

SEE-ERA.NET Plus Joint Call

SEELEGUMES

Sustainable preservation of indigenous South East European legumes and their traditional food and feed Products

Agricultural Institute of Slovenia

Report, September 2012



Agricultural Institute of Slovenia
Crop and Seed Science Department
Hacquetova 17
1000, Ljubljana, SLOVENIA

Role of partner no. 6, KIS

The Crop and Seed Science Department covers research in the field of plant breeding and genetics, physiology and technology of field, fodder and vegetable crop production. Emphasis is given to the environment and to humans and animal friendly production, which includes also the Slovene Plant Gene Bank with the mission to collect, evaluate, map, conserve and propagate Slovene autochthonous varieties, old varieties and wild relatives of species important for agriculture.

Botanical and agronomical data on genetic resources from the SRGB are for certain legume species abundant (for instance for *Phaseolus* beans), on the other hand are fairly scarce for some other species.

The goal of our part of work lies in morphological and genetic analysis of selected in Slovenia underutilized and neglected grain legume species: faba bean (*Vicia faba*), vetches (*Vicia* spp.), vetchlings (*Lathyrus* spp.), lentil (*Lens culinaris*), chickpea (*Cicer arietinum*) and lupins (*Lupinus* spp.).

WP1

Within the WP1 we will organize expeditions to selected regions and check the references in existing national inventory, provide updated information on selected legume species in both wild and agricultural flora. The selected regions of Slovenia and of the territory of SEE may still be rich in plant genetic resources of grain legumes. The effective communications with local farmers requires the representation of plant genetic resources experts. That would help in the more precise documentation of the information on landraces, on their use, and other special characteristics. The joint collecting missions will contribute to the further development of collaboration between the national programmes.

WP2

Within the WP 2 we will perform morphological and molecular evaluation of the collected species. Field and pot trials will be a basis for morphological characterization and evaluation of genetic resources. As we have already characterized most of the Phaseolus beans accessions we will broaden the evaluation with analysis of protein, mineral and other nutritional compound contents. The selected material of collected grain legume species will be evaluated as well using Microsatellite markers, also called Simple Sequence Repeats (SSR).

All the work described above within WP1 and WP2 will be performed at the Agricultural Institute of Slovenia. The collaboration with other partners is foreseen mainly through the joint collecting missions, characterization and evaluation and data sharing and exchange.

Results and milestones

Results of the project will consist of:

- Detailed description of all the existing and newly acquired accessions
- Estimation of genetic diversity of grain legume species using morphological, biochemical and molecular markers
- Protocols for appropriate conservation and preservation of grain legume genetic resources
- Preservation and reintroduction of selected accessions.

Milestone 1: Month 12

- Evaluation of existing databases and national inventories
- Preparation for and collecting genetic resources through collecting missions
- Evaluation and recording of material collected
- Preparation of seed material for planting and consecutive trial establishment
- Botanical and morphological characterisation and evaluation
- Genetic analysis of selected accessions using SSRs
- Presentation of results at the meetings and workshops

Milestone 2: Month 18

- Genetic analysis of selected accessions using SSRs
- Biochemical analysis of selected accessions
- Botanical and morphological characterisation and evaluation
- Estimation of genetic diversity of grain legume species using morphological, biochemical and molecular markers
- Inscription of passport data of newly acquired accessions into existing database

Milestone 3: Month 24

- Final data evaluation and diversity analysis
- Presentation of results and preparation of the final report
- Presentation of results at the meetings and workshops
- Preparation of publications concerning dissemination of results and various protocols

Report WP1

We have made an evaluation of existing databases and national inventories and made wider selection of accessions of different species that are going to be characterised and evaluated during the project.

We have made an inventory of previously visited sites and selected regions for further collecting missions.

We organized expeditions to collect genetic legume resources to selected regions. Basic evaluation and recording of material collected is in progress.

Along with that we are checking the references in existing national inventory and we will provide updated information on collected legume species.

Along with that three (3) expeditions by SEELegumes Institutions were made (Uni and AgInstitute Osijek, Uni and Institue NoviSad).

As well extensive exchange of the material took place with AgInstitute and Uni Skopje, Uni Banja Luka, UniOsijek, AgInstitute Osijek and Institue NoviSad).

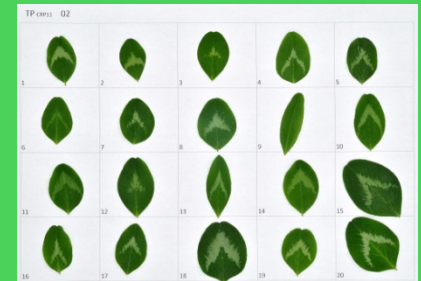
Agricultural crop	Species	Number of accessions
Zrnate stročnice	Phaseolus spp.	1116
	Vicia faba	41
	ostale	7
	Skupaj	1164
Krmne rastline	Trifolium sp.	216
	Medicago sp.	43
	Vicia sp.	42
	Lotus sp.	35
	Lolium sp.	31
	Festuca sp.	102
	Dactylis	116
	Phleum	45
	Travniške zeli	112
	ostale	242
	Skupaj	984
Poljščine	Zea mays	13
	Triticum	6
	Secale	12
	Papaver	26
	Panicum	15
	ostale	63
		Skupaj
Zelenjadnice	Lactuca sp.	227
	Allium cepa	31
	Brassica oleracea	11
	Diplotaxis spp.	18
	ostale (Cichorium, Valerianella, Solanum Lyc.)	16
	Skupaj	303
Krompir	<i>Solanum tuberosum</i> L.	34
Druge vrste krompirja	<i>Solanum</i> sp.	18
	<i>Solanum</i> sp. diploidne populacije	14
	Skupaj	66

Agricultural crop	Species	Number of accessions
Vinska trta (kloni lastnih selekcij)	Vitis	39 (Ivanjkovci, Vrhopolje)
Vinska trta (stare sorte)	Vitis	50 (Amp. vrt BF - Kromberk pri N.G.)
	Skupaj	89
Jablana	Malus sp.	245 (na Brdu)
Jagoda	Fragaria sp.	56 (na Brdu)
	Skupaj	301
Malina	Rubus sp.	29 (na Brdu)
Robida	Rubus sp.	7 (na Brdu)
Črni ribez	Ribes sp.	53 (na Brdu)
Rdeči ribez	Ribes sp.	13 (na Brdu)
Beli ribez	Ribes sp.	6 (na Brdu)
Kosmulja	Ribes sp.	12 (na Brdu)
Ameriška borovnica	Vaccinium corymbosum	50 (na Brdu)
Brusnica	Vaccinium macrocarpon	2 (na Brdu)
	Skupaj	161
	SKUPAJ	3203

Report WP2 a

We performed morphological and molecular evaluation of the collected species and selected accessions.

Field and pot trials will form a basis for morphological characterization and evaluation of genetic resources. Seed material was prepared for planting and consecutive trial establishment. Twenty plants per accession were prepared in styrofoam plates for consecutive planting in pots and in the field (Picture 1). Botanical and morphological characterisation and evaluation for the first year was conducted according to IPGRI(Bioversity International)/FAO descriptors in the greenhouse and in the field (Picture 2 and 3). Herbarium sheets of leaves were prepared for selected species (Picture 4) enabling us to perform morphological measurements (e.g. width and length) later in the season.



Evaluation of red clover accessions

Oznaka	Mesto nabiranja - izvor	n.m.v.	Tip	ZCVE	DGP*	D(mm)	Š(mm)
TP CRP11 01	Rožice	500	4	5.jun.	95	37	21
TP CRP11 02	Otlica	800	6	5.jun.	35	37	24
TP CRP11 03	Predmeja	900	6		5	35	21
TP CRP11 04	Most na Soči	200	3	5.jun.	90	41	22
TP CRP11 05	Trenta (hotel Zlatorog)	620	4		55	34	21
TP CRP11 06	Zadnja Trenta	890	5		5	36	24
TP CRP11 07	Vršič (16 serpentina)	1390	7		0	32	22
TP CRP11 08	Soča 94	1200	7	16.jun.	15	34	22
TP CRP11 09	Rudno polje	1400	7		0	27	21
TP CRP11 10	Logatec -Pusto polje	476	4	6.jun.	75	36	21
TP CRP11 11	Planina pri Jezeru	1450	7		0	31	22
TP CRP11 12	Planina Blato	1147	7		0	30	22
TP CRP11 13	Planina Krasca	1400	6		0	33	22
TP CRP11 14	Pokljuka – Šijec	1200	7		0	35	23
TP CRP11 15	Pod Vel. Draškim vrhom	1895	4		0	29	20
TP CRP11 16	Vrata - Ros	720	4	13.jun.	50	38	23
TP CRP11 17	Trenta - Na Logu	680	3	13.jun.	85	41	27
TP CRP11 18	Loški potok - Draga	805	7	5.jun.	40	35	21
TP CRP11 19	Loški potok - Podpreska	835	6	10.jun.	60	36	22
TP CRP11 20	Loški potok - Žagarji	860	7		30	31	20
TP CRP11 21	Loški potok - Javorje	920	8		15	30	19
TP CRP11 22	Kumrovo, Kočevski rog	865	5		65	37	

* - delež rastlin, ki so v prvem letu prešle v generativno fazo

Evaluation of Faba bean accessions

Bob (*Vicia faba*)

Pronađen u selu Gomiljani (blizu Trebinja N42°42'910"; E018°18'178"; 270 m nadmorske visine, domaćinstvo Milenke i Vase Marića 19.03.2012. Sećaju se da se davno koristio kao stočna hrana i to i kao zelena (košenje i davanje stoci odmah ili kao suvo zrno); ne može da se seje s jeseni već krajem zime, u januaru, tačnije od 25.12. do 15.01., „između Božića i Novih Godina“. Ne sećaju se da se koristio za stočnu hranu. Mada su im neki pričali da je bilo i takvog boba. Koristi se kao varivo od mladih mahuna, ređe kao salata od mladog zrna, a najređe kao varivo od suvog, zrelog zrna isto kao pasulj.

varivo od mladih mahuna (uobičajeno jelo): Mahune treba da budu što mlađe. One se kuvaju u vreloj vodi sve dok se ne skuhaju, a onda se ocede. Obavezno se izdinstaju crni luk (Vaso), pa se dodaju mahune i još malo dinstaju zajedno. Ili svemu tome može da se doda i krompir (predhodno skuhan - Miljenka). Kad bude gotovo „izgnječka se“. Dobro je i samo i sa mesom i sa salatam.

salata od mladog zrna (voli ga samo domaćin Vaso): Isto se kuva u vreloj vodi, voda se prospe, pa se oljušti. Pomeša se sa maslinovim uljem, sirćetom i belim lukom. Dobro je i vruće i hladno.

varivo od suvog, zrelog zrna (najređe ga prave, a rado ga jedu stariji): Sprema se isto kao pasulj: Uveče se potopi u hladnu vodu, koja se ujutru odlije. Kad se prokuva i ta prva voda se prospe. Kuva se dalje sam ili sa suvim mesom, uz dodatak povrća (mrkva, peršun, luk) i obavezno zaprži.

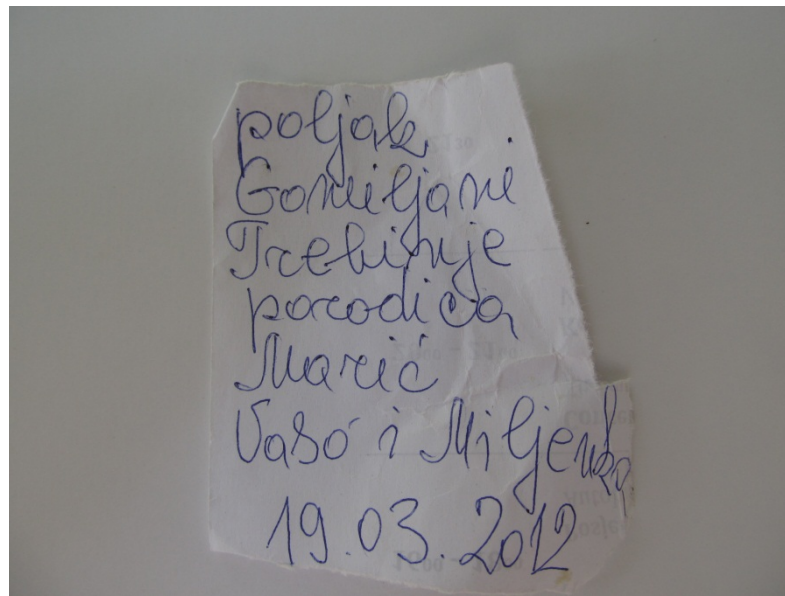
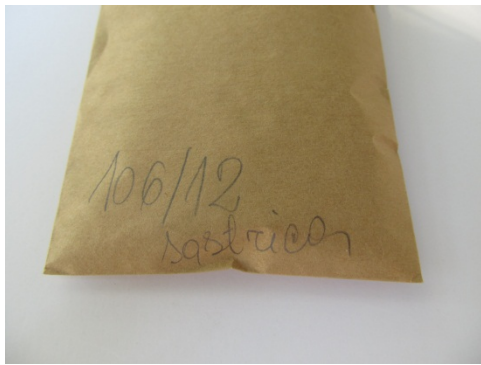


Evaluation of Lathyrus accessions

Sastrica (Lathyrus sp.)

Pronađena u selu Zgonjevo (Petrovo polje, Trebinje; N42°39'11.9"; E018°19'19.8"; 285 m nadmorske visine);
Prije II svjetskog rata sijana na velikim površinama, mijenjana u Konavlima za ulje;
Sad se proizvodi kod manjeg broja proizvođača;
Koristi se manje kao varivo, uglavnom kod oboljelih od dijabetesa i u tom slučaju se melje i kuva kao kafa;





Phaseolus:

Evaluation of morphological traits

lišček marmorirani in temnordeč					
PHA23	zelo lep, zdrav, veliko strokov po celi rastlini, še zeleni, nitka, vlakna	rdeč	ja		ja
PHA29	rahlo virotičen, stroki zeleni, malo debelejši, manj poln, a po celi rastlini	bakreno rdeč, sr. poln	ja.		ja
PHA79	virotičen, nizek, zbit, majhni listi, niti ni do vrha opore	t. rdeč	ne		
PHA133	rahlo virotičen, sr. poln, stroki po celi rastlini, zeleni	pozen, marmorirano rdeč	ja.	ja	ja
PHA279	veliko strokov po celi rastlini, manj listne mase kot 23, t. zeleni listi, zeleni stroki	virotičen, rdeč	ja.		ja
PHA289	poln, po celi rastlini, zdrav, sr. listne mase, zeleni stroki	rdeč, zelo zgoden	ja.	ja	ja
PHA626	ni čisto do vrha opore, rahlo virotičen, stroki po celi rastlini, zeleni	rdeč	ne		
PHA627	lep, poln, veliko strokov po celi rastlini, zdrav, le na nekaj listih malo mehurjivosti	rdeč lišček, lep	ja	ja	ja
PHA628	veliko velikih listov, sr. strokov, rahlo virotičen	rdeč lošček, pozen, kar poln	ja.		ja
PHA629	sr. veliki listi, malo rumenijo, stroki po celi rastlini, rahlo ožig na strokih	rdeč lišček, leši kot 923, a bolj bolan	ne		
PHA923	majhni listi, ne gosti, stroki po celi rastlini, rahli mehurji na strokih,	rdeč lišček, srednje poln		če je edini tip, drugače ne	preveriti
lišček črn					
PHA77	malo olistan, stroki povsod, a precej ožiga in rje	noč in dan	ne		
PHA134	listi že rumenijo, veliki, stroki na 2/3 rastline, ni najbolj zdrav, začetek tje	noč in dan, zgodnejši kot 902	ja.	ja	ja
PHA902	več listov, večji, še veliko cvetnih nastavkov, cvet nima žil, začetek boleznj na strokih	noč in dan, pozen, podoben kot 631, a lepši	ja.		ja
PHA993	virotičen, veliko strokov, ukrivljeni, začetek rje	kratki, zeleni, ukrivljeni stroki, sr. veliko	ne	ja?	ja
lišček koksasti					
PHA631	rahle rdeče proge na strokih, začetek pegavosti, veliki listi in stroki, sr. poln, 2 tipa?	zelo lep, zdrav	ja	ja	ja
PHA640	šibke rastline, malo listov, sr. strokov, rdeče proge, močan ožig,	podoben kot 631 po semenu, a slabše rasti	ne		
PHA642	podoben 640, več strokov, močan ožig	640 in 642 podobna	ne		
PHA922	virotičen, zeleno-vijolični stroki, bolj šibke ratsline	veliko strokov	ne		
lišček rjav					
PHA132	rastline ne čisto do vrha opore, a zelo polno strokov, rumeni z vijoličnimi progami, zdrav	lep, poln, zgoden, za kombinirano uporabo	ja.	ja	ja
PHA137	rumeni stroki po celi rastlini, lepe rastline, do vrha opore, za kombinirano uporabo	malo kasnejši kot 132	ja.	ja	ja
lišček siv					
PHA637	veliko ožiga	sivo rjav	ne	ja	ja
lišček oranžno rjav					
PHA963	močno virotičen	bakrene barve	ne		



GB 00446 – trešnja



Phaseolus vulgaris
VT-2-53



Phaseolus vulgaris
VT-2-53



Phaseolus vulgaris
VT-2-53

Yield evaluation (standard varieties included)

	Total weight (g)	No of plants	Weight (g)/plant
Črešnjevec rdeči			
GB153*	1607	47	34,2
GB729	1039	46	22,6
GB733	541	49	11
GB280	597	45	13,2
Črešnjevec pisani debeli			
GB152	1119	48	23,3
GB283	1375	50	27,5
GB740	884	46	19,2
GB751	754	41	18,3
GB1026	536	47	11,4
Lišček marmorirani in temnordeč			
GB23	1441	41	35,1
GB29	669	34	19,6
GB133	1317	37	35,6
GB279	968	43	22,5
GB289	1519	48	31,6
GB627	1291	50	25,8
Lišček koksasti			
GB631	773	35	22,1
KLEMEN	1483	83	17,8
JABELJSKI PISANEC	1396	85	16,4
SEM22	1867	85	21,9

*planted in 2012 on three locations in Slovenia



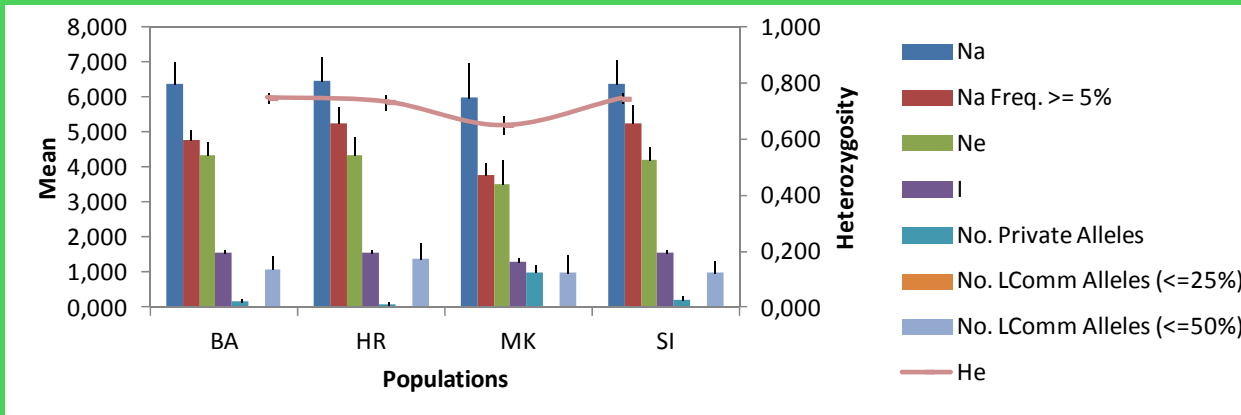
2012 evaluation of selected genotypes in three different locations (standard varieties included)



Genetic linkages between Macedonian, Bosnian, Croatian and Slovenian common bean genotypes

In total, 129 genotypes of common bean were included in genetic analysis using 13 highly polymorphic microsatellite markers. Genetic screening was performed on 73 accessions from Macedonia (MK), 21 accessions from Bosnia and Herzegovina (BA), 15 accessions from Croatia (HR) and 20 accessions from Slovenia (SI).

ALLELIC PATTERNS AMONG POPULATIONS



Na = No. of Different Alleles

Na (Freq >= 5%) = No. of Different Alleles with a Frequency >= 5%

Ne = No. of Effective Alleles

I = Shannon's Information Index

No. Private Alleles = No. of Alleles Unique to a Single Population

No. LComm Alleles (<=25%) = No. of Locally Common Alleles (Freq. >= 5%) Found in 25% or Fewer Populations

No. LComm Alleles (<=50%) = No. of Locally Common Alleles (Freq. >= 5%) Found in 50% or Fewer Populations

He = Expected Heterozygosity

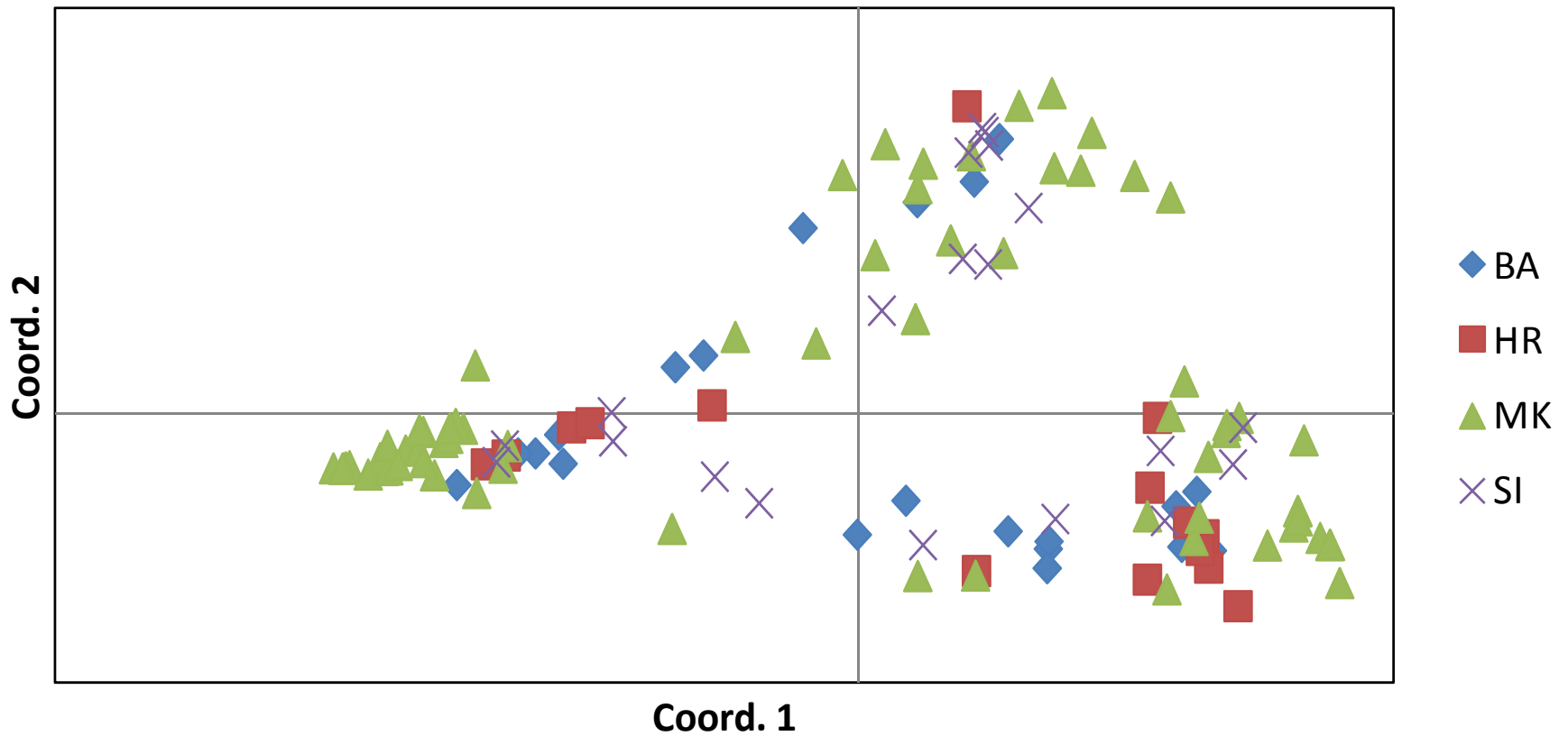
UHe = Unbiased Expected Heterozygosity

PRINCIPAL COORDINATES ANALYSIS (PCA)

via covariance matrix with data standardization

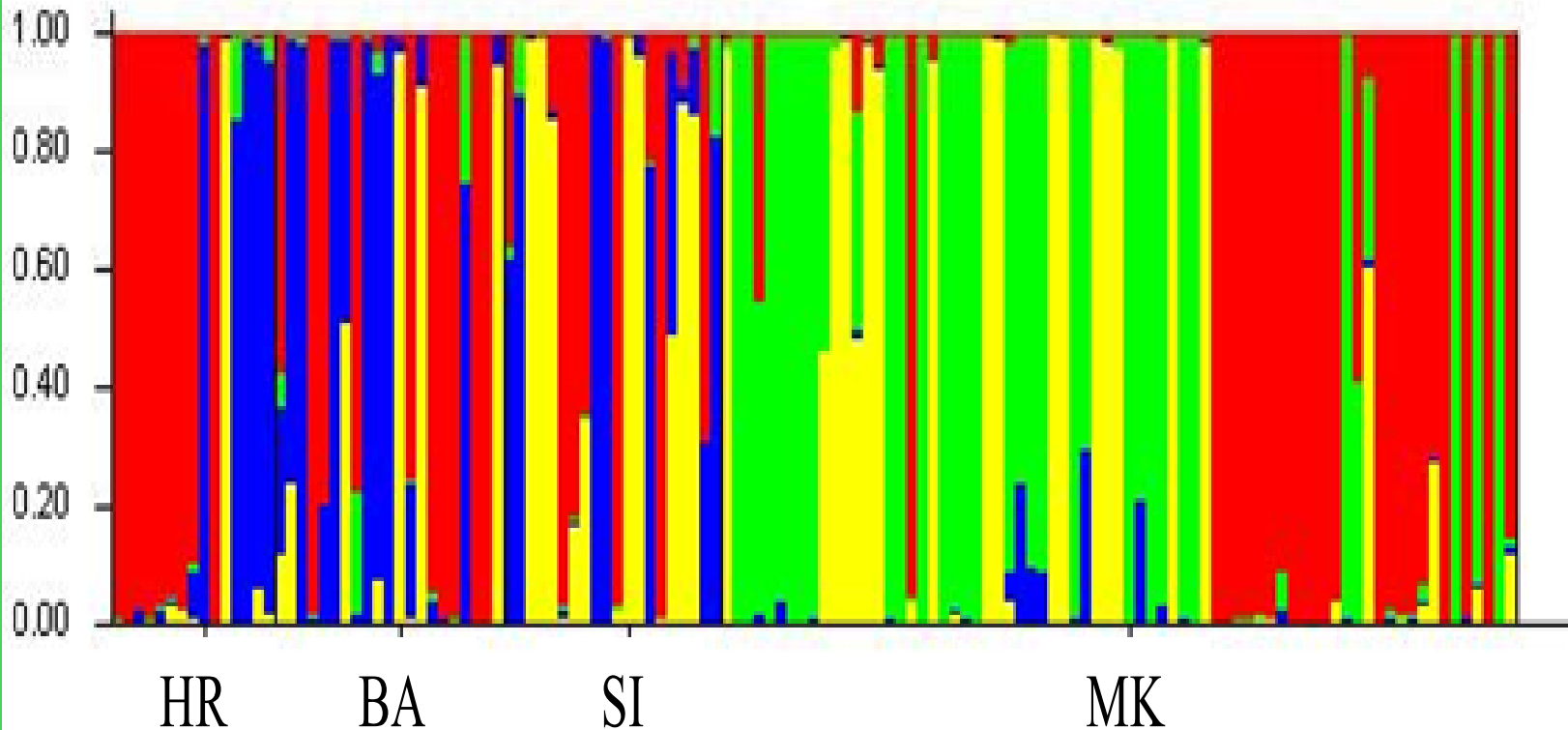
Percentage of variation explained by the first 3 coordinates

Axis	1	2	3
%	46.56	20.17	11.45
Cum %	46.56	66.73	78.18



THE CLUSTER ANALYSIS FOR INFERRING POPULATION STRUCTURE

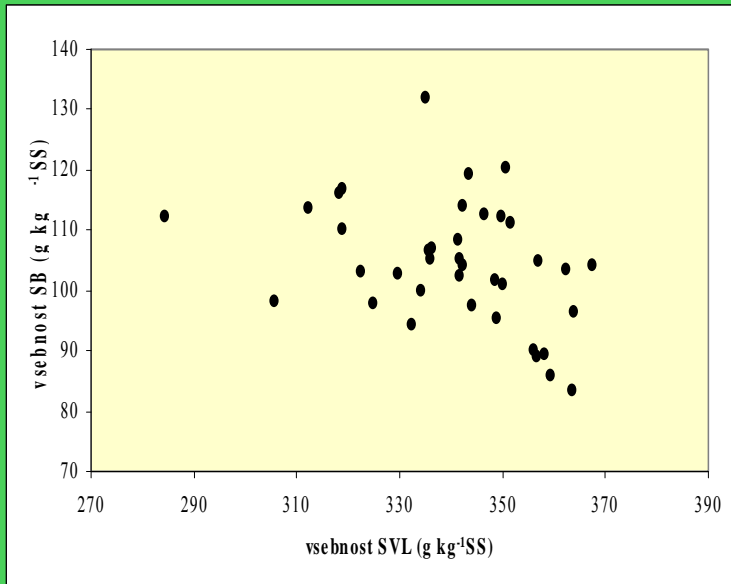
using Bayesian method by computing *ad hoc* statistic, where four genetic clusters were estimated



Report WP2 c

Already characterized Phaseolus accessions were evaluated for protein, mineral and other nutritional compound contents. In order to do that, we have performed initial analysis of two accessions, to validate analytical methods.

Characterisation of red clover accessions (quality, NIR).



Analytical number	2028/2011		2029/2011		
Accession	ČEŠNJEVEC		GB 740		
Parameter		In sample	In DM	In sample	In DM
DM	g/kg	925,2	1000	904,3	1000
Moisture	g/kg	74,8	0	95,7	0
Raw ash	g/kg	39	42	40	45
Kalcij - Ca	g/kg	1,08	1,17	0,91	1,01
Magnezij - Mg	g/kg	1,41	1,53	1,37	1,51
Kalij - K	g/kg	15,1	16,3	16,7	18,5
Fosfor - P	g/kg	5,25	5,67	4,14	4,57
Baker - Cu	mg/kg	8	9	6	7
Železo - Fe	mg/kg	47	51	63	70
Mangan - Mn	mg/kg	10	10	10	12
Cink - Zn	mg/kg	22	24	28	31

Evaluation of chemical characteristics

- 12 Slovene varieties
- 12 accessions from SRGB



Oznaka	Kalij - Ca	Magnezij - Mg	Kalij - K	Fosfor - P	Baker - Cu	Železo - Fe	Mangan - Mn	Cink - Zn
	g/kg v.s.s.	g/kg v.s.s.	g/kg v.s.s.	g/kg v.s.s.	mg/kg v.s.s.	mg/kg v.s.s.	mg/kg v.s.s.	mg/kg v.s.s.
PHA 15 - Jabelski stročnik	0,81	1,81	17,6	5,78	7,6	112,8	10,4	27,2
PHA 23 - Lišček	1,78	1,54	14,5	5,44	8,9	114,4	13,0	36,0
PHA 283 - Češnjevce	1,07	1,71	16,2	5,51	9,7	75,9	10,7	32,4
PHA 153 - Češnjevce	1,04	1,70	15,7	5,80	9,8	69,2	11,1	33,2
PHA 133 - Lišček	1,41	1,69	14,9	5,10	8,4	76,6	10,8	31,6
JABELJSKI PISANEC	1,10	1,75	14,5	5,61	8,8	110,6	11,7	36,7
PHA 449 - Tetovec	1,36	1,93	16,4	5,91	8,4	85,2	13,7	28,4
SEM 22 - Semenarna	0,78	1,85	16,9	6,35	10,5	83,7	11,4	37,6
KLEMEN	1,10	1,70	17,3	6,50	9,6	86,6	11,7	33,0
ZORIN	1,19	1,69	15,5	5,43	7,5	76,6	14,6	28,3
STAROZAGORSKI	1,11	1,50	17,9	6,09	8,3	89,1	16,0	39,8
PHA 486 - Ribničan	1,02	1,60	17,0	6,48	8,9	67,5	11,6	31,3
PHA 7	1,85	1,76	15,4	5,83	8,9	250,5	13,2	28,5
PHA 15	0,84	1,70	17,0	5,50	8,4	145,3	8,1	27,8
PHA 29	1,05	1,71	17,0	6,05	9,6	118,3	8,0	32,5
PHA 59	1,68	1,71	18,4	6,43	5,8	158,5	11,3	28,7
PHA 153	0,84	1,50	17,5	5,70	7,0	77,4	7,4	25,6
PHA 316	1,89	1,49	16,6	5,89	5,6	91,1	10,4	26,5
PHA 358	0,94	1,76	16,5	5,86	8,0	127,2	10,0	31,4
PHA 363	1,19	1,79	18,7	6,17	8,6	130,8	9,7	32,6
PHA 418	1,22	1,34	15,8	6,35	11,4	93,2	9,4	32,2
PHA 639	0,98	1,65	15,5	6,56	7,3	68,7	8,0	30,0
PHA 642	0,86	1,55	16,2	5,79	7,4	57,4	7,2	27,3
PHA 717	1,86	1,60	17,2	5,54	6,0	62,8	10,5	28,1



Evaluation of sensorial traits

Preparation of the sample:

- Soaking conditions, boiling procedure, optimum boiling point and preparation of the samples for the tasting.

Elaboration of the sensory profile:

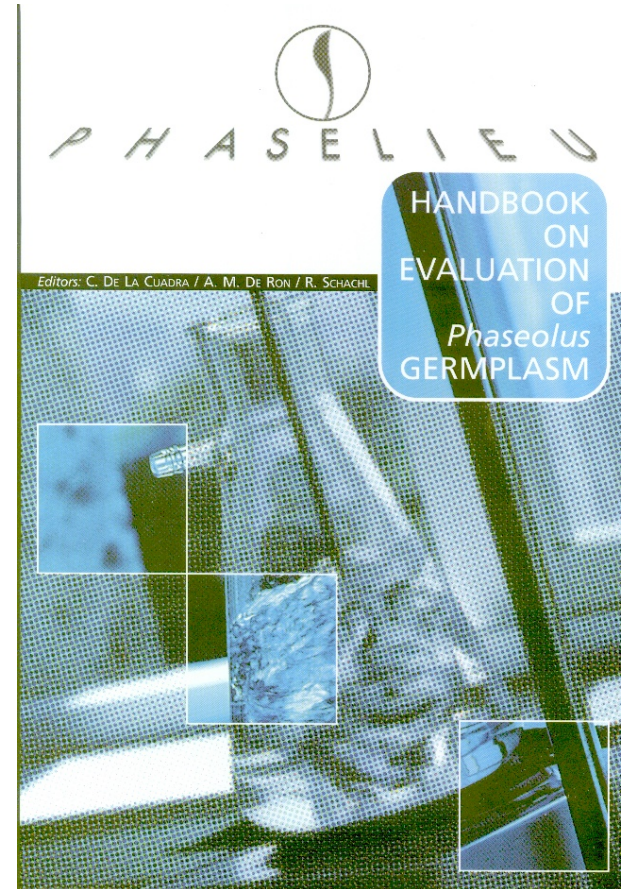
- Recruitment and selection of the judges,
- Training,
- Tasters selection.

Deskriptors selection:

- Visual aspect (seed coat izgled: entire, broken),
- Characteristic of surface,...

Descriptors definition:

- Whole grain (no broken grains), loos of seed coat, smooth surface grain,



Evaluation of sensorial traits

Evaluation of the grain integrity (aspect of the sample is observed in the plate):

-1 (broken) - 5 (whole)

Evaluation of the texture of the seed (sample is introduced into the mouth and without biting it is analysed with the tongue):

- 1 (smooth surface) - 5 (rough surface)

Evaluation of seed coat and albumin hardness (sample is introduced into the mouth and removing albumen):

-1 (soft) - 5 (hard)

Evaluation of characteristics of buttery, granular, mealy, stickiness, astringency (sample is introduced into the mouth and is assigned according to a scale):

-1 (nothing) - 5 (much)



Evaluation of sensorial traits

	Celost zrnja (1-5)	Tekstura plašča (1-5)	Trdota plašča (1-5)	Trdota albumna (1-5)	Maslenost (1-5)	Znatost (1-5)	Kepavost (1-5)	Moknatost (1-5)	Lepljivost (1-5)	Vieča skupaj (1-5)
Črešnjevce rdeči										
GB153	2	1	4	4	2	4	3	3	1	1
GB729	2	2	3	2	1	2	1	2	1	1
GB733	5	2	2	2	1	1	2	2	1	1
GB280	4	1	4	4	2	4	4	3	1	1
Črešnjevce pisani debeli										
GB152	5	1	3	3	2	4	3	3	1	1
GB283	5	2	4	4	1	5	4	2	1	1
GB740	2	2	2	2	3	3	3	2	1	1
GB751	4	1	3	3	3	3	3	3	1	1
GB1026	5	2	4	4	3	3	4	4	1	1
Lišček marmorirani in temnordeč										
GB23	2	2	1	2	2	2	2	1	1	1
GB29	3	2	2	3	3	3	2	2	1	1
GB133	2	1	2	5	2	4	4	2	1	1
GB279	1	1	2	4	2	4	3	2	1	1
GB289	3	1	1	1	3	1	1	2	1	1
GB527	1	1	2	2	3	2	2	2	1	1
Lišček koksasti										
GB631	1	2	4	3	1	3	3	2	1	1
Standardi										
KLEMEN	5	1	3	4	1	4	4	2	1	1
JABELJSKI PISANEC	4	1	4	4	1	3	4	4	1	1
SEM22	1	1	3	3	1	2	3	3	1	1
Legenda:	1-pocen 5-cel	1-gladka 5-hrapava	1-mehak 5-trd	1-mehak 5-trd	1-ni prisotno, 5-zelo prisotno					

