

**Efficient management of resources for smart  
legumes utilization**

**(SMARTLEG)**



**January 2017 – December 2017**

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*Phaseolus coccineus* L. ©KIS

**February 2023**

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# Efficient management of resources for smart legumes utilization (SMARTLEG)

## Activity Report

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### INTRODUCTION

#### Background

The project came in the context of the International Year of Pulses, and aimed to underline the importance of pulses as a primary source of protein and other essential nutrients. The importance of pulses is found both on the agenda of governments that work to find solutions in terms of providing sufficient and healthy food for the world population, and on the agenda of researchers from various fields (conservation, breeding, food, agriculture), thanks to their multiple benefits for human and environmental health. In the last decade, several thousand accessions were collected in different parts of Europe and are stored in national genebanks. At present, the *Phaseolus* Database as part of the EURISCO web catalogue contains over 50,000 records, including more than 40,000 characterization and evaluation records and 585 photos (ECPGR, 2018). The four SMARTLEG project partner Institutions (Agricultural institute of Slovenia, Slovenia, Suceava Genebank, Romania, Institute of Field and Vegetable Crops, Serbia and Genetic Resources Institute, Bosnia and Herzegovina) are housing in total 5,696 *Phaseolus* accessions and from those, 5,389 common bean (*P. vulgaris*) and 307 runner bean (*P. coccineus*) accessions. In addition, the observer countries (Italy, Croatia, North Macedonia, Austria, Slovakia) are contributing information on 3,368 *Phaseolus* sp. accessions to EURISCO (Table 1).

**Table 1.** Status of *Phaseolus* genetic resources holdings in SMARTLEG partners and observers institutions

Country	Gene bank	Institution	<i>P. vulgaris</i>	<i>P. coccineus</i>	Total
Slovenia*	Slovene Plant Gene Bank	Agricultural institute of Slovenia (AIS), Ljubljana	980	53	1,033
Romania*	Suceava National Gene Bank	Suceava Genebank, Suceava	3,177	159	3,336
Serbia*	Serbian National Gene Bank	Institute of Field and Vegetable Crops, Novi Sad	750	17	767
Bosnia and Herzegovina*	BiH National Collection	Genetic Resources Institute, University of Banja Luka	150	15	165
Italy		Facoltà di Agraria, Ancona (Istituto del Germoplasma)	332	63	395
Croatia	Croatian Plant Genetic Resources	Agricultural college, Križevci	12	3	15
North Macedonia	Macedonian National Collection	Faculty of Agricultural Sciences and Food, Ss Cyril and Methodius University, Skopje	1,046	50	1,096
Austria	Austrian Genebank	AGES – Österreichische Agentur für Gesundheit und Ernährungssicherheit, Wien	798	134	932
Slovakia	Gene Bank of the Slovak Republic	National Agricultural and Food Centre – Research Institute of Plant Production, Piešťany	1,271	54	1,325
<b>Total number of accessions</b>			<b>8,516</b>	<b>548</b>	<b>9,064</b>

\*partner institution

Genetic diversity of common bean from Central Europe was studied at the Agricultural Institute of Slovenia (AIS) by AFLP and microsatellite markers (Šuštar-Vozlič et al., 2006; Maras et al., 2013, 2015; Meglič et al., 2017; Pipan et al., 2017). The surveys revealed that extensive diversity resides in common beans cultivated in this area and includes variation beyond the two gene pools, Andean and

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Mesoamerican. As revealed by the analysis of a large set of European common bean landraces using chloroplast microsatellites (cpSSRs) and two unlinked nuclear loci (phaseolin, Pv-shatterproof1), a relatively high proportion of the European bean germplasm might have derived from hybridization between the two gene pools (Angioi et al., 2010, Gioia et al., 2013). Recently, 300 Croatian common bean landraces have been evaluated by phaseolin and microsatellite markers. The majority of the studied landraces belong to the Andean gene pool. A similar result was found for Slovenian landraces (Šuštar-Vozlič et al., 2006).

Genetic diversity of runner bean has been less extensively studied. The largest set of European landraces, more than 300, was evaluated by cpSSRs and a smaller set was studied also for phenotypic traits (Rodriguez et al, 2013).

However, the characterization data are still lacking or/and are not easily accessible both for common as well as for runner bean. A very large number of accessions (ca. 6,000) are still of unknown origin. In the project, multi-crop passport descriptors and seed characteristics of *P. vulgaris* and *P. coccineus* from different collections were used by project partners to establish a set of several hundreds of accessions with diverse phenotypes and distinct geographical origins. With regards to the runner bean germplasm from Central Europe, significantly less information is available since *P. coccineus* is represented only by a small number of accessions in the databases.

### Aims of the activity

One of the main goals of the SMARTLEG project was proper identification and phenotypic characterization of European *Phaseolus* accessions with emphasis on *P. coccineus* germplasm providing new data to EURISCO for integration of information and extending genomic information on *ex situ* material. During the project, we provided three different types of datasets including morpho-agronomic traits of plants, morphometric seed characteristics and genetic profiles of *P. coccineus* accessions from eight geographic origins from Central Europe.

The aim of the project was also to establish collaboration with the ERA-CAPS project Bean\_Adapt (non-ECPGR), to continue with the characterization of *P. coccineus* germplasm applying GBS (Genotyping By Sequencing) and to obtain subsets of genotypes for phenotyping (field and growth chamber) and for a deeper genomic–transcriptomic–metabolomic characterization to identify genes/QTLs that control important agronomic and adaptive traits.

### Expected outcomes related to ECPGR objectives

- SSR (Short Sequence Repeat) marker data on a commonly agreed set of 159 European *Phaseolus coccineus* accessions (Outcome 1, output 1.2., activity 1.2.3: Monitoring of the management of AEGIS accessions by the AMs in accordance with the principles of AEGIS).
- Increasing the number of AEGIS *Phaseolus* sp. accessions (Outcome 1, output 1.1., activity 1.1.2.: Establishment of proper documentation of AEGIS accessions).
- Increasing quality and quantity of data in EURISCO (Outcome 1, output 1.2., activity 1.2.2.: Verification of the proposed AEGIS accessions).
- Acquired information will be useful for end users (e.g. breeders) (Outcome 1, output 1.2., activity 1.2.1.: Identification of eligible accessions to be proposed for registration as AEGIS accessions; Outcome 1, output 1.5.; activity 1.5.3.: Services for characterization, evaluation and/or phenotyping of AEGIS accessions provided to AMs; Outcome 2, output 2.1.; activity 2.1.2. Collaboration between NFPs and collection holding institutes strengthened; Outcome 5, output 5.4., activity 5).
- Research partnerships established between genebanks and researchers, including cooperation for establishing links for future EU project proposals.

### List of partners involved

Project partner institutions (Agricultural institute of Slovenia, Ljubljana, Suceava Genebank, Suceava, Institute of Field and Vegetable Crops, Novi Sad, Genetic Resources Institute, University of Banja

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Luka and Food Science Department of the Faculty Agriculture, Ancona) and five observer Institutions (Agricultural College, Križevci, Faculty of Agricultural Sciences and Food, Ss Cyril and Methodius University, Skopje, AGES – Österreichische Agentur für Gesundheit und Ernährungssicherheit, Wien, National Agricultural and Food Centre – Research Institute of Plant Production, Piešťany and the University of Udine, Department of Agricultural Sciences, Udine). The contact details for individual partners are given in Appendix 1.

## MATERIALS AND METHODS

### Study material

Seeds of *P. coccineus* accessions were obtained from participating and self-funded partner collections (Appendix 2). The accessions geographically originated from Slovenia, Romania, Bosnia and Herzegovina and Serbia as SMARTLEG partners; and from Italy, Slovakia, North Macedonia and Austria as self-funded participant countries. Additionally, reference/standard varieties ('Bonela', 'Darko' and 'Emergo') were identified and distributed among partners. Morphometric evaluation and genetic analysis were performed for all collected accessions at the genetic laboratory of the AIS; morpho-agronomic traits of plants were assessed only by SMARTLEG partners in their individual countries and also in North Macedonia.

### Morpho-agronomic traits of plants

At the introductory meeting (AIS, Ljubljana), the methodology for morphological evaluations and the reference genotypes were agreed upon, and descriptors for *Phaseolus* were adjusted (ECPGR\_PhaseChar, Austria). The final version of the evaluated morpho-agronomic characteristics of plants is available in Appendix 3. During the growing season, each participating partner evaluated morphological traits in the field. Results of the field trials were obtained for Slovenia, Romania, Bosnia and Herzegovina, Serbia and North Macedonia for the growing season 2017. In addition, evaluation of certain traits was performed also for *P. vulgaris* accessions included.

The study focused on two *Phaseolus* species; *P. coccineus* and *P. vulgaris*. Partners from Slovenia, Bosnia and Herzegovina, North Macedonia, Serbia and Romania participated and altogether 153 accessions were examined for their morphological traits; 137 accessions of *P. coccineus* and 16 accessions of *P. vulgaris*. Additionally, three varieties of *P. coccineus* were used as a control – 'Bonela' from Austria, 'Darko' from Serbia and 'Emergo' from Slovenia.

Samples were sown separately in every participating country between 9 May and 24 June 2017. Days were counted from the sowing date to emergence, budding, appearance of first flower, 50% flowering and full flowering. The time needed to reach 50% flowering was between 24 and 70 days.

### Morphometric characteristics of seeds

Before sowing, the morphometric characterization of *P. coccineus* seeds was performed according to the adopted Community Plant Variety Office-Technical Protocol (CPVO-TP) and Phaselieu/AIS descriptors for 14 different seed characteristics. Morphometric characterization covers seed length (L) [mm], width in longitudinal cross-section (T) [mm], 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 [g] and seed colour and coat pattern. In addition, a high-resolution photo of each accession was taken.

### Genetic analysis

All *P. coccineus* accessions (#159) from eight geographic origins were germinated in a greenhouse and DNA was extracted from first trifoliolate leaves using DNeasy Plant Mini kit (250) (Qiagen) under optimized manufacturer instructions. The quantification of isolated DNA was performed on Nanodrop 1000 Spectrophotometer.

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Since there is no SSRs specifically developed for *P. coccineus*, a set of 12 already proven and reliably cross-species amplified SSR markers (Yu et al. 2000; Blair et al 2003; Gaitan-Solis et al. 2002, Garcia et al. 2011) from *P. vulgaris* and *P. coccineus* genomes was provided according to Garcia et al. (2011), Spataro et al. (2011) and Mercati et al. (2015) applications. Polymerase chain reactions were performed under optimized protocols and temperature conditions for each primer combination. Fragment analysis was conducted on Genetic Analyser ABI3130XL. Exact allele lengths were determined in GeneMapper 4.0 comparing to internal size standard ROX-500. Bioinformatics was performed by applying different programmes and software packages including GenAIEx, Structure, Structure Harvester, GenePop, Arlequin, Identity, Populations, Genetix, TreeView, MSToolkit to provide information about the genetic background and other genetic/molecular diversity parameters of the accessions.

### Establishment the collaboration with the Bean\_Adapt project

The coordinator of the ERA-CAPS project Bean\_Adapt devised a strategy to obtain self-derived progeny from *P. coccineus* (cross-pollinated plant species) and a DNA extraction method, suited for GBS. For this purpose, all accessions were planted in the greenhouse at the AIS; for North Macedonian accessions on the fields of the Faculty of Agricultural Sciences and Food. At the stage of first trifoliate leaves, leaf tissue was sampled for DNA extraction. Self-pollination was performed in July 2017 and only fully developed and physiologically mature self-pollinated pods were sampled and dried. Obtained seeds (from self-pollinated pods) were then germinated in a greenhouse at AIS (November 2017) and first trifoliate leaves were sampled for DNA extraction. DNA was extracted according to the prescribed protocol for GBS. The Nanodrop measurements of DNA quality and quantity had to be within required limits after the optimization of the extraction protocol for DNeasy Plant Mini kit 250 (Qiagen). Nevertheless, the 260/280nm ratio had to reach the 1.8 limit in a quantity of 1,000ng of DNA as a template for GBS.

### Virus screening

During the growing season, all Slovenian accessions were visually screened in the field for bean common mosaic virus (BCMV) and the four most common viruses in beans. Only symptomatic plants were sampled and put to the enzyme-linked immunosorbent assay (ELISA) for BCMV, bean common mosaic necrosis virus-BCMNV, bean yellow mosaic virus-BYMV, cucumber mosaic virus-CMV and alfalfa mosaic virus-AMV. Additionally, the leaf samples of all 159 accessions from eight geographic origins grown in a greenhouse were sampled and put on hold for further viral screening to BCMV, BCMNV, AMV and CMV.

## RESULTS

### Morphologic characteristics of plants

Morpho-agronomic traits of plants were observed in four SMARTLEG partner countries (Slovenia, Romania, Bosnia and Herzegovina and Serbia) and North Macedonia. The data for Serbian accessions are incomplete due to unfavourable weather conditions including hail at flowering time; therefore further morphologic evaluation is just representatively described.

Observations of the colour of the flower banner and flower wings showed large variability; from plain white and white with lilac edge or red stripes to greenish, dark lilac, carmine red, purple and orange. The following leaf morphology parameters were measured: leaf area, length and width were measured and leaf shape and presence of colour of anthocyanin were observed. Leaves could be found in triangular, quadrangular or round shapes; they vary between 4 and 14cm long and between 4 and 12.4cm wide.

The samples from *P. vulgaris* were mostly determinate bushes and reached a height from 35 to 38cm, while samples of *P. coccineus* mostly grew in the form of indeterminate climbing with a height between 69 and 197cm.

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Pod morphology parameters were: pod colour of immature and mature pods, presence of pod suture strings, length and width of pods, shape of pods in cross-section, their curvature and number of seeds per pod. Immature pods showed different shades of green (normal, shiny, dull green, in some cases with red stripes) or yellow-golden colour, while mature pods ranged from beige to yellow. Strings were present in small or moderate quantities in all observed accessions of *P. coccineus*, whereas they were absent in *P. vulgaris*. Measurements ranged from 4.5 and 17.9cm in length and 1.2 and 2.3cm in width. The large majority of pods were pear-shaped in cross-section, but there were also round elliptic or very flat ones; the latter being much more common in *P. vulgaris* accessions. In one accession (*P. coccineus*), pods shaped as a figure of eight were recorded. Most of the *P. coccineus* accessions had slightly curved pods with some showing medium curvature and they contained two or three seeds (with one exception containing six). On the other hand, pods of *P. vulgaris* were mostly straight, some of them slightly curved, and contained five to seven seeds. Pods of *P. coccineus* reached 90% maturity after 94 to 170 days, whereas pods of *P. vulgaris* matured after 62 to 75 days.

#### Slovenia

In Slovenia, 47 accessions and additional three control accessions were evaluated. The time needed to emerge was between 10 and 20 days and between 41 and 57 days to reach 50% flowering. Flowers showed two colour varieties; white and red to dark lilac. Pods reached 90% maturity after 94 to 143 days. Colour was observed in immature pods, where it was either normal green or green with pale red stripes. All of the pods were moderately stringy and big majority of them slightly curved in shape. In all of the samples, plant growth habit was indeterminate climbing.

#### Bosnia and Herzegovina

In Bosnia and Herzegovina, 11 sample accessions and two control accessions were germinated and many of them faced drought while growing. They needed between 36 and 41 days to reach 50% flowering and the flowers showed a big variety in colour; they could be seen in white (plain, with lilac edge or with red stripes), dark lilac or different shades of red. Leaves were found in triangular or round shapes, often both were present in the same accession. The majority of samples grew in the form of indeterminate climbing, while a few of them were determinate bushes or showed determinate climbing. They grew from 69 to 197cm high. Seeds were observed for coat colour, pattern and brilliance and they showed great variety in all of these categories. Colours of seed hilum and seed hilum ring were less variable, mostly present in white with occasional beige, ochre, violet or black samples.

#### North Macedonia

In North Macedonia, 57 accessions and two control accessions originating from different parts of North Macedonia were evaluated in the study. Colour of flowers ranged from white (plain or with red stripes), greenish or lilac to carmine red and even different shades of orange, which is unique for North Macedonian accessions. Additionally, the size and position of the raceme were recorded and the latter showed a big variety – from facing downward to completely upward and all the positions in between. In many samples colour of anthocyanin was present in leaves and stems, which was very rare in samples from other countries.

#### Serbia

The study included 12 accessions collected on 8 different locations in Serbia or Bosnia and Herzegovina and 3 control accessions. They grew mostly in the form of indeterminate climbing and two accessions as determinate bushes. Flowers were of white, lilac, red or apricot colour.

#### Romania

In Romania, 26 sample accessions (14 accessions of *P. vulgaris* and 12 accessions of *P. coccineus*) and 3 control accessions (*P. coccineus*) were evaluated. *P. vulgaris* accessions needed less time to reach 50% flowering (28 to 36 days) in comparison with *P. coccineus* (45 to 70 days). Both species had flowers in white and red shades, while lilac and purple were present only in *P. vulgaris*. Leaves were recorded in all three observed shapes (triangular, quadrangular and round), but triangular ones were present only in *P. coccineus* samples. Mature pods showed no variety in colour, all of them being beige, but they did differ greatly in cross-section shape and curvature, seed colour, shape, pattern and

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brilliance. Variety in colour of seed hilum was smaller, most of them being white; however, samples of *P. vulgaris* showed quite a big variety in colour of seed hilum ring.

### Morphometric evaluation of seeds

The morphometric characterization of *P. coccineus* seeds was performed for 166 accessions including 17 composite accessions, which were obtained from four participating and four self-funded countries. Altogether we assessed 207 accessions from Austria (16), Bosnia and Herzegovina (18), Italy (7), North Macedonia (32), Romania (12), Serbia (12), Slovakia (57) and Slovenia (53). Three commercial varieties from different geographic origins of *P. coccineus* ('Bonela' from Austria, 'Darko' from Serbia and 'Emergo' from Slovenia) were included as standards. A high-resolution photography of each accession is available from the activity coordinator of the SMARTLEG project.

The average seed length among all 207 accessions is 20.32mm and varies between 12.97mm (ACC-01270 from Slovakia) and 29.97mm (MK1086 from North Macedonia); the average width in longitudinal cross-section of seeds is 8.56mm, the variation is between 4.66mm (PHA949 from Slovenia) and 11.50mm (MK826 from North Macedonia). The seed shape of the median-cross section varies between 8.80mm (ACC-01270 from Slovakia) and 17.29mm (MK1086 from North Macedonia), the average value for all observed genotypes is 12.53mm. The highest value of seed weight (56.40g) was measured for accession from North Macedonia (MK1086), and the lowest one (3.80g) was detected for first component of ACC-00774/1 from Slovakia. The average value of 10 seed weight is 14.78g. From all collections, 33.8% of genotypes are with only one colour on the seed coat without secondary colour. The secondary colour is distributed around the hilum for only two accessions; for 134 accessions from all eight geographic origins, the secondary colour is distributed on the entire grain. A weak veining pattern was proven for 155 accessions, medium veining has 33 accessions and a strong veining pattern was observed in 19 accessions. 25.6% of all *P. coccineus* seeds are oval-circular to elliptic; 48.3% have shape cuboid-elliptic; 15.9% are kidney shaped and only 10.1% are truncated. The majority are bi-coloured seeds of pinto type (38.6%), followed by white-coloured seeds (30.4%), constant mottled seeds (20.3%), broad striped seeds (7.2%) and black seeds (3.4%). Global principal component analysis (PCA) extracted four components which cumulatively explain 74,6% of the morphometric variability of *P. coccineus* seeds (Figure 1).

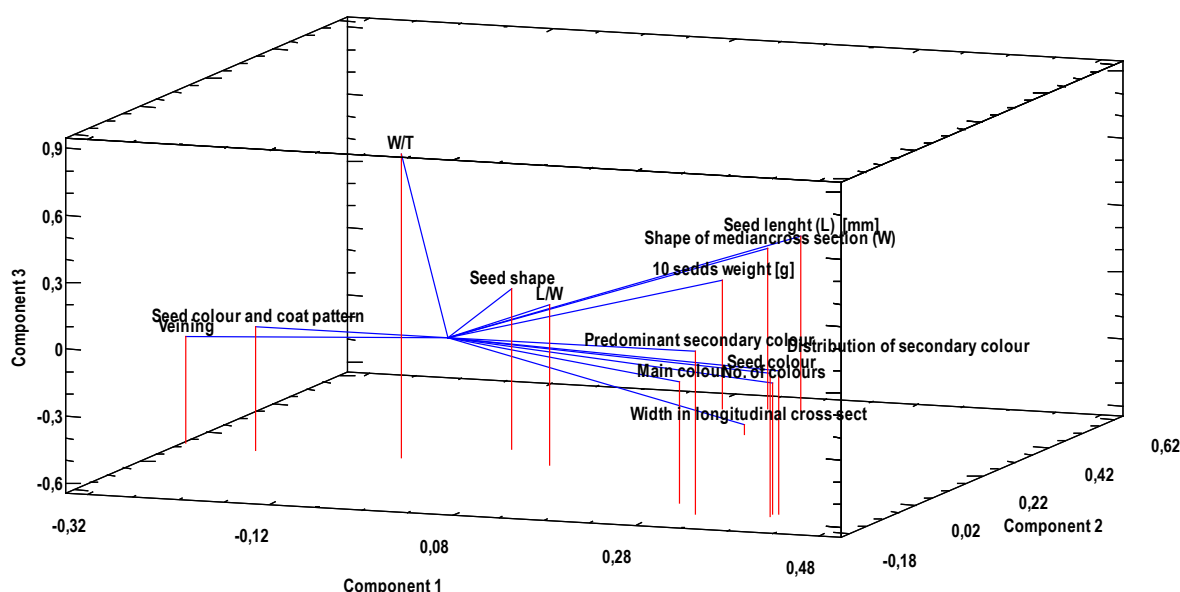


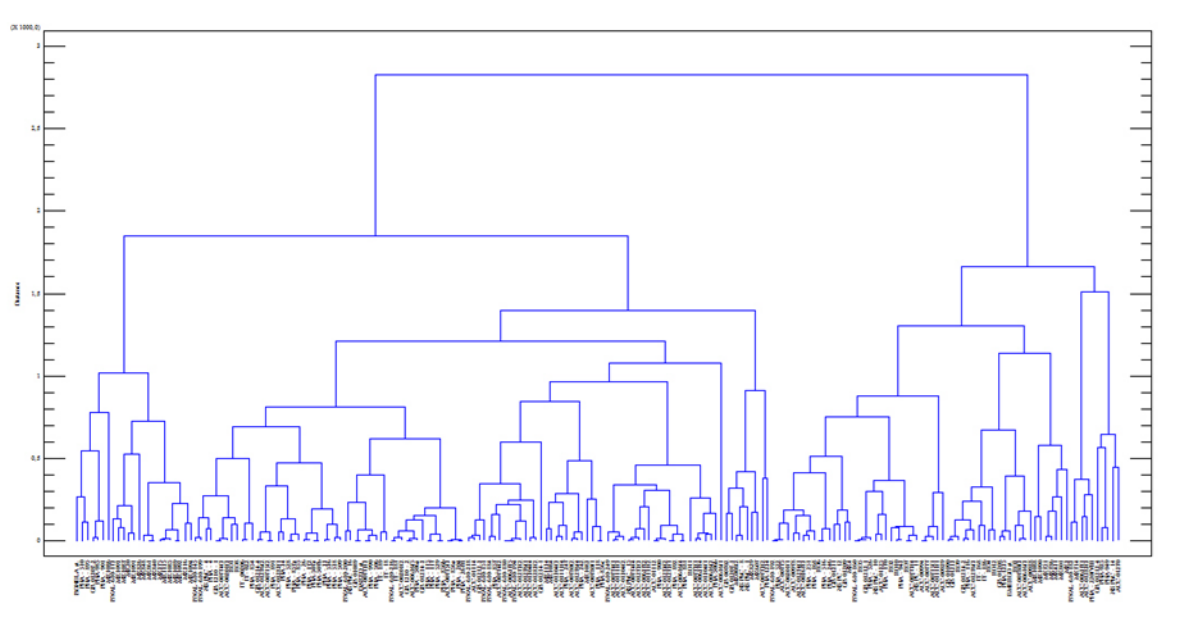
Figure 1. PCA distribution of accessions for morphometric descriptors.



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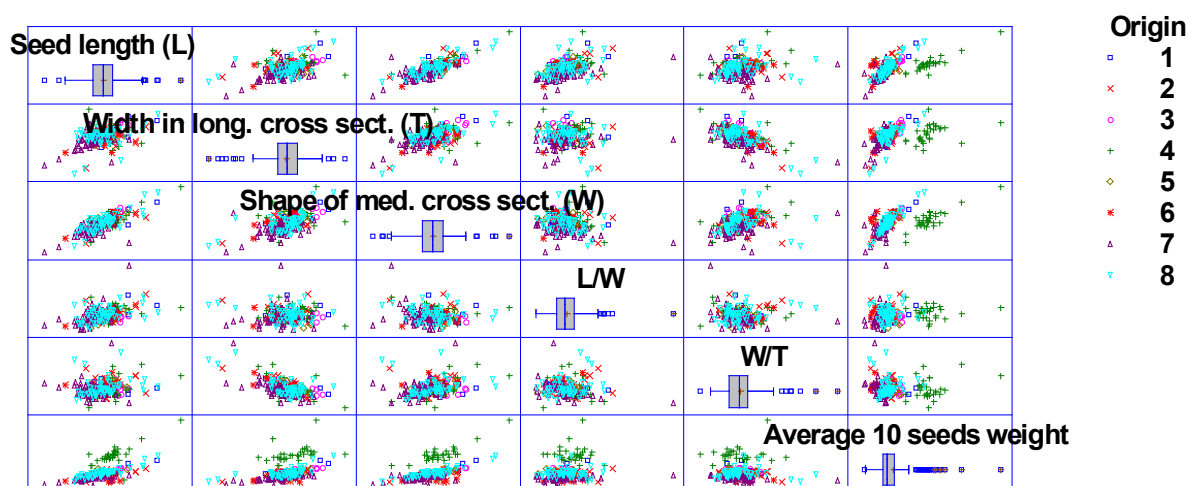
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Cluster analysis of all 207 accessions among 14 seed descriptors applying Ward's clustering method and squared Euclidian distance generate two main groups of *P. coccineus* (Figure 2).



**Figure 2.** Dendrogram of *P. coccineus* accessions.

Multivariate analysis for quantitative measured characteristics regarding the geographic origin of the accessions is presented in the following figure, depicting a matrix plot for quantitative seed characteristics depending on geographic origin (1-Austria, 2-Bosnia and Herzegovina, 3-Italy, 4-North Macedonia, 5-Romania, 6-Serbia, 7-Slovakia, 8-Slovenia).



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

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### Genetic structure

Altogether 159 viable *P. coccineus* accessions from eight participating countries were involved in morphometric seed evaluation and genotyping. Including standard varieties, there were 16 accessions from Bosnia and Herzegovina, 16 accessions from Austria, 7 accessions from Italy, 32 accessions from North Macedonia, 11 accessions from Romania, 9 accessions from Serbia, 24 accessions from Slovakia and 44 accessions from Slovenia. A list of genotyped accessions is presented in Appendix 2.

Parameters of variability among loci (Table 2) showed that the selected set of 12 SSR markers is informative and enables the provision of adequate genetic information on observed loci among *P. coccineus* genome. The most efficient for *P. coccineus* genetic diversity studies was proven to be locus BM137 and the least informative was locus PVSTBR137.

**Table 2. 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	3.957x10 <sup>-13</sup>	0.7131	0.8374	0.6776	0.0617

Analysis of molecular variability (AMOVA) 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 ( $p > 0.01$ ). The pairwise population matrix of Nei's genetic identity is presented in Table 3.

**Table 3. Pairwise comparisons regarding Nei's genetic identity**

	BiH	Austria	Italy	N. Macedonia	Romania	Serbia	Slovakia	Slovenia
<b>BiH</b>	1.000							
<b>Austria</b>	0.937	1.000						
<b>Italy</b>	0.892	0.866	1.000					
<b>N. Macedonia</b>	0.942	0.937	0.937	1.000				
<b>Romania</b>	0.854	0.852	0.836	0.906	1.000			
<b>Serbia</b>	0.915	0.878	0.867	0.914	0.869	1.000		
<b>Slovakia</b>	0.919	0.879	0.851	0.915	0.868	0.937	1.000	
<b>Slovenia</b>	0.893	0.896	0.791	0.880	0.826	0.880	0.901	1.000

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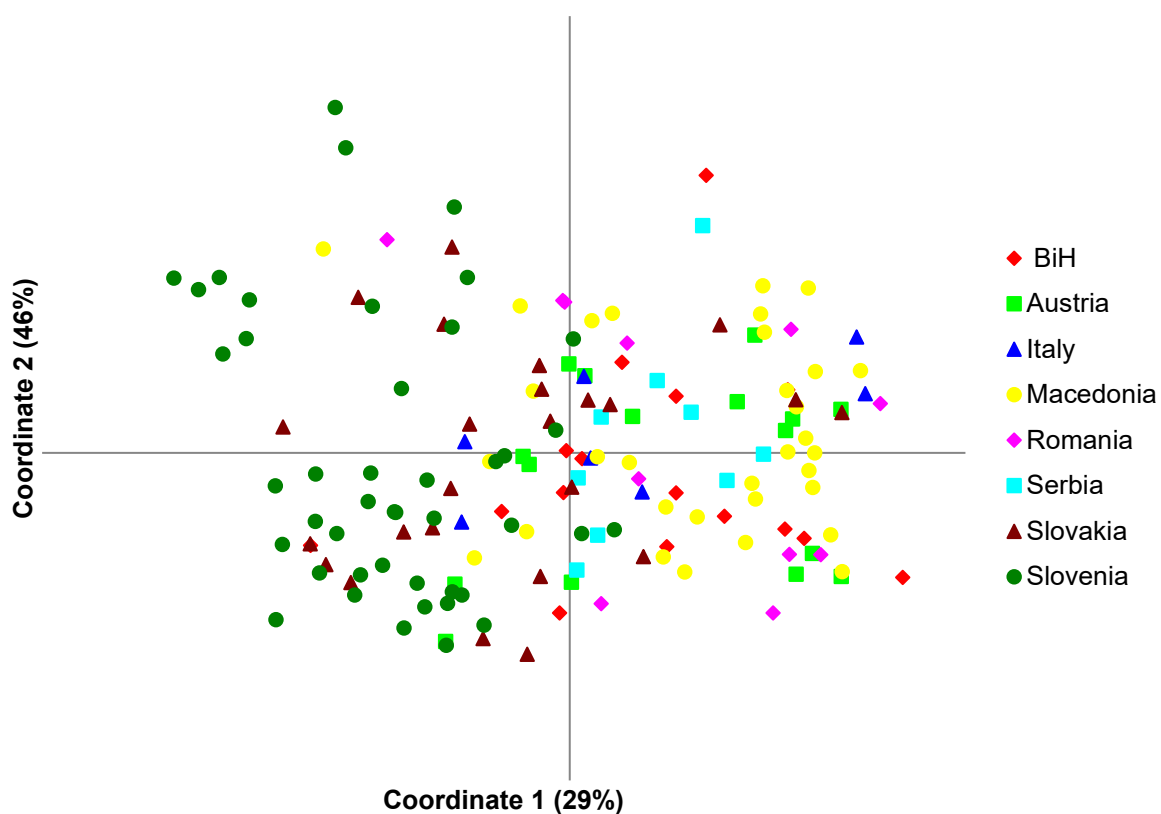
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The most genetically distinct origins are observed between Slovenian and Italian accessions; the most closely related are accessions from North Macedonia and Bosnia and Herzegovina.

The first three coordinates in principal coordinate analysis (PCoA) cumulatively explain 61% of molecular variability among accessions. The distribution of genotypes from each geographic origin is presented in Figure 4.



**Figure 4.** PCoA plot of genotypes from different geographic origins.

Genetic structure of the *P. coccineus* germplasm from eight geographically distinct collections was proposed and three genetic clusters were formatted (Figure 5). The average genetic distance between genotypes in cluster one (blue colour) is 0.592, for cluster two (green colour) is 0.623 and for cluster three (red colour) is 0.816.

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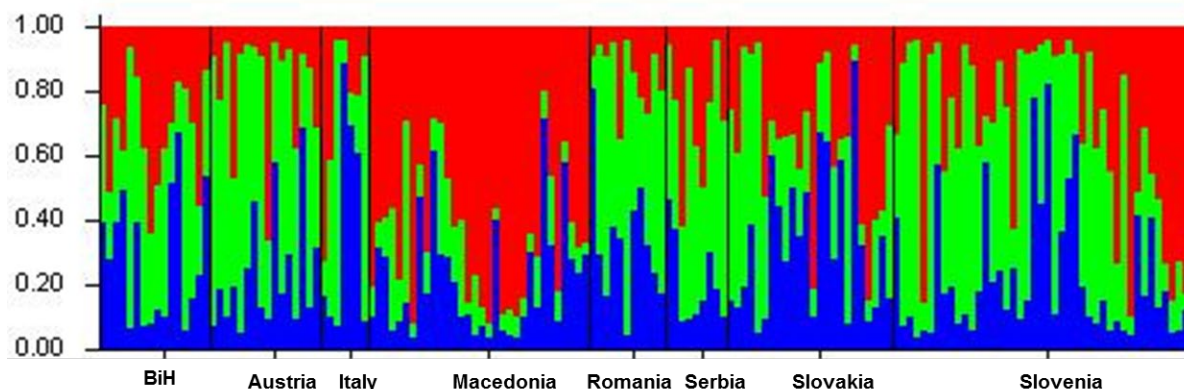


Figure 5. Overall structure plot of *P. coccineus* accessions according to origin.

### Samples for the Bean\_Adapt project

Due to the cross-pollination nature of *P. coccineus* and its high level of self-incompatibility, it was difficult to obtain viable self-pollinated seeds. Out of 159 successfully germinated accessions, 72 self-pollinated pods were obtained, mostly from North Macedonian accessions (33). For other geographic origins, we harvested from 2 to 9 self-pollinated pods per participating country. Altogether, 159 DNA samples from original accessions and 72 DNA samples of self-pollinated progenies from original accessions were prepared according to demanding protocols and sent to the GBS.

### Results of viral screening

In the field, 20 symptomatic plants from Slovenian accessions were sampled for ELISA and only three plants were positive. Two accessions have virus infection with BCMV and one accession was positive for both BCMV and CMV, respectively.

## CONCLUSIONS

Under field conditions, morpho-agronomic traits were observed only for SMARTLEG partner countries (Slovenia, Romania, Bosnia and Herzegovina and Serbia) and North Macedonia. All collected accessions from eight geographic origins (partner, self-funded and observer partner collections) were morphometrically evaluated (seeds) and genotyped using SSR molecular marker analysis to provide information about the genetic background and other genetic/molecular diversity parameters of the accessions. The morpho-agronomic evaluation shows differentiation between *P. coccineus* accessions even for standard varieties under geographically distinct field conditions. Results of morphometric and genetic characterization indicate a common origin of *P. coccineus* accessions from different collections within Central Europe; on the other hand, a high level of variability among accessions from the same geographic origin occurs. Overall results obtained in this study reflect numerous variations of phenotypic traits, particularly seed characteristics and genetic background of *P. coccineus* germplasm. During the project, three different types of datasets including morpho-agronomic traits of plants, morphometric seed characteristics and genetic profiles of *P. coccineus* accessions from eight geographic origins from 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, providing valuable material for use in breeding, scientific research, educational and other programmes. Regarding activities started within the SMARTLEG project, ongoing collaboration with the Bean\_Adapt project is established.

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**APPENDIX 1. LIST OF PARTNERS INVOLVED IN THE PROJECT**

<b>ECPGR-funded participants</b>	<b>Observers</b>
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<p>Mirjana Vasić Institute of Field and Vegetable Crops Maksima Gorkog 30 21000 Novi Sad <b>Serbia</b> Tel (381) 21 4898359 Fax (381) 21 4898355 mirjana.vasic@ifvcns.ns.ac.rs</p>	<p>Jelka Šuštar-Vozlič Kmetijski inštitut Slovenije Hacquetova ulica 17 1000 Ljubljana <b>Slovenia</b> Tel (386) 1 2805262 Fax (386) 1 2805255 jelka.vozlic@kis.si</p>
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<p><b>Self-funded participants</b></p> <p>Roberto Papa Dipartimento di Scienze degli Alimenti - Facoltà di Agraria 60131 Ancona <b>Italy</b> Tel (39) 0712204280/4617 Fax (39) 0712204858 rpapa@univpm.it</p>	<p>Zdravko Matotan Podravka d.d. Ante Starčevića 32 48 000 Koprivnica <b>Croatia</b> Tel (385) 48 651 144 Fax (385) 48 622 518 zdravko.matotan@podravka.hr</p>

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#### APPENDIX 2. LIST OF GENOTYPED ACCESSIONS

Lab label	ACC #	Additional ACC info	Country of origin
2	GB01090		BiH
4	GB00702		BiH
6	GB01213		BiH
7	GB01089		BiH
8	GB01210-2		BiH
9	GB01212-3		BiH
10	GB01210-1		BiH
17	GB01207-1		BiH
20	GB01207-2		BiH
21	GB01212-1		BiH
23	GB01214-1		BiH
24	GB01214-4		BiH
66	GB01209		BiH
67	GB01208		BiH
68	GB01214-3		BiH
70	GB01214-2		BiH
28	BVAL610160		Austria
30	BVAL-610180		Austria
35	BVAL-610200		Austria
38	BVAL610145		Austria
40	BVAL-610151		Austria
41	BVAL-610183		Austria
42	BVAL-610147		Austria
48	Bonela'	standard Austria	Austria
72	BVAL-610173		Austria
76	BVAL-610192		Austria
77	BVAL-610218		Austria
82	BVAL-610184		Austria
87	BVAL-610216		Austria
88	BVAL-610194		Austria
89	BVAL-610249		Austria
90	BVAL-610199		Austria
27	ITA-ACC187		Italy
31	ITA-ACC188		Italy
34	ITA-ACC00206		Italy
78	ITA-ACC022		Italy
79	ITA-ACC186		Italy
83	ITA-ACC114		Italy
86	ITA-ACC189		Italy



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129	MK1088	1088 white	Macedonia
130	MK714	714	Macedonia
131	MK848	848	Macedonia
132	MK104	104	Macedonia
133	MK1134	1134	Macedonia
134	MK829	829 brown/cream	Macedonia
135	MK1090	1090	Macedonia
136	MK375	375	Macedonia
137	MK020	020	Macedonia
138	MK861	861 violet/d.violet	Macedonia
139	MK1091	1091 d.brown/cream	Macedonia
140	MK721	721	Macedonia
141	MK826	826	Macedonia
142	MK1093	1093	Macedonia
143	MK1083	1083 d.viol/pink small	Macedonia
144	MK316	316 cream/brown	Macedonia
145	MK1089	1089 cream/brown	Macedonia
146	MK1084	1084d.violet/pink	Macedonia
147	MK801	801	Macedonia
148	MK840	840	Macedonia
149	MK607/1	607/1 d.violet/pink	Macedonia
150	MK003	003	Macedonia
151	MK786	786	Macedonia
152	MK1094	1094 violet/d.violet	Macedonia
153	MK431	431	Macedonia
154	MK1086	1086	Macedonia
155	MK1135	1135	Macedonia
156	MK1087	1087d.violet/pink	Macedonia
157	MK1092	1092	Macedonia
158	MK1085	1085 cream/brown (red flowers)	Macedonia
159	MK004	004	Macedonia
160	MK008/2	008/2	Macedonia
25	RO-11		Romania
32	RO-1		Romania
45	RO-3		Romania
49	RO-4		Romania
51	RO-5		Romania
55	RO-6		Romania
58	RO-12		Romania
73	RO-8		Romania
74	RO-7		Romania
80	RO-9		Romania
84	RO-10		Romania

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26	NSPhc-8		Serbia
29	NSPhc-5		Serbia
33	NSPhc-16		Serbia
36	NSPhc-14		Serbia
37	NSPhc-12		Serbia
39	NSPhc-7		Serbia
44	Darko'	standard Serbia (NS-16)	Serbia
81	NSPhc-1		Serbia
85	NSPhc-4		Serbia
91	SVK00978	425/97 19/4	Slovakia
92	SVK00928	372/97 480/8	Slovakia
93	SVK00929	Gracia	Slovakia
94	SVK00616	479/97 60/1	Slovakia
95	SVK01274	700/97 32/5	Slovakia
96	SVK01111	637/97 104/1	Slovakia
97	SVK00201	0114 H	Slovakia
98	SVK01283	777/97 116/1	Slovakia
99	SVK00715	930/99 288	Slovakia
100	SVK00777	586/97	Slovakia
101	SVK01114	676/97 102/3	Slovakia
102	SVK00637	Strakata	Slovakia
103	SVK01304	1199/00 1	Slovakia
104	SVK01276	43/960 5408	Slovakia
105	SVK00773	1160/99 219	Slovakia
106	SVK00780	1122/99	Slovakia
107	SVK01273	1087/99 33	Slovakia
108	SVK00774	1164/99 186	Slovakia
109	SVK01270	686/97 19	Slovakia
110	SVK00994	941/99 29	Slovakia
111	SVK00775	1167/99 21	Slovakia
112	SVK01325	Albena	Slovakia
113	SVK01112	643/97 40	Slovakia
1	SRGB00828	PHA892	Slovenia
3	SRGB00926	PHA990	Slovenia
5	SRGB00791	PHA856b	Slovenia
11	SRGB00222	PHA220	Slovenia
12	SRGB00502	PHA516	Slovenia
13	SRGB00248	PHA246	Slovenia
14	SRGB00249	PHA247	Slovenia
15	SRGB00004	PHA4= 'Emergo' from SRGB	Slovenia
16	SRGB00171	PHA170	Slovenia
18	SRGB00837	PHA901	Slovenia
19	SRGB00495a	PHA 506a	Slovenia

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22	SRGB00921	PHA985	Slovenia
43	SRGB00879	PHA943	Slovenia
46	SRGB00117	PHA115	Slovenia
47	SRGB00120	PHA118	Slovenia
50	SRGB00170	PHA168	Slovenia
52	SRGB00793	PHA857	Slovenia
53	SRGB00504	PHA522	Slovenia
54	SRGB00206	PHA204	Slovenia
56	SRGB00788	PHA854	Slovenia
57	SRGB00173	PHA172	Slovenia
59	SRGB00789	PHA855	Slovenia
60	SRGB00035	PHA34	Slovenia
61	SRGB00495	PHA506	Slovenia
62	SRGB00505	PHA526	Slovenia
63	SRGB00827	PHA891	Slovenia
64	SRGB00034	PHA33	Slovenia
65	SRGB00511	PHA536	Slovenia
69	SRGB00500	PHA510	Slovenia
71	SRGB00792	PHA856c	Slovenia
75	SRGB00027	PHA26	Slovenia
115	Emergo'		Slovenia
116	SRGB00119	PHA117	Slovenia
117	SRGB00817	PHA881	Slovenia
118	SRGB00118	PHA116	Slovenia
119	SRGB00507	PHA528	Slovenia
120	SRGB00499	PHA509	Slovenia
121	SRGB00508	PHA529	Slovenia
122	SRGB00268	PHA267/ I	Slovenia
123	SRGB00836	PHA900	Slovenia
125	SRGB00244	PHA242	Slovenia
126	SRGB00253	PHA251	Slovenia
127	SRGB00790	PHA856a	Slovenia
128	SRGB00513	PHA538	Slovenia
114	SVKunknown		Slovakia

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### APPENDIX 3. DESCRIPTORS LIST FOR PHASEOLUS

#### Descriptors list for *PHASEOLUS* / Bean / Bohne

*ECPGR\_Phaseolus.csv / NI\_Phaseolus.csv*

#### GENERAL ACCESSION INFORMATION / Allgemeine Informationen zur Akzession

<u>Year of characterization</u> <b>Charakterisierungsjahr</b>	(ERNTE)
<u>Accession number</u> <b>Akzessionsnummer</b>	(ACCENUMB)
<u>Accession name</u> <b>Akzessionsname</b> (aus Passportdaten)	(ACCENAME)
<u>Genus/Species</u> <b>Gattung/Art</b> (dt+en; aus Passportdaten)	(GENUS+SPECIES +SPAUTHOR)
<u>Subtaxa</u> (dt+en; aus Passportdaten)	(SUBTAXA+SUBTAUTHOR)
<u>Cropname</u> (aus Passportdaten)	(CROPNAME)
<b>Kulturart</b> (dt; aus Passportdaten)	(CROPNAME_DE)

#### INFLORESCENCE / Blütenmerkmale

<u>Days from sowing to 50% flowering</u> <b>Tage bis zur Blüte</b>	(DAYSBLOS)
Tage vom Säen bis zu der Zeit, wo 50% der Pflanzen blühen.	
<u>Colour of flower standard (banner)</u> <b>Farbe der Blütenfahne (oberstes Blütenblatt)</b>	(BLOSVEX)
1 white <b>weiß</b>	
2 greenish <b>grünlich</b>	
3 lilac <b>lila</b>	
4 white with lilac edge <b>weiß mit lila Rändern</b>	
5 white with red stripes <b>weiß mit lila Streifen</b>	
6 dark lilac with purple outer edge <b>dunkellila mit violetten Rändern</b>	
7 dark lilac with purplish spots <b>dunkellila mit violetten Flecken</b>	
8 carmine red <b>karminrot</b>	
9 purple <b>violett / lila</b>	
99 other (specify in descriptor NOTES) <b>anders (siehe unter NOTES)</b>	

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<b>Colour of flower wings</b>	<b>Farbe der beiden seitlichen Blütenflügel</b>	(BLOSWING)
1 white <b>weiß</b>		
2 greenish <b>grünlich</b>		
3 lilac <b>lila</b>		
4 white with carmine stripes <b>weiß mit roten Streifen</b>		
5 strongly veined in red to dark lilac <b>rot bis dunkellila geadert</b>		
6 plain red to dark lilac <b>rot bis dunkellila</b>		
7 lilac with dark lilac veins <b>lila mit dunkellila geadert</b>		
8 purple <b>violett / lila</b>		
99 other (specify in descriptor NOTES) <b>anders</b>		

### LEAF DATA / Blattmerkmale

<b>Leaf shape Blattform</b>	(LEAFSHAPE)
1 triangular <b>dreieckig</b>	
2 quadrangular <b>quadratisch</b>	
3 round <b>rund</b>	

<b>Leaf colour of anthocyan Anthocyanfärbung des Blattes</b>	(LEAFANTHO)
0 absent <b>fehlt</b>	
9 present <b>vorhanden</b>	

<b>Leaflet length [cm] Blättchenlänge</b>	(LEAFLENGTH)
Length of the upper (middle) leaflet of the third fully developed trifoliate leaf (leaves are measured from the bottom – from stem node)	
Länge des obersten (mittleren) Blättchens des dritten, zusammengesetzten (dreiblättrigen) Blattes von unten - vom Stängelknoten weg gemessen	

### PLANT DATA / Pflanzenmerkmale

<b>Plant growth habit Wuchstyp</b>	(TYPE)
1 determinate bush <b>Buschbohne begrenzt wachsend</b>	
2 indeterminate bush <b>Buschbohne unbegrenzt wachsend</b>	
3 indeterminate prostrate but not climbing <b>unbegrenzt wachsend, aber nicht rankend</b>	
4 indeterminate climbing <b>unbegrenzt rankend (Stangenbohne)</b>	
5 determinate climbing <b>begrenzt rankend (Strauchbohne)</b>	
6 mixture <b>uneinheitliches Wachstum</b>	

<b>Plant height [cm] Wuchshöhe in cm</b>	(HEIGHT)
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**POD DATA / Hülsenmerkmale**

**Pod colour from fully expanded immature pod Farbe der unreifen / „grünen“ Hülse**  
(PODCOLIMM)

- 1 dark purple dunkelviolett
- 2 carmine red karminrot
- 3 purple stripe on green grün, violett gestreift
- 4 carmine red stripe on green grün, karminrot gestreift
- 5 pale red stripe on green grün, hellrot gestreift
- 6 dark pink (rose) dunkelrosa
- 7 normal green grün
- 8 shiny green hellgrün
- 9 dull green to silver gray dumpfgrün bis silbergrau
- 10 golden or deep yellow golden oder tiefgelb
- 11 pale yellow to white blaßgelb bis weiß
- 99 other (specify in descriptor NOTES) anders (siehe unter NOTES)

**Pod suture strings Fädigkeit der Hülsennaht einer grünen ausgewachsenen Hülse**  
(STRING)

- 0 stringless kein Faden
- 3 few strings wenig Faden
- 5 moderately stringy mittel fädig
- 7 very stringy stark fädig

**Pod colour at physiological maturity Hülsenfarbe bei physiologischer (Gelb-)Reife**  
(PODCOLMAT)

(e.g. yellow pods not yet dried!!!)  
(z.B. gelbe Hülse nicht trocken !!!)

- 1 dark purple dunkelviolett
- 2 red rot
- 3 pink rosa
- 4 yellow gelb
- 5 pale yellow with coloured mottling stripes blassgelb mit farbigen Streifen
- 6 persistent green grün bleibend
- 9 other anders

**Pod length [cm] Hülsenlänge bei physiologischer (Gelb-)Reife in cm** (PODLENGTH)  
(at physiological maturity (yellow) mature)

**Pod width [mm] Hülsenbreite bei physiologischer (Gelb-)Reife in mm** (PODWIDTH)  
(at physiological maturity (yellow) mature)

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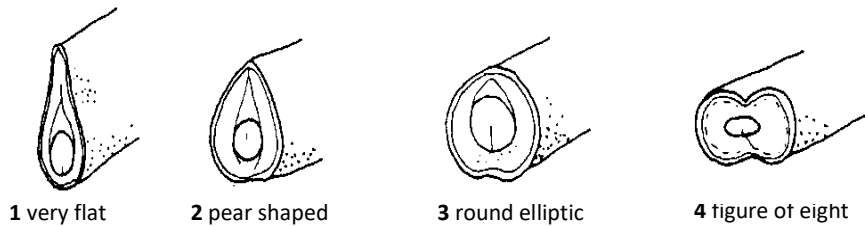
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#### **Pod cross-section** **Hülsenquerschnitt vor physiologischer (Gelb-)Reife** (PODCROSS)

(before physiological maturity / green - yellow mature)

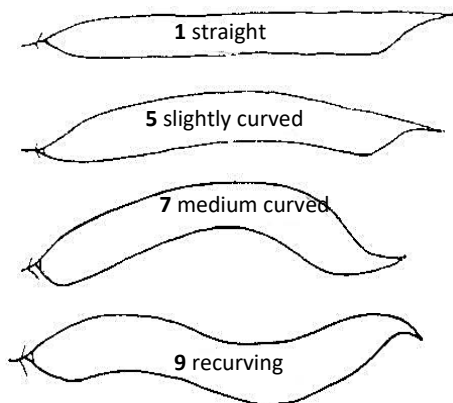
- 1 very flat **sehr flach**
- 2 pear shaped **birnenförmig**
- 3 round elliptic **elliptisch-rund**
- 4 figure of eight **achterförmig**
- 9 other **anders**



#### **Pod curvature** **Hülsenkrümmung bei physiologischer (Gelb-)Reife** (PODCURV)

(at physiological maturity (yellow) mature)

- 1 straight **gerade**
- 5 slightly curved **leicht gebogen**
- 7 medium curved **gebogen**
- 9 recurving **wellig**



#### **Days to 90% pod maturity** **Tage bis zur Totreife von 90 % der Hülsen** (MATURITY)

wird aus Anbaudatum und Enddatum der Ernte berechnet

#### **Locules per pod** **Anzahl der möglichen Samenanlagen** (PODLOCULES)

#### **Seeds per pod** **Anzahl der ausgebildeten Samen pro Hülse** (PODSEED)

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# Efficient management of resources for smart legumes utilization (SMARTLEG)

## Activity Report

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### SEED DATA / Samenmerkmale

#### Seed coat colour **Samengrundfarbe(n)** (SEEDCOLOR)

The main colours are listed below. More than one colour can be expressed by using semicolons (e.g.: 3;5;8)

Die Hauptfarben sind unten gelistet. Hat der Same mehr als eine Farbe, wird die zweite bzw. dritte Färbung mit einem Strichpunkt getrennt hintangereiht (e.g.: 3; 5; 8)

- 1 white **weiß**
- 2 cream **cremeweiß**
- 3 yellow **gelb**
- 4 brown **braun**
- 5 pink **rosa**
- 6 red **rot**
- 7 purple **violett / lila**
- 8 black **schwarz**
- 99 other (specify in descriptor NOTES) **anders**

#### Seed coat pattern **Art der Zeichnung** (SEEDPATCHAR)

- 0 no pattern **keine Zeichnung**
- 1 mottled **marmoriert**
- 2 striped **gestreift**
- 3 speckled **locker gesprenkelt, gepunktet**
- 4 spotted **getupft**
- 5 blotched **gefleckt (großflächig)**
- 99 other (specify in descriptor NOTES) **anders**



1 mottled



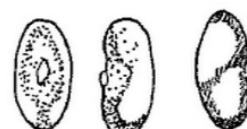
2 striped



3 speckled



4 spotted



5 blotched

#### Brilliance of seed **Glanz des Samens** (BRILLIANCE)

- 3 dull **matt**
- 5 medium **halbmatt**
- 7 shiny **glänzend**

#### Seed length [mm] **Samenlänge in mm** (SEEDLENGTH)

#### Seed height [mm] **Samenhöhe in mm** (SEEDHEIGHT)

#### Seed width [mm] **Samenbreite in mm** (SEEDWIDTH)



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#### Seed shape longitudinal **Samenform längs**

(SEEDSHAPEL)

- 1 circular **rund**
- 2 oval **oval**
- 3 cuboid **rechteckig**
- 4 kidney shaped **nierenförmig**
- 5 markedly truncate **merklich abgeflacht**



#### Colour of seed hilum **Farbe des Samennabels**

(SEEDHILUM)

- |                          |                                |
|--------------------------|--------------------------------|
| 1 white <b>weiß</b>      | 6 carmine red <b>karminrot</b> |
| 2 beige <b>beige</b>     | 7 violet <b>violett</b>        |
| 3 ochre <b>ockergelb</b> | 8 black <b>schwarz</b>         |
| 4 brown <b>braun</b>     | 9 other <b>anders</b>          |
| 5 red <b>rot</b>         |                                |

#### Colour of seed hilumring **Farbe des Nabelringes**

(SEEDHILUMRING)

Main colours are listed below. More than one colour can be expressed by using semicolons (e.g.: 3;5;8)

Die Hauptfarben sind unten gelistet. Bei mehr als einer Farbe, wird die zweite bzw. dritte Färbung mit einem Strichpunkt getrennt hintangreift (e.g.: 3; 5; 8)

- |                          |                                |                         |
|--------------------------|--------------------------------|-------------------------|
| 1 white <b>weiß</b>      | 4 brown <b>braun</b>           | 7 violet <b>violett</b> |
| 2 beige <b>beige</b>     | 5 red <b>rot</b>               | 8 black <b>schwarz</b>  |
| 3 ochre <b>ockergelb</b> | 6 carmine red <b>karminrot</b> | 9 other <b>anders</b>   |

#### 100-seed weight [g] **Hundertkorngewicht in g**

(SEEDHKM)

Weight of 100 seeds to the first decimal place at a moisture content of 12-14%

#### Percentage seed protein [%] **Proteingehalt in %**

(PROTEIN)

#### Phaseolin type **Phaseolin-Typus**

(PHASEOLIN)

The phaseolin types should be indicated by a letter, e.g. T, C, S, as it has been indicated in specialized publications such as Toro O, CH Ocampo & DG Debouck, 2007. 'Phaseolin: variability and reference materials in wild and cultivated common bean'. Annual Rept. *Bean Improvement Cooperative* (USA) 50: 69-70. Once the phaseolin type has been indicated by a conventional letter, then a digital image of the gel with the particular accession under study can be added

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# Efficient management of resources for smart legumes utilization (SMARTLEG)

## Activity Report

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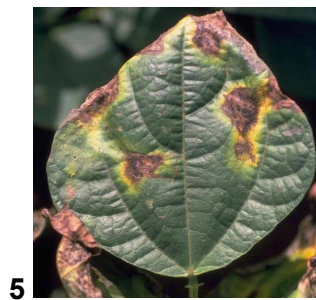
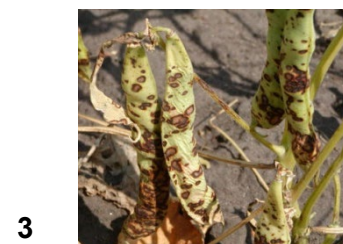
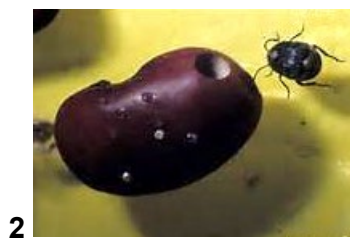
### ABIOTIC STRESSES / Abiotische Stressmerkmale

Drought **Dürre**

(DROUGHT)

### BIOTIC STRESSES / Biotische Stressmerkmale

1. **Bruchid** (*Acanthoscelides obtectus*) **Bohnenkäfer** (*Acanthoscelides*) (ACANTHO)
2. **Bruchid** (*Zabrotes subfasciatus*) **Bohnenkäfer** (*Zabrotes*) (ZABROTOS)
3. **Anthraco** (*Colletotrichum lindemuthianum*) **Anthraco** (ANTHRACNOSE)  
Pilzinfektion
4. **Halo blight** (*Pseudomonas syringae* pv. *phaseolicola*) **Fettfleckenkrankheit** (PSEUDOMON)  
bakteriell
5. **Bacterial blight** (*Xanthomonas campestris* pv. *phaseoli*) **Aderschwärze** (XANTHO)  
bakteriell
6. **Bean common mosaic virus** (BCMV) **Bohnenmosaikvirus** (MOSAIC)



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## Efficient management of resources for smart legumes utilization (SMARTLEG)

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#### NOTES / Anmerkungen

(NOTES)

Give additional information where descriptor is noted as 99 Other. Also include here any further relevant information (diseases, drought, etc.).

Aufgetretene Stressfaktoren (Krankheiten, Dürre, etc.) und eventuelle Maßnahmen notieren  
Anmerkungen und Erklärungen der Merkmale, die mit **99 other (anders)** bewertet wurden