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Previous achievements of the Grain Legumes WG

SMARTLEG project

Efficient management of resources for smart legumes utilization

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dr. Barbara Pipan

Bucharest, 10th April, 2019

About the project…

ECPGR Activity Grant Scheme Proposal Form
Third Call

Activity Proposal

Activity	
Full title	SMARTLEG
Acronym (or short title)	Efficient management of resources for smart legumes utilization
Duration of Activity (in months)	12
Starting date	Jan 1st, 2017

Applying Working Group(s)

Working Group	Indicate name and surname of Working Group Chair
1.Grain Legumes	Creola BREZEANU

Activity Coordinator

Activity Coordinator	
Name and Surname	Vladimir MEGLIČ
Nationality	Slovenian
Current position	Research Programme Leader
Institute	Kmetijski inštitut Slovenije (Agricultural Institute of Slovenia)
Country	Slovenia

- Main objective of the SMARTLEG project was proper phenotypic and genetic evaluation of European Phaseolus accessions **with emphasis to *Phaseolus coccineus* germplasm** providing new data to EURISCO and AEGIS.
- **Four SMARTLEG project partner** institutions

Partner ID No.	Name and Surname	Institute	Country
1	Mr Vladimir Meglič	Kmetijski Inštitut Slovenije (Agricultural Institute of Slovenia)	Slovenia
2	Ms Creola Brezeanu	Vegetable Research and Development Station, Bacau, University of Agricultural Science and Veterinary Medicine, Iasi	Romania
3	Ms Marina Antić	University of Banja Luka, Genetic Resources Institute	Bosnia and Herzegovina
4	Ms Mirjana Vasić	Institute of Field and Vegetable Crops	Serbia

- **Four self-founded** institutions from ***Italy, Macedonia (FYR), Austria and Slovakia***
- The SMARTLEG project meeting was held in April 2017 at the Agricultural Insitute of Slovenia in Ljubljana. The coordinator of the ERA-CAPS project **Bean_Adapt**, Roberto Papa, gave an overview of the project also in **relation with SMARTLEG project**.

Introduction

- In the last decade several thousand accessions were collected in different parts of Europe and are stored in national gene banks; at present the Phaseolus Database as part of the EURISCO Web catalogue contains over 46000 records.
- During the project, we provided **four different types of datasets/results** including:

I. **Morphometric seed characteristics** (partner and self-funded countries)

II. **Morphoagronomic traits of plants** (project partner institutions only+Macedonia (FRY)).

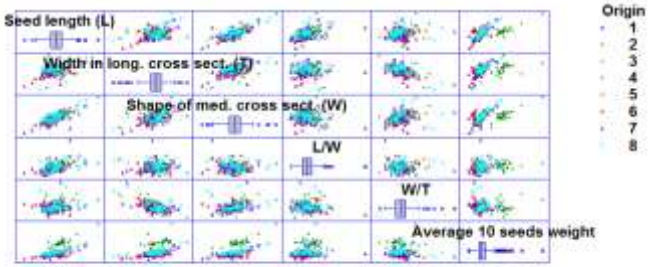
III. **Genetic evaluations** from **eight Central European geographic origins** (partner and self-funded countries).

IV. **Preparing materials for Bean_Adapt project.**



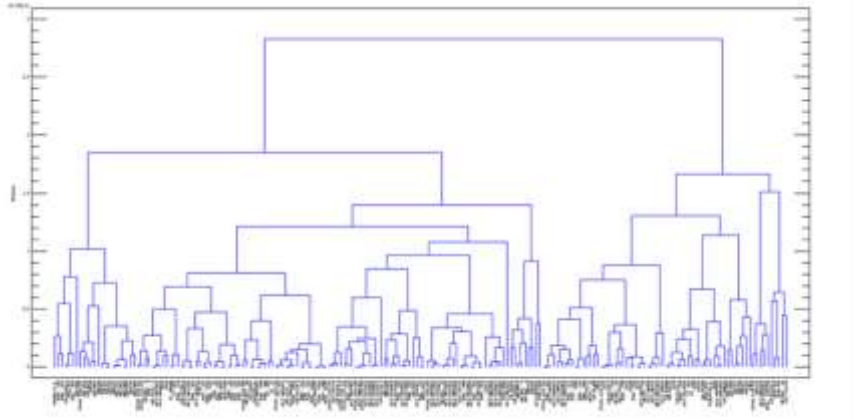


I. Morphometric seed characteristics

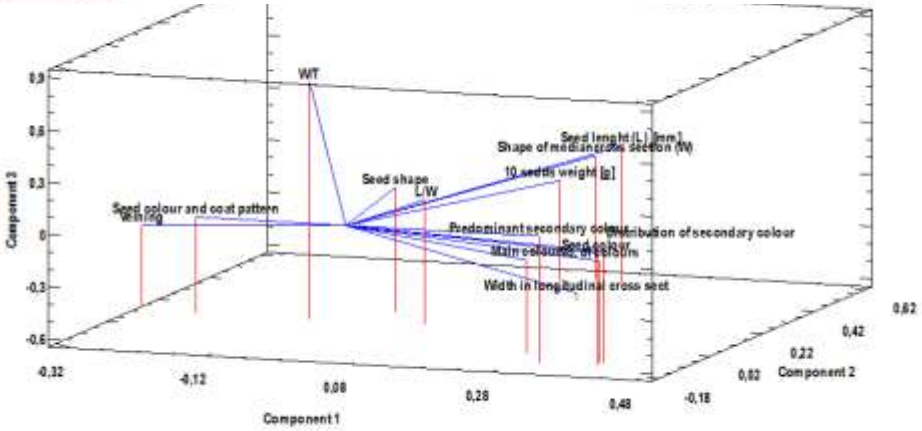


Matrix plot for quantitative seed characteristics depending on geographic origin
(1-Austria, 2-Bosnia and Herzegovina, 3-Italy, 4-Macedonia, 5-Romania, 6-Serbia, 7-Slovakia, 8-Slovenia)

- Performed before sowing, at AIS.
- *P. coccineus* seeds was assessed according to **adopted *CPVO-TP and Phaselieu/AIS descriptors* observing 14 different seed characteristics.**
- Altogether **we assessed 207 ACCs** from Austria (16), Bosnia and Herzegovina (18), Italy (7), Macedonia (FYR) (32), Romania (12), Serbia (12), Slovakia (57) and Slovenia (53).
- Morphometric characterization covered: Seed length (L), Width in longitudinal cross section (T) Shape of median-cross section (W) [mm], L/W and W/T ratios, Seed colour, No. of colours, Main colour, Predominant secondary colour, Distribution of secondary colour, Veining, Seed shape, Average 10 seeds weight and Seed colour and coat pattern.
- Global principal component analysis extracted four components which cumulatively explain 74.6% of morphometric variability of *P. coccineus* seeds.



Dendrogram of *P. coccineus* accessions



PCA distribution of accessions regarding to morphometric descriptors

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II. Morhoagronomic traits of plants

- The morphological evaluations were performed **upon adjusted descriptors for Phaseolus (ECPGR_PhasChar from Austria) under field conditions** in Slovenia, Romania, Serbia, Macedonia and Bosnia and Herzegovina.
- Three commercial varieties from different geographic origins of P. coccineus ('Bonela 'Darko' and 'Emergo') were included as standards.
- Morpho-agronomical evaluation shows differentiation between *P. coccineus* accessions even for standard varieties under geographically distinct field conditions.
- Time needed to reach 50% flowering was between 24 and 70 days.
- Colour of flowers showed large variability; from plain white and white with lilac edge or red stripes to greenish, dark lilac, carmine red, purple and orange.
- Leaves were found in triangular, quadrangular or round shape.
- Large majority of pods were pear shaped.

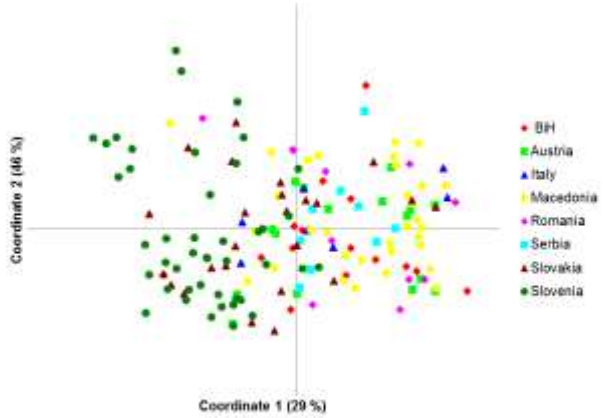
III. Genetic evaluations

- Since there are no SSRs specifically developed for P. coccineus, a set of 12 already proven and reliable cross-species amplified SSR markers were chosen.
- Altogether 159 viable *P. coccineus* accessions from eight participating countries were involved in genotypization to assess genetic background and genetic/molecular diversity.
- Analysis of molecular variability on the basis of allelic patterns reflects 3% of the molecular variability among P. coccineus germplasm from eight different geographic origins, which means that 97% of germplasm is common to all accessions (p>0.01). Regarding the genetic structure of the *P. coccineus* germplasm from eight geographically distinct collections, three genetic clusters were formatted; the average genetic distance between genotypes in each cluster varies between 0.592 and 0.816.

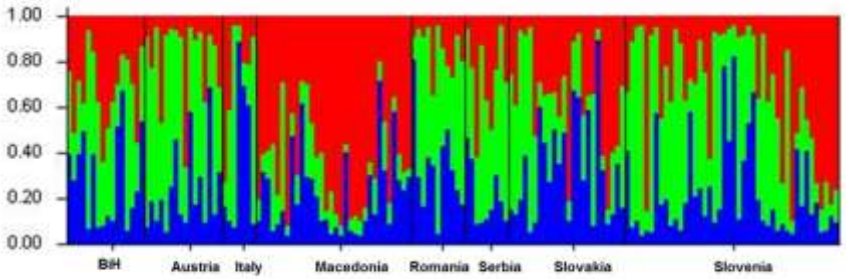
Pairwise comparisons regarding to Nei's genetic identity

	BiH	Austria	Italy	Macedonia	Romania	Serbia	Slovakia	Slovenia
BiH	1.000							
Austria	0.937	1.000						
Italy	0.892	0.866	1.000					
Macedonia	0.942	0.937	0.937	1.000				
Romania	0.854	0.852	0.836	0.906	1.000			
Serbia	0.915	0.878	0.867	0.914	0.869	1.000		
Slovakia	0.919	0.879	0.851	0.915	0.868	0.937	1.000	
Slovenia	0.893	0.898	0.791	0.880	0.826	0.880	0.901	1.000

The most genetically distinct origins are observed between Slovenian and Italian accessions; the most closely related are accessions from Macedonia and Bosnia and Herzegovina.



PCoA plot of genotypes from different geographic origins



Overall structure plot of *P. coccineus* accessions according to origin

Summary statistics among loci

Locus	No. of alleles	Probability of identity	Expected heterozygosity	Observed heterozygosity	Polymorphic information content	Fst
PVag001	11	0.107	0.743	0.911	0.701	0.021
BM137	26	0.019	0.898	0.826	0.886	0.032
PVat007	23	0.026	0.882	0.961	0.866	0.043
BM158	6	0.183	0.625	0.701	0.582	0.069
PVBR60	42	0.029	0.871	0.921	0.856	0.046
BM187	12	0.183	0.624	0.654	0.581	0.043
Pv-cct001	8	0.301	0.554	0.987	0.451	0.009
PVESTBR006	7	0.115	0.739	0.987	0.691	0.011
Pv-ag004	32	0.090	0.758	0.987	0.725	0.025
PVESTBR017	8	0.084	0.779	0.926	0.748	0.076
PVSTBR258	28	0.052	0.819	0.912	0.796	0.250
PVSTBR137	6	0.554	0.265	0.275	0.251	0.115
average	17.4	3.957×10^{13}	0.7131	0.8374	0.6776	0.0617

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IV. Preparing materials for Bean_Adapt project

- Due to cross-pollination nature of Pc and high level of self-incompatibility it was quite hard difficult to obtain viable self-pollinated seeds.
- Out of 159 successfully germinated accessions, 72 self-pollinated pods were obtained, mostly from Macedonian accessions (33).
- For other geographic origins, we harvested from 2 to 9 self-pollinated pods per each participating country.
- Altogether, 159 DNA samples form original accessions and 72 DNA samples of self-pollinated progenies from original accessions were prepared according to demanding protocols and sent to the GBS (to prof. Jackson‘s lab, UGA). For 231 Pc ACC fastq results are available!

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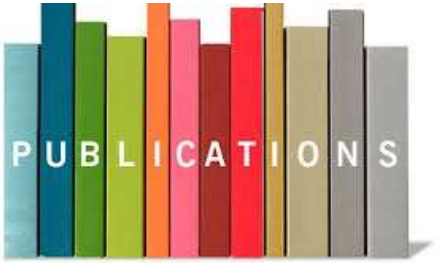
Conclusion

- Overall results obtained in this study **reflect numerous variations** of phenotypic **traits**, particularly seed characteristics and genetic background of **European *P. coccineus* germplasm**.
- During the project, four different types of datasets including morhoagronomic traits of plants, morphometric seed characteristics and genetic profiles of *P. coccineus* accessions **from eight geographic origins form Central Europe** were presented to contribute new data to EURISCO and new bean accessions to AEGIS.

- **SMARTLEG will add value by enrichment of the Phaseolus collection**, improvement of the documentation, study of the accessions for valuable **breeding traits** in a large area – according to the team, provide valuable material for use in breeding, **scientific research**, educational and other programmes.
- Regarding to activities started within **SMARTLEG** project, **collaboration** with **Bean_Adapt** project was established.

Dissemination of the project



- PIPAN, Barbara, MEGLIČ, Vladimir. Characterization and utilization of Phaseolus European germplasm for sustainable crop improvement : Bean Adapt, Ancona, 11th September, 2018.
- PIPAN, Barbara, ŠUŠTAR VOZLIČ, Jelka, TODOROVIĆ, Vida, ANTIĆ, Marina, BREZEANU, Creola, IVANOVSKA, Sonja, VASIĆ, Mirjana, KAINZ, Wolfgang, MICELI, Fabiano, HAUPTVOGEL, Pavol, PAPA, Roberto, MEGLIČ, Vladimir. Comperative evaluation of Phaseolus coccineus L. germplasm originating from eight Central European collections. V: SKOČAJ, Matej (ur.). Genetika 2018 : book of abstracts, 8th Congress of the Genetics Society of Slovenia [and] 8th Meeting of the Slovenian Society of Human Genetics, September 19-21, 2018. Ljubljana: Slovensko genetsko društvo. 2018, str. 144. http://genetika2018.alfa-faktor.si/.
- Pipan B., Šuštar-Vozlič J., Todorović V., Antić M., Brezenau C. , Ivanovska S., Jankulovska M., Savić A., Vasić M. & Meglič V.: EVALUATION OF THE BALKAN *PHASEOLUS COCCINEUS* L. GENETIC RESOURCES, 7th Balkan Botanical Congress, University of Novi Sad, 2018.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Research Article

Morphological Seed Characterization of Common (*Phaseolus vulgaris* L.) and Runner (*Phaseolus coccineus* L.) Bean Germplasm: A Slovenian Gene Bank Example

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Thank you for your attention