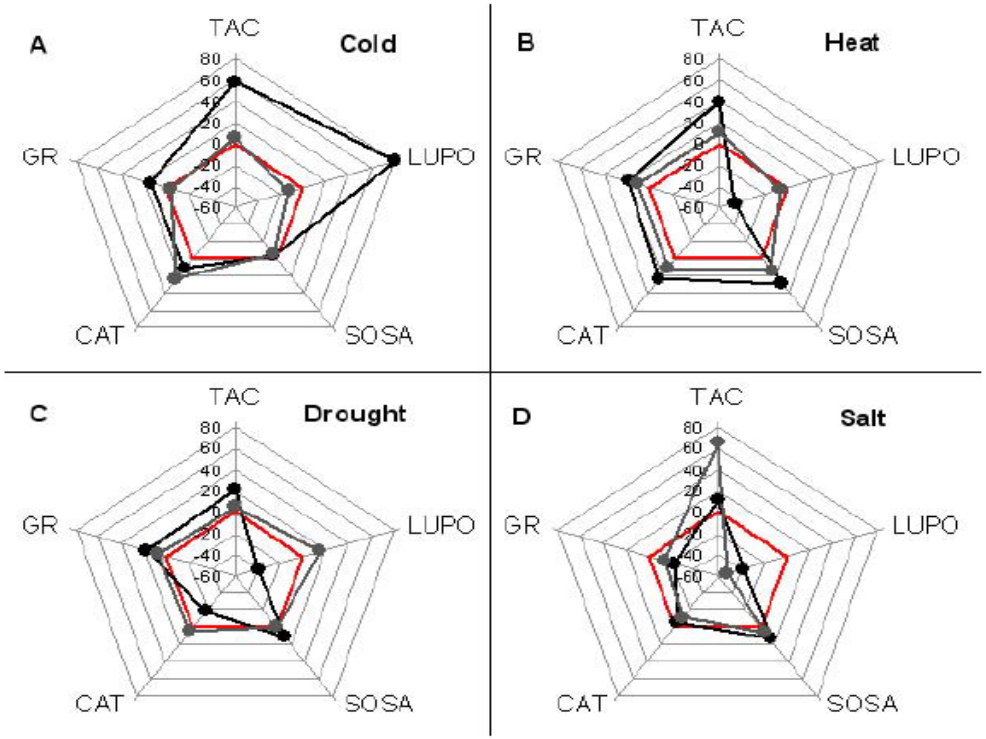

Figure 4
Fingerprints of antioxidative activities in *Lepidium sativum* after abiotic stress. The percentage change in the five