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Legumes of Southeast Europe

The hitchhikers along an ancient Eurasian crop highway

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The SEELEGUMES project logo by Aleksandar Mikić

FOR AUTHORS

Legume Perspectives is an international peer-reviewed journal aiming to interest and inform a worldwide multidisciplinary readership on very different aspects of the research and use of all kinds of legume plants and crops. Its scope ranges from biodiversity, genetics and breeding to agronomy, animal production, human nutrition and health and economy.

The issues of *Legume Perspectives* are usually thematic and devoted to specific crop or topic. They are edited by guest editor, chosen by the Editor-in-Chief and Assistant Editors, who solicit the articles, mostly reviews, from selected authors. The original research articles are also welcome, but are considered for publication by the Editorial Board.

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Please send your prepared manuscripts for publishing in *Legume Perspectives*, as well as any ideas or questions, to Technical Editor (aleksandar.mikic@nsseme.com).

EDITORIAL

Welcome to the Issue 5 of Legume Perspectives! Unlike the previous four that were devoted to individual legume crops, this one brings you the articles resulting mostly from one project. Its name is Sustainable preservation of indigenous South East European legumes and their traditional food and feed products (SEELEGUMES) and it was carried out during 2011 and 2012 within the SEE-ERA programme of the European Union and under the auspices of its Seventh Framework Programme (FP7). The articles in this issue are authored by majority of the project participants and deal with diverse legume plants and crops, as well as with various research topics. We deeply believe that this issue of Legume Perspectives, deliberately illustrated to a perhaps greater extent than usual, will bring you an impression of the genuine beauty and richness of the legume flora of Southeastern Europe. Enjoy your reading!

Branko Ćupina and Aleksandar Mikić
Managing Editors of Legume Perspectives Issue 5

CONTENTS

CARTE BLANCHE

4 Branko Ćupina, Aleksandar Mikić: Where Europe meets Asia and the Present mingles with the Past

RESEARCH

5 Aleksandar Medović, Aleksandar Mikić: Archaeobotanical findings of annual and other legumes in Serbia

7 Roman Hovsepyan: Pulses cultivated in prehistoric periods at the territory of Armenia: Short review of present archaeobotanical data

9 Petr Smýkal, Aleksandar Medović, Živko Jovanović, Nemanja Stanisavljević, Bojan Zlatković, Branko Ćupina, Vuk Đorđević, Aleksandar Mikić: Molecular analysis of ancient DNA isolated from charred pea (*Pisum sativum*) seeds found at an Early Iron Age settlement in southeast Serbia

11 Bojan Zlatković, Stefan Bogosavljević, Aleksandar Mikić, Branko Ćupina: Legumes diversity in the gorges of Carpathian-Balkan Mountain arc in Serbia

14 Aleksandar Simić, Savo Vučković: Pasture and meadow legumes in Serbia

16 Sokrat Jani, Agim Canko, Milto Hyso: Sustainable preservation of indigenous Albanian legumes and their traditional use

19 Aleksandar Mikić, Branko Ćupina, Bojan Zlatković, Petr Smýkal, Svetlana Antanasović, Vuk Đorđević, Aleksandar Medović, Đorđe Krstić, Vojislav Mihailović: Collecting, characterisation and evaluation of 'tall' pea (*Pisum sativum* subsp. *elatius*) in southeastern Serbia

23 Branko Đurić: Reintroduction of faba bean (*Vicia faba* L.) in the Republic of Srpska

24 Siyka Angelova, Mariya Sabeva, Yana Guteva: Local plant genetic resources of faba bean (*Vicia faba*) in Bulgaria

26 Cengiz Toker, Jens Berger, Abdullah Kahraman, Abdulkadir Aydogan, Canan Can, Bekir Bukun, R. Varma Penmetsa, Eric J. Von Wettberg, Douglas R. Cook: *Cicer reticulatum* Ladizinsky, progenitor of the cultivated chickpea (*C. arietinum* L.)

28 Vladimir Meglič, Jelka Šuštar-Vozlič, Marko Maras: South East European (SEE) autochtonous *Phaseolus* bean germplasm genetic diversity and reintroduction of traditional landrace

30 Lana Zorić, Jadranka Luković, Dunja Karanović, Aleksandar Mikić: Lignified tissues in vegetative organs of wild-growing *Trifolium* L. forage species

32 Margarita Vishnyakova, Alla Solovyova, Andrey Sabitov, Pavel Chebukin, Marina Burlyaeva: Economic value of perennial vetchlings (*Lathyrus* L.) species from the Russian Far East

34 Janna Akopian, Andrey A. Sinjushin, Ivan Gabrielyan, Gayane G. Shaboyan: On some biomorphological peculiarities of seedlings of *Vavilovia formosa* (Stev.) Fed. (Fabaceae)

36 Sergio J. Ochatt, Catherine Conreux: *In vitro* biotechnology approaches now available for 'beautiful' vavilovia (*Vavilovia formosa*)

37 Tihomir Čupić, Marijana Tucak, Aleksandar Mikić: Nutritive value of grass pea (*Lathyrus sativus*)

39 Nemanja Stanisavljević, Živko Jovanović, Tihomir Čupić, Jovanka Lukić, Jovanka Miljuš Đukić, Svetlana Radović, Aleksandar Mikić: The effect of cooking and enzymatic digestion on phenolic content of grass pea seeds flour extracts

41 Đorđe Malenčić, Biljana Kiprovski, Branko Ćupina, Aleksandar Mikić: Screening for dietary phenolics and antioxidant capacity of faba bean (*Vicia faba*) and vetches (*Vicia* spp.)

PROJECTS

42 Branko Ćupina, Aleksandar Mikić: SEELEGUMES: One genuine piece in the jigsaw puzzle of the European legume biodiversity

EVENTS

43 Second International Legume Society Conference, Tróia, Portugal, 12-14 October 2016

IN MEMORIAM

47 Professor Reid G. Palmer

Carte blanche to…

…Branko Ćupina[1] and Aleksandar Mikić[2]

Where Europe meets Asia and the Present mingles with the Past

"Above East and West" is an old religious, phylosophic and poetic term denoting what in a more political language, is often denoted as southeastern Europe or, from a geographic point of view, the Balkan Peninsula. This region was truly, as designated in the subtitle of this issue of Legume Perspectives*, one of the main routes Neolithic came to Europe, some ten thousand years ago, when Europe had broke free from the cold dungeon of the last Ice Age.*

Indeed, the first farmers, coming from the parts of the famous Fertile Crescent in Near East via Asia Minor, crossed over into our home continent in present Thrace and quickly sped forward up the flow of the river Danube into other regions. They bore with themselves their revolutionary culture of tilling soil and cultivating plants. According to the present knowledge on these earliest days of crops in Europe, along with three cereals, namely einkorn, emmer and barley, and flax, there were four pulses: chickpea, lentil, pea and bitter vetch. In parallel, the wild flora, that once had been present and that had perished under the glaciers and their lethal breath, began to return to the places it had inhabited eons ago, enriching the landscapes of Southeastern Europe once more and making them both beautiful and genuine. In the end, the crops from other continents, such as Phaseolus *beans, found their new home here and became indispensable in human diets. All this biodiversity, both agricultural and wild, have always comprised a considerable number of legumes and endured the passing millennia.*

Today, it is none else but the Man himself who is the main threat to the survival and diversity of all those countless legume crops and their wild relatives. Travelling along this ancient Eurasian highway, one meets the legume hitchhikers less often and their milestones have become rather rare. An uncontrolled development of industry and urbanisation pay their toll every day by making further life of one legume taxon nearly every day. Our task, as agronomists, geneticists, breeders, botanists, biologists or archaeobotanists, is to work persistently and as hard as possible towards one common goal: to preserve and conserve a treasury we have been blessed with and multiply its benefits for the health and the wealth of our own children. ■

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Archaeobotanical findings of annual and other legumes in Serbia

by Aleksandar MEDOVIĆ[1]* and Aleksandar MIKIĆ[2]

Abstract: The oldest archaeological findings of chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), pea (*Pisum sativum*) and bitter vetch (*Vicia ervilia*) date back as early as from 9th millennium BC and are mostly in Syria and Israel. Together with few cereals and other crops, grain legumes entered Europe via Asia Minor. Serbia is rather rich in archaeobotanical remains of cultivated and wild legumes. Lentil and pea may be regarded as the most important in this region transgressing from the Balkan to Central Europe, being present in most of the excavated material and with Gomolava as one of the most ancient, dated back to 6th millennium BC. The most interesting find of grain legume crops in Serbia comes from the fortified hill of Hissar, near present Leskovac, dated to 11th century BC, with several thousands of the charred seeds of both bitter vetch and pea.
Key words: archaeobotany, charred seeds, crop history, pulse crops, Serbia

Chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.) and bitter vetch (*Vicia ervilia* (L.) Willd.) are among the first domesticated plant species in the world. The oldest archaeological findings from 9th millennium BC are mostly in Syria and Israel (3). From Near East, agriculture began to spread in all directions. Together with few cereals and other crops, grain legumes entered Europe via Asia Minor.

Serbia is rather rich in archaeobotanical remains of cultivated and wild legumes, with the territory of its northern Province of Vojvodina as rather well-studied (Fig. 1). As may be seen, chickpea was mostly absent at the majority of the studied archaeological sites. This may be explained by a probable loss of winter-hardy genotypes during the chickpea distribution to more northern latitudes, although this crop, as the other cool season legumes, has a considerable tolerance to the intensity and length of low temperatures. All this most likely resulted in a much more uncertain cultivation than those of pea or lentil (Fig. 2).

On the other hand, lentil and pea may be regarded as the most important in this region transgressing from the Balkan to Central Europe, being present in most of the excavated material and with Gomolava as one of the most ancient, dated back to 6th millennium BC. Faba bean (*Vicia faba* L.) and common vetch (*Vicia sativa* L.) appeared during the Early Iron Age, grass pea (*Lathyrus sativus* L.) during La Tène and fenugreek (*Trigonella phoenum-graecum* L.) in the Roman period. As for the weedy legume flora, the oldest remains belong to the genera *Coronilla* L. and *Vicia* L.

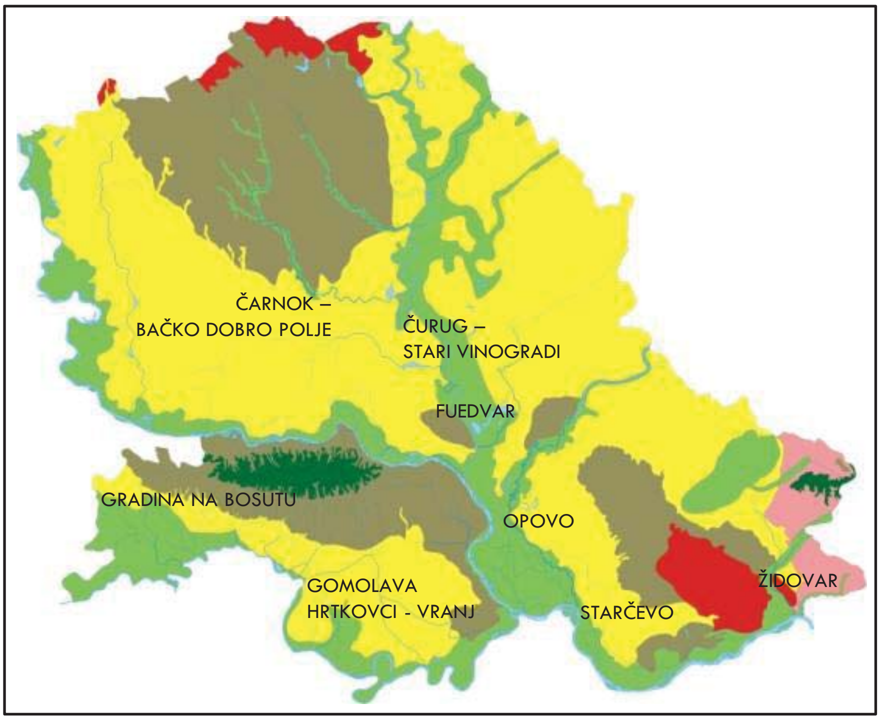

Figure 1. Soil map of the Serbian Province of Vojvodina, with the most important archaeobotanical finds of pulse crops: alluvial deposit (light green), typical loess (brown), terrace loess (yellow), aeolian sand (red), gravel, sand and clay (pink) and mountains (dark green)

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Site	Period	*Cicer arietinum*	*Lathyrus sativus*	*Lens culinaris*	*Pisum sativum*	*Vicia ervilia*	*Vicia faba*	*Vicia sativa*	*Trigonella foenum-*	*Coronilla varia*	*Coronilla-Type*	*Lathyrus nissolia*	*Trigonella*	*Trifolium pratense*	*Trifolium-Type*	*Vicia-Type*	*Vicia/Lathyrus-*
Čarnok - Bačko Dobro Polje	La Tène														X		
Čurug - Stari vinogradi	Roman period (Barbaric)					X				X			X		X	X	
Feudvar	Bronze & Early Iron Age	X	X	X	X	X	X				X	X		X	X		X
Gomolava	Neolithic			X	X											X	
	Eneolithic			X		X					X					X	
	Early Iron Age			X	X	X	X				X					X	
	La Tène		X	X	X	X	X							X		X	
Gradina na Bosutu	Early Iron Age		X	X	X	X	X	X		X					X		
Hrtkovci - Vranj	Roman period					X	X		X						X	X	
Kalakača	Early Iron Age			X													
Opovo	Neolithic			X												X	
Starčevo	Neolithic				X												
Židovar	Bronze Age			X	X	X									X		
	La Tène			X			X								X		

Figure 2. Presence of the ancient Eurasian pulse crops in the archaeological sites in Vojvodina, Serbia

The most interesting find of grain legume crops in Serbia, namely pea and bitter vetch, comes from the fortified hill of Hissar, near present Leskovac, dated to 11th century BC (1). Unlike the vast majority of archaeological sites, where cereals are much more dominant, here several thousands of the charred seeds of both bitter vetch and pea were identified (2) (Fig. 3). The reason why the Hissar population was so fond of pulses in their diets remains unanswered, although this tradition of the excellence in preparing pulse-based meals in the region have survived until today.

Figure 3. Charred seeds of pea (left) and bitter vetch (right) from Hissar, souteastearn Serbia, 11th century BC

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Pulses cultivated in prehistoric periods at the territory of Armenia: Short review of present archaeobotanical data

by Roman HOVSEPYAN

Abstract: The most common cultivated pulses in the territory of Republic of Armenia (South Caucasus) in prehistoric times were lentil (*Lens culinaris*) and pea (*Pisum sativum*). These crops are recorded already in early agricultural period, namely in the Late Neolithic - Early/Middle Chalcolithic (end of 7th millennium - beginning of 5th millennium cal BC) settlements in the Ararat valley. There are three main types of archaeobotanical findings of pulses: charred seeds from soft archaeological sediments (soil), the impressions of pods valves and sometimes seeds in the clay-made walls of dwellings and structures. Charred seeds from archaeological sediments are generally interpreted as remains of food, and impressions of pods valves are remains of crop threshing waste used as tempering material in building clay. Findings of pulses (pea, lentil, grass pea) from Late Chalcolithic period (43rd-33rd centuries BC) are represented with charred seeds and they are much less than cereals remains.

Key words: Armenia, archaeobotany, charred seeds, crop history, pulse crops

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Lentil (*Lens culinaris* Medik.) and pea (*Pisum sativum* L.) were the most common cultivated pulses in the territory of Republic of Armenia (South Caucasus) in prehistoric times. In this territory pulses are recorded already in early agricultural, Late Neolithic – Early/Middle Chalcolithic period (end of 7th millennium - beginning of 5th millennium cal BC) settlements in the Ararat valley. In the earliest stage of agriculture in the territory of Armenia, pulses, such as small-seeded lentil, bitter vetch and possibly pea (Figure 1), had important role in food economy and agrarian culture of local population: remains of pulses are very frequent and with large quantities, often prevailing over cereals. There are three main types of archaeobotanical findings of pulses in early agricultural sites of the Ararat valley: charred seeds from soft archaeological sediments (soil), the impressions of pods valves and sometimes seeds in the clay-made walls of dwellings and structures. Charred seeds from archaeological sediments are generally interpreted as remains of food, and impressions of pods valves are remains of crop threshing waste used as tempering material in building clay.

Some decline in pulses cultivation is recorded for the Late Chalcolithic period, 43rd-33rd centuries B.C. Findings of pulses (pea, lentil and grass pea) are represented with charred seeds and they are much less than the cereals remains.

Cultivation of pulses has not been a continuous practice in the South Caucasus. There are practically no records of pulses for the entire Bronze Age through the Early Iron Age period, after the second half of 4th to the beginning of 1st millennia cal BC (Table 1), when agriculture in the territory of Armenia appears monotonic and specialized on cereals cultivation. Similar tendency was recorded in the other parts of the Old World as well (3, 4), but in the territory of Armenia and in general in the South Caucasus Early Bronze Age agriculture seems to be based only on cereals cultivation.

Starting from Middle Iron Age (Van Kingdom / Urartu), 9th-6th centuries BC, the cultivation of a series of pulses re-started, including lentil, bitter vetch, pea, chickpea and faba bean. Often hoards of pulses are found from the Van Kingdom settlements (1, 2).

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Figure 1. Neolithic lentil and bitter vetch from the territory of Armenia: impression of lentil pod left valve (Masis blur, T.M10/1, L332, F9) in building clay (left); charred seeds of lentil (right, above) and bitter vetch (right, below), Aknashen, T5, UF10b, F16), scale size 2 mm

Table 1. Summary on cultivated pulses presence/absence in archaeological periods in the territory of Armenia

Period	Neolithic	Chalcolithic	Early Bronze Age	Middle Bronze Age	Late Bronze Age & Early Iron Age	Middle Iron Age (Van Kingdom / Urartu)
Sites examined (2)	Aknashen, Masis Blur, Aratashen	Godedzor, Areni-1, Getahovit-2	Gegharot, Aragatsi-berd, Margahovit, Lorut, Tsaghkasar-1, Aparan III, Voskevaz (Akhtamir), Shengavit, Sotk-2, Norabak-1, Aygevan	Nerkin Naver, Aghavnatun, Aygevan, Shaghat I	Gegharot, Aragatsi-berd, Tsaghkahovit, Horom, Hnaberd, Teyshebaini, Shaghat III, Metsamor, Uyts (VP site 21), Jujevan	Horom, Yenokavan-2, Dvin, Aramus, Argishtikhinili, Horom, Karmir Blur, Sev-sev kareri blur, Tsaghkahovit, Shaghat 1
Cereals (*Poaceae*)	+	+	+	+	+	+
Pulses (*Fabaceae*)	+	+				+
Lentil (*Lens culinaris* ssp. *microsperma*)	+	+				+
Bitter vetch (*Vicia ervilia*)	+					+
Pea (*Pisum sativum*, *Pisum* spp.)	+	+				+
Chickpea (*Cicer arietinum*)						+
Faba bean (*Vicia faba*)						+
Grass pea (*Lathyrus sativus*)		+				
Vetches (*Vicia* spp., wild?)	+	+	+	+	+	+

Molecular analysis of ancient DNA isolated from charred pea (*Pisum sativum*) seeds found at an Early Iron Age settlement in southeast Serbia

by Petr SMÝKAL[1]*, Aleksandar MEDOVIĆ[2], Živko JOVANOVIĆ[3], Nemanja STANISAVLJEVIĆ[3], Bojan ZLATKOVIĆ[4], Branko ĆUPINA[5], Vuk ĐORĐEVIĆ[6] and Aleksandar MIKIĆ[6]

Abstract: Archaeobotanically, the bulk of the 2,572 charred pea (*P. sativum*) seeds recovered at the 12th century BC settlement Hissar, near the modern town of Leskovac in southeastern Serbia, belongs to the cultivated pea (*P. sativum* L. subsp. *sativum*). As wild or semi-wild pea species, such as *P. sativum* subsp. *elatius* still grow in the area, it could be hypothesised that such seeds could be possibly collected. To better identify the origin of sample, we subjected two samples to molecular analysis using informative chloroplast DNA sequences. In addition to phylogenetically informative single polymorphism sites (SNPs) providing relationship to extant pea forms and species, there were also additional substitutions likely attributed to damage of DNA. On the basis of these molecular data, we conclude that the material of the study was not so much wild pea, but rather an early domesticated pea. This is the first report of successful aDNA extraction from any legume species so far.

Key words: ancient DNA, charred seeds, legumes, paleogenetics, pea

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Although pea (*Pisum sativum* L.) is one of the first crops cultivated by man, a lucky find of 2,572 pea seeds in only one sample from the 12th century BC settlement Hissar, near the modern town of Leskovac in southeastern Serbia, represents a unique example in the archaeobotany of South East Europe. The pea from Hissar was a distinct crop, stored separately from other crops (3). Archaeobotanically, the bulk of pea recovered at Hissar belongs to the cultivated pea (*P. sativum* L. subsp. *sativum*). Several morphological characteristics indicate this: smooth surface of the seed coat, "coffee-bean-shaped" hilum, broad ellipsoid seed shape and small size range difference between seeds and high weight of charred seeds. On the other hand, there existed an uncertainty about the determination pea seeds lacking testa and hillm in the largest portion of the sample.

As wild or semi-wild pea species, such as 'tall' pea (*P. sativum* subsp. *elatius* (Steven ex M. Bieb.) Asch. & Graebn.), still grow in the area (6) it could be hypothesized that such seeds could be possibly collected by the Hissar population. The cultivation of sole wild, 'tall' pea is highly unprofitable: high net yield loss due to poor establishment caused by wild-type low germination rates and pod dehiscence (1).

To better identify the genetic origin of the Hissar charred pea seeds, we subjected two samples to molecular analysis using informative chloroplast DNA sequences (4). The four selected chloroplast DNA loci, *trnSG, trnK, matK* and *rbcL*, amplified in six fragments of between 128 bp and 340 bp with a total length of 1,329 bp were successfully recovered in order to distinguish between the cultivated and wild gathered pea. In addition to the phylogenetically informative single polymorphism sites (SNPs) providing relationship to extant pea forms and species, there were also additional substitutions likely attributed to damaging DNA. Since the majority of these 16 substitutions were of the type 2 transitions, it supports the evidence of analysis of genuine ancient pea DNA. This results from the deamination of cytosine (and 5-methyl cytosine) to uracil (and thymine), shown to be associated with post-mortem damage (2).

On the basis of the obtained molecular data (Fig. 1), we may conclude that the material of the study was not so much wild pea, but rather an early domesticated pea. Consequently, based on a combination of morphological and molecular data, we may also consider the material an early domesticated pea. We speculate that the Early Iron Age pea from Hissar could have coloured flower and pigmented testa, similar to field pea (*P. sativum* subsp. *sativum* var. *arvense* (L.) Poir.), today used mostly for forage, and possibly of winter type (5).

It may be said that our results are the first report of a successful extraction of ancient DNA (aDNA) from any legume species so far (Fig. 2).

Acknowledgments

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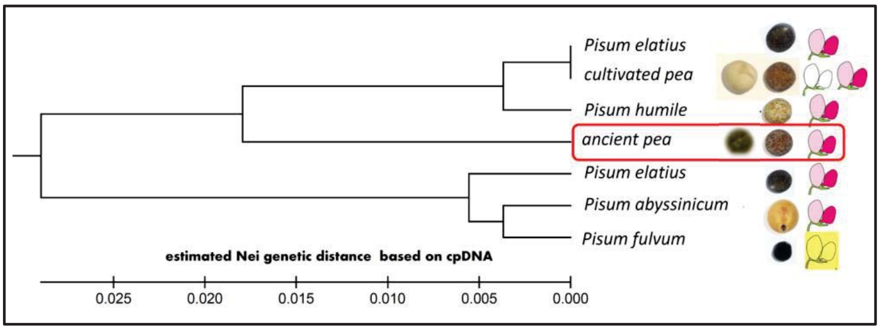

Figure 1. The phylogenetic position of the Early Iron Age pea from Hisar and its relationship with the other *Pisum* taxa

Figure 2. Some of the authors after the press conference announcing the first known successful extraction of ancient DNA from charred legume seeds and a series of expeditions in search for *Pisum elatius* in southeastern Serbia, Museum of Vojvodina Novi Sad, Serbia, late May 2011

Legumes diversity in the gorges of Carpathian-Balkan Mountain arc in Serbia

by Bojan ZLATKOVIĆ[1]*, Stefan BOGOSAVLJEVIĆ[2], Aleksandar MIKIĆ[3] and Branko ĆUPINA[4]

Abstract: According to its taxonomical diversity, legume family is one of most represented families in the rich floras of Serbia and Balkan Peninsula. Wild-growing relatives of cultivated species in the flora of the gorges of mentioned areas may represent a valuable source of desirable traits for the introgression into cultivated species, such as the tolerance to abiotic and biotic stress is of special relevance.

Key words: Carpathian-Balkan Mountains, floristic analysis, gorges, Leguminosae, Serbia

Introduction

The limestone gorges and canyons of the Balkan Peninsula are classified as one of the most significant, realistic and potential centres of the floristic diversity in Serbia (7). These regions are characterised by a peculiar floristic diversity and diverse habitats, enabling the survival of the plants with different ecology, origin and age. The gorges and canyons play the role of refugia and therefore have a great richness, originality and antiquity of their floras (4). Numerous botanical researches from the middle of the last century until today have significantly contributed to the knowledge on their flora and vegetation. However, the specific aspect of the richness and taxonomic variability of the family Leguminosae in their flora have not been studied yet to the satisfactory extent.

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This paper aims at determining the diversity fo the family Leguminosae in the floras of the seven most important gorges of the Carpathian-Balkan Mountain arc, including their comparative analysis (Fig. 1). The checklists of the Leguminosae taxa related to their flora have been extracted from the available botanical literature (1, 2, 3, 5, 6, 12) and the *in situ* observations, while the data on the family Leguminosae in the whole flora of Serbia were obtained from the modern literary resources (8).

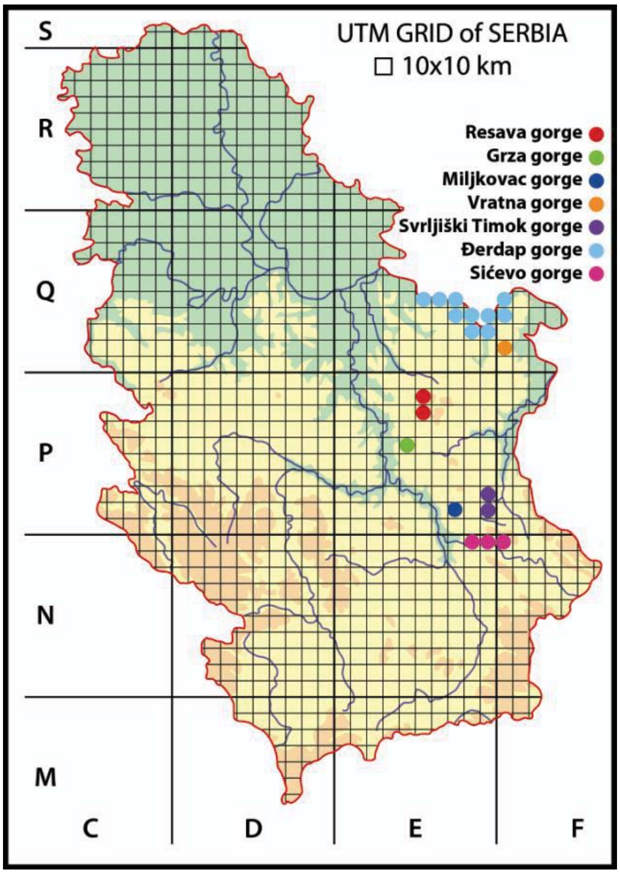

Figure 1. Geographic position of examined gorges of the Carpathian-Balkan arc in Serbia

Legumes in gorges of eastern and northeast Serbia

The flora of the predominantly limestone gorges and canyons of the southern part of the Carpathian-Balkan Mountains, spreading through eastern and northeast Serbia, is relatively rich in the Leguminosae species and is characterised by rare and endemic taxa. Here is present 135 species and subspecies of Leguminosae in total, being 51% in comparison to whole Serbia, with 266 taxa.

Out of 33 genera in total in the flora of the country, there is 24 legume ones (73%). In a taxonomic sense, the richest are the genera *Trifolium* L. (31 taxa), *Lathyrus* L. and *Vicia* L. (18 taxa), *Cytisus* Desf. (13 taxa), *Medicago* L. (10 taxa) and *Astragalus* L. (8 taxa). Most of these genera comprise economically important crops, thus making the species of the examined gorges and canyons potentially useful in applied genetics and plant breeding. The richest in the Leguminosae species is the Sićevo gorge, with 102 taxa, followed by Đerdap, with 70 taxa, and Svrljiški Timok, with 50 taxa. The smallest number is found in the smaller gorges, such as Grza (23 taxa), Resava (20 taxa), Vratna (20 taxa) and Miljkovac (18 taxa). The Sićevo gorge is the richest one if the indexes of floristic diversity of Leguminosae are compared, expressed as a ratio between the number of species and the area of the location (*Log*S(*L*) / *Log*A). It is followed by Svrljiški Timok, while the Đerdap gorge, despite having the largest area, is the third one (Table 1). The gorges with the most original and characterstic Leguminosae representatives are Sićevo, with 37, Đerdap, with 15, and Resava, with 4 taxa.

As the southernmost of all seven examined gorges, Sićevo is dominated by the Mediterranean species, with 37%, followed by Eurasian, with 22%, and Pontic and Central European, with 13% each. In Đerdap, there is 26% Mediterranean, 21% Eurasian and 20% and Central European legume taxa. In the Svrljiški Timok gorge, the Eurasian taxa are prevalent (30%) over the Mediterranean (26%) and somewhat higher proportion of the Pontic species (18%) than in other gorges. Among the Pontic species of this gorge, those such as *Lathyrus pallescens* (M. Bieb.) K. Koch and *Vicia sparsiflora* Ten. are of specific interest. The other, smaller, gorges of eastern Serbia are characterised by an increased number of the cosmopolitan species. Of a peculiar importance are so-called differential species, that is, the taxa that are present in only one location. In Sićevo, these are the Mediterranean taxa, such as *Lens nigricans* (M. Bieb.) Godr., *Pisum sativum* subsp. *elatius* (Steven ex M. Bieb.) Asch. & Graebn.), *Trifolium micranthum* Viv., *T. scabrum* L. or *T. vesiculosum* Savi, that are important crop wild relatives and gene pools of desirable traits that may be introgressed into the cultivated species. In Đerdap, the differential species are *Laburnum anagyroides* Medik., *Lathyrus inconspicuus* L. and *Trifolium patens* Schreb.

Table 1. General (*Log*S / *Log*A) and Leguminosae (*Log*S(L) / *Log*A) diversity among the gorges of Carpathian-Balkan arc in Serbia; S - number of taxa, A - area, L - *Leguminosae* species and subspecies

Area	A (km^2)	Number of taxa	Number of legume taxa	Share of total flora (%)	LogS / LogA	LogS(L) / LogA
Đerdap	603.08	1013	70	6.91	0.3423	0.2101
Grza	49.40	340	23	6.76	0.3290	0.1770
Miljkovac	27.50	330	18	5.45	0.3385	0.1687
Resava	69.50	297	20	6.73	0.3153	0.1659
Sićevo	100.00	1275	102	8.00	0.3882	0.2511
Svrljiški Timok	66.20	689	50	7.24	0.3629	0.2172
Vratna	4.90	310	20	6.45	0.3724	0.1945
Serbia	88631.00	3662	266	7.26	0.3256	0.2215

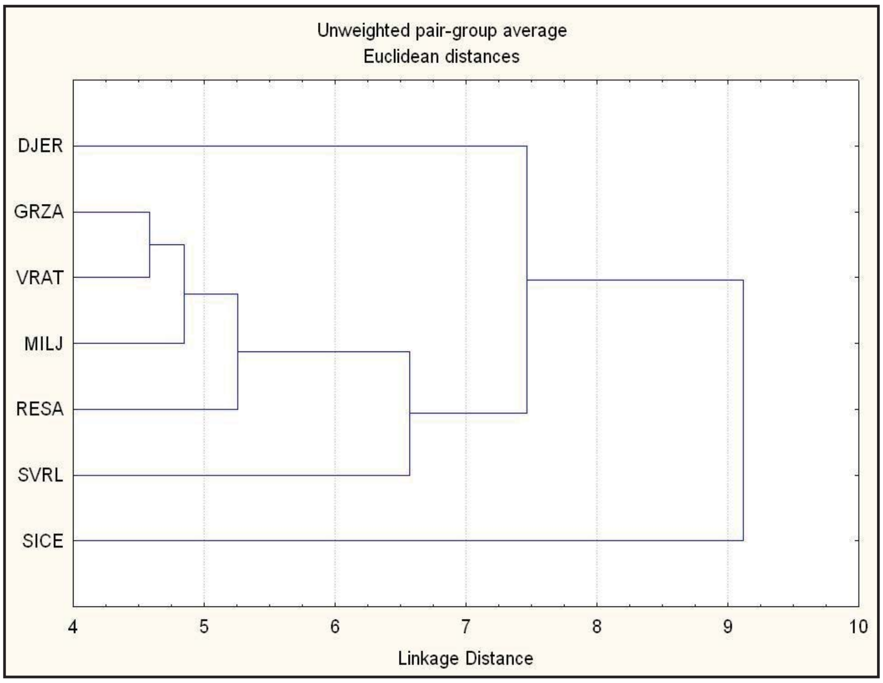

Figure 2. Cluster diagram of taxonomic similarity of *Leguminosae* flora in the gorges of Carpathian-Balkan arc in Serbia

The limestone gorges and canyons, being the refugia of flora and vegetation, are extremely important for preserving the biodiversity, especially for the species requiring a stable environment, as well as for those with a narrow distribution and endemic taxa (8). The local Leguminosae endemics are not present in the gorges and canyons of Carpathian-Balkan Mountains in Serbia, but there may be found the Balkan endemites, that are, the endemics not recorded out of the Balkan Peninsula (9, 11).

Out of 492 Balkan endemic taxa, typical for the floras of Central Serbia and Kosovo (10), 26 belongs to the family of Leguminosae (5.3%). In the gorges and canyons of Carpathian-Balkan Mountains, 4 Balkan endemic taxa were recorded, namely *Cytisus jankae* Velen., *Genista subcapitata* Pančić, *Trifolium dalmaticum* Vis. and *T. medium* L. subsp. *balcanicum* Velen. The gorges of Sićevo and Đerdap have 3 Balkan endemites each, Svrljiški Timok 2, while Miljkovac has 1 (Fig. 3).

Conclusion

The legume flora of limestone gorges and canyons of Carpathian-Balkan Mountain arc in Serbia, are comparatively rich in native legume species, representing an important genetic and breeding genepools for grain and forage legume crops. The analysis emphasizes the gorges in the south, floristically impacted by the Pontic and Mediterranean regions, as the centres of biodiversity for Leguminosae and the flora as a whole. A specific value of the examined plant groups is the presence of the Balkan endemic species, stressing the importance of gorges and canyons in preserving local flora.

Acknowledgements

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Figure 3. The Balkan endemic and rare *Leguminosae* species in the gorges of Carpathian-Balkan arc in Serbia: *Cytisus jankae* (left, above), *Genista subcapitata* (left, below) and *Ononis pusilla* (middle, above) from Sićevo, *Trifolium dalmaticum* from Miljkovac (middle below) and *Trigonella gladiata* from Svrljiški Timok (right)

Pasture and meadow legumes in Serbia

by Aleksandar SIMIĆ and Savo VUČKOVIĆ

Abstract: Perennial legumes are characterized with high content of nutritive substances, especially protein and represent the most important protein source in livestock nutrition. Livestock feed obtained from these plant species can be used in several ways, from grazing as most efficient and economical way, to preparation of hay and high quality silages and haylages. The legumes could provide forage for livestock and help improve soil fertility and pest management, because they fix nitrogen over an extended portion of the year. The distribution of good-quality species of legumes in phytocenoses of Serbian grasslands is low, as opposed to the high share of other plants. State of biodiversity of grasslands and overall in Serbia is still on high level.

Key words: flora, legumes, meadow, pasture, Serbia

Introduction

Grain and forage legumes are grown on some 180 million ha or from 12% to 15% of the global arable area (2). Forage legumes have been the foundation for dairy and meat production for centuries. When properly managed, they are rich sources of protein, fiber and energy. Even in intensive animal and milk production, where grain crops are major feed sources, forage legumes are required to maintain animal health. Alfalfa (*Medicago sativa* L.) is the prevalent forage legume in temperate climates, while other important temperate pasture species include clovers (*Trifolium* spp.), birdsfoot trefoil (*Lotus corniculatus* L.), and vetches (*Vicia* spp.).

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Legumes are the major element of grassland ecosystems on which the forage quality depends. Grazing tolerance and persistence of the legume components in the pastures are closely related to mechanism of self-maintenance of the legumes in the pastures. The inclusion of a nitrogen-fixing legume component in the sward corresponds to the principles of sustainable agriculture and extensive agricultural production (3). They also have a great importance due to their contribution to an even distribution of the yield produced by a pasture by seasons. These species will often naturally reseed themselves and can be an important, high-quality component of summer pastures. In southeastern Europe, the growth of legumes is seriously limited by the ability of each species to grow during usually cold winters.

Role and potential of legumes in the Serbian grasslands

It is estimated that the permanent grasslands occupy approximately 830,000 ha in Serbia (**5**). Perennial legumes in the country are grown as sole crops on more than 350,000 ha. Economically, the most important and most present are alfalfa, red clover (*Trifolium pratense* L.), birdsfoot trefoil and white clover (*Trifolium repens* L.), although during the last decade sainfoin (*Onobrychis viciifolia* Scop.) and alsike clover (*Trifolium hybridum* L.) have become very important (6).

Alfalfa, with its well-developed and deep root system, enables overcoming dry conditions and even in the years with very low precipitations it produces satisfactory yields. The legume possessing the highest grazing tolerance in Serbia is white clover.

Apart from being an important source of good-quality fodder, natural grasslands are also significant in terms of soil protection from erosion and biodiversity or gene fund preservation. They are also of an exceptional importance for the development of livestock production in the upland regions since they often represent the main or the only source of livestock feed. Presently, many natural meadows and pastures are in rather bad condition and are being increasingly subjected to various degradation processes. This is due to the absence of melioration measures, primarily fertilisation, but also due to unfavourable environmental conditions and improper exploitation. There, the distribution of legumes ranges from 6.73% to 34.12%, depending on plant nutrition (1). The grasslands with a higher share of legumes and lower percentage of other plants are characterised by a higher crude protein content.

In the Serbian pastures, perennial and annual legumes play a special role as a pasture components because of their high quality, production and ability to fix atmospheric nitrogen (Fig. 1). On the other hand, the problems are the lack of leguminous species, their low share and the longevity, especially in perennial legumes, since it limits a successful forage production. Widely adapted forage legumes are increasingly important, but there are some constraints: alfalfa, in Serbia mainly used for hay production, does not tolerate acid soils, that is, pH values lower than 6.2, red clover has a short life span than most other pasture legume species, birdsfoot trefoil possesses a very weak competitive ability, while white clover has a low-growth habit and is associated with a low dry matter yield (4). When used as fresh forage, legumes, except birdsfoot trefoil, may cause bloat.

Generally, the most important forage legumes on the Balkan Peninsula belong to genus *Trifolium* L., *Medicago* L:, *Lathyrus* L. and *Vicia* L.. Clovers and alfalfa are common field crops, but vetches and vetchlings (*Lathyrus* spp.), especially annual ones, have not been tested adequately in pastures so far.

Annual legumes

Recent examinations of natural pastures in Serbia are focused to mark natural grasslands rich in annual legumes and determining species suitable for livestock. The emphasis is expected to shift from traditional self-regenerating species, such as alfalfa and clovers, to new species that are adapted to short periods, namely 1 or 2 years, of pasture which can be used in between extended cropping phases or phase pastures.

Naturalized ecotypes of annual pasture legumes have been sources of well-adapted commercial cultivars. Annual legumes represent one of the most significant crops in the global agriculture today, with a prominent place in both animal nutrition and animal feeding. The conservation of annual pasture legumes seeds has two important purposes: 1) the long term preservation of germplasm and 2) the availability of a basic material to obtain new varieties well-adapted to specific soil and climatic conditions.

One of the main goals of the programmes related to genetic resources of annual forage legumes is the conservation of a remarkable genetic variability of these crops. They are carried out in the form of establishing and maintaining collections of accessions of diverse origin, with an emphasis on local populations and aimed at using in pasture improvement programmes. One of main goals is to find and record the hotspots, rich in annual legumes important for pastures and hay production. These are biogeographic regions with a rich biodiversity endangered by the human activities.

There is a lot of identified annual vetches in the central Balkans. The territory of Serbia is a reservoir of the *Vicia* biodiversity. The most widespread vetches are *Vicia sativa* L., *V. hirsuta* (L.) Gray, *V. tetrasperma* (L.) Schreb., *V. villosa* Roth, *V. grandiflora* Scop., *V. lutea* L. and *V. narbonensis* L.. Among vetchlings, the most widespread are *Lathyrus sativus* L., *L. aphaca* L., *L. hirsutus* L. and *L. cicera* L.. All species are native to Europe, while some are typical for South Europe. *L. cicera* is grown mainly in South Europe and the Mediterranean for forage and grain production. Also, it is identified in the flora of Serbia. *L. cicera* may be a promising species in the Balkan environments, regarding its use as a multipurpose crop for feed grain in cattle and sheep, fodder, hay and green manure in numerous countries, such as Australia. In contrast to *L. sativus*, the grain of *L. cicera* contains low amounts of ODAP, while other plant parts have even more insignificant concentrations of this neurotoxin.

Grazing may the main use of annual vetches and vetchlings in pastures, but they are easily damaged by trampling. There is a general recommendation not to graze in late spring when the pods are developed.

The collected seeds and herbaria of annual legumes could be a source for conservation of these species in the form of collections of accessions of diverse origin. It is possible to use these local genetic resources for future forage crop breeeding programmes.

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Figure 1. Some of the legumes present in the grassland communities in Serbia: (from left to right) *Lathyrus hirsutus, Lathyrus nissolia, Vicia hirsuta* and *Vicia tetrasperma*

Sustainable preservation of indigenous Albanian legumes and their traditional use

by Sokrat JANI[1]*, Agim CANKO[2] and Milto HYSO[3]

Abstract: The diversity of indigenous legumes in Albania is seriously eroded as a result of the multiplicity of environmental, political and socio-economic factors. This paper discusses the developments related to the identification, collection, evaluation and preservation of indigenous forms of leguminous crops, such as common beans (*Phaseolus* spp.), chickpea (*Cicer arietinum*) and *Lathyrus* spp., that are threatened by extinction in the Central Albania, as well as the traditional knowledge on the production and their use for own consumption of the farmers in villages. The paper also suggests that the sustainable use of plant biodiversity requires community-driven *in situ* and on-farm initiatives supported through knowledge dissemination, marketing efforts, publicity, and cooperation with research and governmental structures.

Key words: Albania, collection, conservation, exploration , genetic erosion, indigenous legumes

Introduction

Albania is well-known for its diverse environment and for high variability in cultivated plants. During the long history of Albanian agriculture, local farmers carefully selected plants and seeds for planting and developed numerous farmer-selected varieties, which are well-adapted to local conditions. So, it is a country with a wide diversity of plant genetic resources. There are numerous primitive cultivars, indigenous landraces and wild species, especially of legume plants. Primitive cultivars and indigenous landraces are cultivated especially in farmers' orchards, even in the most remote rural villages (3).

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Since 1963, the collecting, evaluation and conservation of legume crops has been organized by the Agricultural Research Institute (ARI) of Lushnja, with a clear plan to select and produce the commercial seeds for daily needs of agricultural cooperatives. Until the end of 1980s of the last century, 180 accessions of dry beans (*Phaseolus vulgaris* L.) were collected, including 53 landraces and old cultivars. During 1990-2000, another project was accomplished, where several collecting missions took place, especially in the south-eastern regions of the country (1, 4, 5). After this period, the collecting missions were reduced, due to lack of financial funds.

The process of agricultural diversity reduction, which was observed globally in 20th century, severely affected the Albanian agriculture. Not only was the plant diversity reduced, but also the level of utilization of the indigenous crops.

In order to give our modest contribution to the purpose of preserving indigenous populations of legume plants, the SEELEGUMES project was launched.

Main causes of losing the agrobiodiversity in Albania

The Albanian agricultural sector was developed during the communist period, in the form of state and collective farms. This specialization had a negative impact on indigenous crop varieties. In a period of 40 years, introduced varieties predominated in these farms while endemic, rare and threatened varieties were restricted mainly to family plots and agricultural research centers. Consequently, information about local varieties became restricted to the technical staff of research centers and the few families that kept indigenous crop varieties (2).

The process of the agrobiodiversity loss became more intensive after the collapse of the communist state, with the beginning of a democratic processes in the 1990s, because a lot of people started to leave their native land in rural zones and settled in new places, especially in urban zones, in order to have better possibilities of life. This movement of rural people was more emphatic in the mountain zones. The Central Albanian zone, which is distinguished for legume plants variations, was also part of this movement. It was consequently associated with the loss of possibility to cultivate landraces of legume plants in the future (5).

Methodology

Within SEELEGUMES, collecting missions of legume plant in the Central Albania were organised. In order to complete this project, a working plan was designed, and exploration and collecting missions were undertaken for legume plants such as common bean, lentil (*Lens culinaris* Medik.), chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.), vetchlings (*Lathyrus* spp.) or pea (*Pisum sativum* L.).

All this process has been conducted by sub-project staff and ATTC, with the cooperation of the Agricultural Regional Services. Guidelines were followed referring to these main fields: consulting and gathering of the information about the villages where we could be able to get the best variation of legume plants, exploration and collection through missions in the field, characterization and evaluation of the collected populations based on the minimum descriptors list, recording the local information about species growing and traditional knowledge for the production practices and traditional utilization by interviewing farmers, and other persons. Seed samples were collected only when farmers declared their materials have been cultivated for ages in their families without exchanging seed or buying it on the

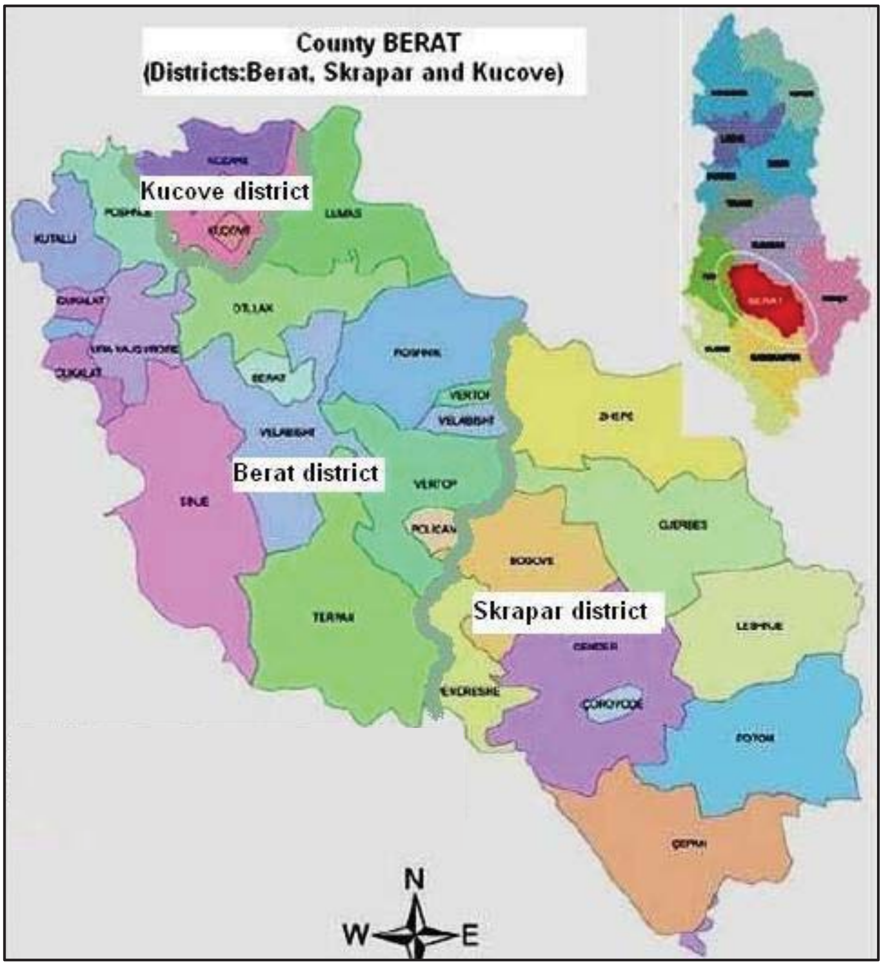

Figure 1. The map of the Berat County with its municipalities of Berat and Skrapar, where the grain legume landraces were collected within the SEELEGUMES project

Project outcomes

The project has achieved some considerable successes. Notably, important landraces have been identified, collected, and morphologically characterized and a demonstration plot has been established. At the end of this project, 27 samples of common bean altogether were collected, including 24 from the Skrapar district, two from the Berat district and the last one from Lushnja (Fig 1). There were also found 2 samples of vetchlings and one sample of chickpea. So, the total number of collected samples was 30. All of the 24 samples from the Skrapar district were collected in 9 villages. Ten samples were taken only in one village, Liqeth (Table 1).

The conservation of all the collected populations is interesting for the future because of their special characteristics, displaying a good variation among them. Now we can demonstrate the variation of the seed, or grain, or kernel, descriptors, especially for their shape, size, color and type of seed coat. We realized that the people of the mountain zones prefer colored common bean. So, from the 24 populations of collected common bean, only 9 of them have white seed (37.5%). The biggest part populations (26 populations) of common bean are used as dry grain and only one of them is used as fresh pod. According to the method of growing, 16 populations needed to be support by a stick, having a climbing plant type, and 11 populations are cultivated without it, being bush type.

All the samples of the legume populations collected and their field information were deposited and landraces seed are preserved in the ATTC gene bank. In addition, seeds maintained in collections will be regularly renewed on the seed's multiplication plot.

The use of landraces with their ability to produce good harvests without need for expensive chemical inputs, and their tolerance to drought, plant pests and diseases has significantly reduced the farmers' exposure to risk. Investing is low and the crops ideally suit to the growing conditions. Most of the farmers use local varieties for their own consumption (1, 6). The reintroduction of the landraces has also improved the nutritional intake of farmers with the addition of a greater range of pulses. Local farmers appear to prefer the landraces for their subsistence needs. Although yields are lower for the landraces, they attract a higher price.

The project collected and documented traditional knowledge on the uses of indigenous crops. A recipe book will be published and widely distributed to raise consumer awareness as soon as possible. In addition, dishes prepared from local varieties have been promoted through food tasting events and media. As a result, demand for indigenous varieties is growing at the local market.

Conclusions

As mentioned above, the project did not imply the protection of the entire spectrum of plants important to agriculture that are threatened by extinction. Otherwise, the project approach was the development of a replicable model of agricultural biodiversity protection for a group of the selected local varieties (legumes) in one region of Albania, which could be used as a strategy in other regions or for other crops and varieties.

Two years of project implementation have shown that the sustainable use of plant biodiversity requires community-driven in situ and on-farm initiatives supported through knowledge dissemination, marketing efforts, publicity, and cooperation with research and governmental structures. The approaches and instruments developed by the project will be tested in other regions of Albania.

Table 1. District and villages and bean landraces collected

District	Village	Number of collected landraces	Dry bean or fresh pod		Plant type	
			Dry bean	Fresh	Climbing	Bush
Berat	Lapardha	2	2	-	1	1
Skrapar	Gjerbes	5	5	-	4	1
	Gjergjove	1	1	-	-	1
	Kovacanj	1	1	-	-	1
	Liqeth	10	9	1	7	3
	Luadh	1	1	-	-	1
	Nishove	1	1	-	-	1
	Potom	2	2	-	2	-
	Strore	2	2	-	2	-
	Trebel	1	1	-	-	1
Lushnje	Hajdaraj	1	1	-	-	1
3	11	27	26	1	16	11

Figure 2. A visit to a household in the village of Potom in the municipality of Skrapar

Figure 3. Gathering *in situ* information on the traditional local practices in cultivating grain legumes from the farmers

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Collecting, characterisation and evaluation of 'tall' pea (*Pisum sativum* subsp. *elatius*) in southeastern Serbia

by Aleksandar MIKIĆ[1]*, Branko ĆUPINA[2], Bojan ZLATKOVIĆ[3], Petr SMÝKAL[4], Svetlana ANTANASOVIĆ[2], Vuk ĐORĐEVIĆ[1], Aleksandar MEDOVIĆ[5], Đorđe KRSTIĆ[1] and Vojislav MIHAILOVIĆ[1]

Abstract: In 2011 and 2012, several expedition in the upper flow of the river Pčinja, southeastern Serbia, rediscovered a complex wild population of *Pisum elatius* in the forest on the slopes of the Mount Kozjak An *in situ* analysis of his pea taxon showed a considerable potential for forage and grain production of very good quality. It has also shown a good tolerance to both length and intensity of low temperatures. *P. elatius* is regarded as able to bring more light onto the pea crop domestication.
Key words: crop wild relatives, *in situ* evaluation, Pčinja, pea, *Pisum sativum* subsp. *elatius*, southeastern Serbia

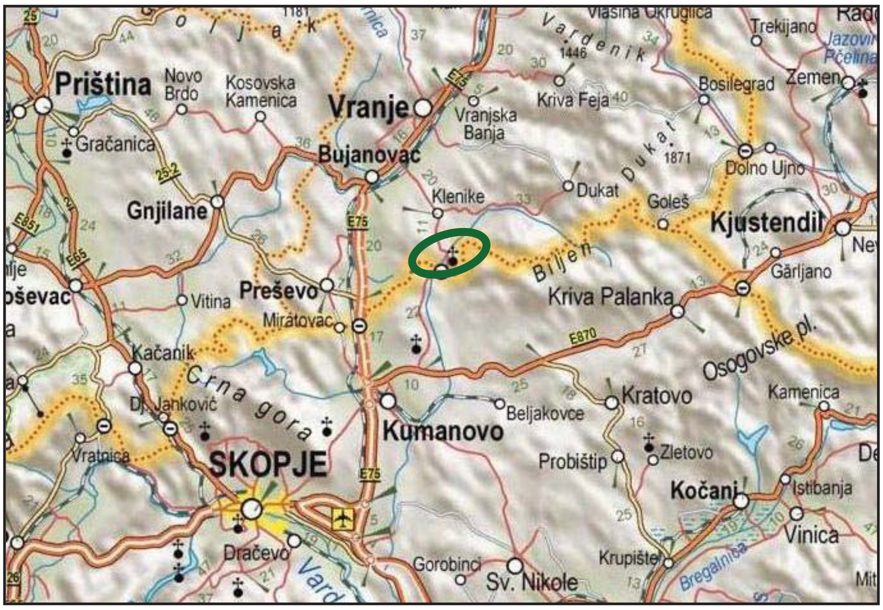

Figure 1. The area in southeastern Serbia where a wild population of *Pisum elatius* (green-outlined elipse) was collected in 2011 and 2012

Figure 2. The monastery of Saint Prohor of Pčinja, 11th century, with the Mount Kozjak in the background and the locations where wild populations of *P. elatius* were discivered in 2009 (greater yellow-outlined elipse) and in 2011 and 2012 (smaller yellow-outlined elipse)

Introduction

So-called 'tall' pea or simply *Pisum elatius* (*P. sativum* L. subsp. *elatius* (Steven ex M. Bieb.) Asch. & Graebn.) is regarded as a direct progenitor of cultivated pea (5). Ecologically, *P. elatius* is natively distributed in Albania, Algeria, Azerbaijan, Bulgaria, Croati a, Cyprus, Egypt, France, FYR of Macedonia, Georgia, Greece, Iran, Israel, Ital y, Lebanon, Libya, Montenegro, Morocco, P ortugal, Russia, Romania, Serbia, Spain, Syria, Tunisia, Turkey and the Ukraine (3).

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The first records on *P. elatius* in the flora of Serbia were made in the second half of 19th century by one of the most eminent Serbian botanists, Josif Pančić (4). There are also curious reports on a white-flowered variety *albiflorum* (2). In 2009, a usually pink-flowered population of *P. elatius* was rediscovered in the the upper valley of the Pčinja river, one the very border of the between Serbia and Former Yugoslav Republic of Macedonia (Fig. 1). There, *P. elatius* grows in the lower zone of northerly exposed slopes of the Mount Kozjak, on the left riverbank and amidst the termophilous submediterranean forests and scattered scrub vegetation on siliceous rocky soil (6).

The SEELEGUMES expeditions

First expeditions were made in 2011 aiming at collecting samples for the *in situ* evaluation of the *P. elatius* population from the region of upper flow of the river Pčinja and the monastery of Saint Prohor Pčinjski (Fig. 2), with emphasis on agronomic characteristics, such as yield components and chemical composition (1).

The first expedition was carried out on 28 May 2011, targeting the same area where *P. elatius* was rediscovered in 2009. One group of several plants was found on the edge between the foothills of the Mount Kozjak, between the forest and the meadow in the very vicinity of the Pčinja river and (Fig. 3 and Fig. 4.) Above this group, up the steep slopes of the Mount Kozjak and in the dense wood of oak and beech trees and shrubs, two more populations of *P. elatius* were identified, with plants either creeping over the rocky ground or climbing up and even hanging from the trees branches more than 1 m high. Several plants were detected on the completely inaccessible slopes between two upper and the lower population. All the plants were in the stages of full bloom and developing first pods (Fig. 5).

The second expedition, performed on 25 June 2011, visited the same population. All its three groups were in full maturity of the oldest pods, with few already shattering their seeds. Unlike its immature hanging pods or mature pods in *P. sativum* subsp. *sativum*, the mature ones were erect, following the peduncle direction, and with the seeds of irregular shape and seed coat colour (Fig. 6).

Both expeditions resulted in the samples of all plant organs for analysing agronomic characteristics and chemical composition of both forage and grain (1).

Figure 3. The lowest of three groups of the *Pisum elatius* population from the upper flow of the river Pčinja, southeastern Serbia, late May 2011

Figure 4. Happy authors with the sample of the lowest group of the *Pisum elatius* population from the upper flow of the river Pčinja, southeastern Serbia, late May 2011

Figure 5. Flowers (left) and hanging immature pods (right) in the plants of the *Pisum elatius* population from the upper flow of the river Pčinja, southeastern Serbia, late May 2011

Table 1. *In situ* forage yield components of the lowest of three groups of the *Pisum elatius* population from the upper flow of the river Pčinja, southeastern Serbia, late May 2011 (1)

Stages of full bloom and developing first pods	Stem length (cm)	Number of stems (plant^{-1})	Stem mass (g plant^{-1})	Leaf mass (g plant^{-1})	Plant mass (g plant^{-1})	Stem proportion	Leaf proportion
Minimum	31	1	4.48	7.70	12.18	0.13	0.48
Maximum	192	8	35.14	63.42	98.56	0.52	0.87
Average	68	3.8	11.04	22.44	33.48	0.33	0.67

Table 2. *In situ* grain yield components of the lowest of three groups of the *Pisum elatius* population from the upper flow of the river Pčinja, southeastern Serbia, late May 2011 (1)

Stage of full maturity of first pods and grains	Stem length (cm)	Number of stems (plant^{-1})	Number of pods (plant^{-1})	Number of grains (plant^{-1})	Stem mass (g plant^{-1})	Leaf mass (g plant^{-1})	Pod mass (g plant^{-1})	Grain mass (g plant^{-1})	Plant mass (g plant^{-1})	Harvest index
Minimum	52	1	2	12	2.82	1.28	0.61	2.46	7.17	0.34
Maximum	168	7	11	74	14.34	5.25	2.35	15.71	37.65	0.42
Average	72	4.1	4.3	27.3	5.52	2.38	1.24	5.60	14.74	0.38

Table 3. *In situ* nutritive value of the forage and grain dry matter (g kg^{-1}) of the lowest of three groups of the *Pisum elatius* population from the upper flow of the river Pčinja, southeastern Serbia, late May 2011 (1)

	Crude protein	Crude fat	Crude fibre	Neutral detergent fibre	Acid detergent fibre	Lignin	Crude ash	Nitrogen-free compounds
Stages of full bloom and developing first pods								
Stem	115	8	483	619	532	136	61	333
Leaf	244	25	265	353	320	62	76	391
Plant	201	19	337	441	390	86	71	372
$LSD_{0.05}$	32	6	123	45	35	15	6	29
Stage of full maturity of first pods and grains								
Stem	69	12	528	674	587	128	88	303
Leaf	146	41	291	324	307	76	131	390
Pod	98	5	437	573	415	93	70	391
Grain	343	-	188	242	205	19	-	-
$LSD_{0.05}$	67	11	211	69	55	27	33	31

Figure 6. A mature pod, regularly following the peduncle direction (left), and the mature seeds (right) of the *Pisum elatius* population from the upper flow of the river Pčinja, southeastern Serbia, late June 2011

The analysis of the fresh aboveground plant biomass in the stages of full bloom and developing pods suggests that this *P. elatius* may be a potential forage crop (Table 1).

The average seed yield of the collected *P. elatius* population (5.60 g plant^{-1}) could be considered high (Table 2). Its harvest index is also considerably high for a wild relative of a crop plant (0.38).

The average crude protein content in the *P. elatius* forage and grain dry matter may be regarded as rather high (Table 3).

Figure 7. ***In situ*** **comparison of a plant that germinated in the autumn 2011 and survived the winter, with a degraded and withered seed, the new shorter stems and smaller leaves and the longer stems and learger and partially damaged leaves during winter (left one) and a plant that germinated in the spring 2012 (right one), both belonging to the** ***Pisum elatius*** **population from the upper flow of the river Pčinja, southeastern Serbia, late March 2012**

Figure 8. ***In situ*** **root with nodules of a** ***Pisum elatius*** **plant from the population from the upper flow of the river Pčinja, southeastern Serbia, late March 2012**

Figure 9. More of the happy authors visiting again one of the upper ***Pisum elatius*** **populations from the upper flow of the river Pčinja, southeastern Serbia, examining its microenvironment and taking soil samples, early June 2012**

Figure 10. ***Ex situ*** **testing of the** ***Pisum elatius*** **population from the upper flow of the river Pčinja, southeastern Serbia, at Rimski Šančevi, northerm Serbia, late June 2012**

Figure 11. Even more of the happy authors in the Archaeobotanical Garden of the Musem of Vojvodina, Novi Sad, with the ***Pisum elatius*** **population from the upper flow of the river Pčinja, southeastern Serbia, in the company of Priapus, Greek god of fertility, April 2013**

The third expedition was done in mid-November 2011, discovering that the shattered seeds produced on the same year germinated most likely during October in a large number, confirming that *P. elatius* is not necessarily characterised by a full seed dormancy.

The fourth expedition in late March 2012 brought forth several important findings, with an emphasis on the winter survival of the *P. elatius* plants to a great extent, despite the absolute minimum temperature of -20 C in January 2012, and a good spring regeneration (Fig. 7) and early development of root nodules (Fig. 8).

The fifth and the last expedition to the slopes of the Mount Kozjak was done in June 2012 (Fig. 9). The collected seeds of the *P. elatius* population were regenerated and multiplied for further *ex situ* research at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi (Fig. 10), as well in an exhibition plot of the Archaeobotanical Garden of the Museum of Vojvodina in Novi Sad in 2011 (Fig. 11).

Acknowledgments

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Reintroduction of faba bean (*Vicia faba* L.) in the Republic of Srpska

by Branko ĐURIĆ

Abstract: Faba bean in Srpska can be found mainly in eastern Herzegovina and the regions close to the Adriatic coast. These varieties are forused mainly for human consumption. In the cooperation with the Institute for Field and Vegetable crops from Novi Sad, Serbia, 13 landraces of fodder faba bean were tested for five years in the Banja Luka region. The results indicate that faba bean may be an important annual forage and grain legume, although it is underrepresented in the sowing structure and cropping system.

Key words: crop reintroduction, faba bean, genetic resources, landraces, Republic of Srpska

Social and scientific importance of plant genetic resources for the whole humanity, community and every individual is priceless. Genetic resources have a special economic and breeding importance for breeding activities for a certain plant species.

The richness of the biodiversity in Bosnia and Herzegovina was noted more than 100 years ago (1), but the significant organized and long-term measures to protect this wealth have never been taken. Bosnia and Herzegovina, that is, the Republic of Srpska, has favorable agroecological conditions, characterized by the abundance of forage legumes and grasses.

Through the project SEELEGUMES, with the University of Banja Luka as one of the partners, a small collection of faba bean (*Vicia faba* L.) landraces was established. Faba bean in Srpska can be found mainly in eastern Herzegovina and the regions close to the Adriatic coast (2). These varieties are forused mainly for human consumption, while faba bean varieties for animals are negligible (Fig. 1).

In the cooperation with the Institute for Field and Vegetable Crops from Novi Sad, Serbia, 13 landraces of fodder faba bean were tested for five years in the Banja Luka region (Fig. 2). The aim of the experiment was to investigate the faba bean yield and adaptability of the cultivars in this region. The results indicate that faba bean may be an important annual forage and grain legume, although it is underrepresented in the sowing structure and cropping systems. Due to a number of favorable characteristics, a greater presence of faba bean in our arable land is recommended. Further step would be the establishment of demonstration trials on selected farms in this region.

Figure 1. The seed of a faba bean landrace from Herzegovina

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Figure 2. A trial with local faba bean landraces from Serbia and Srpska, Banja Luka, 2008

University of Banja Luka, Faculty of Agriculture, Banja Luka, Republic of Srpska, Bosnia and Herzegovina

Local plant genetic resources of faba bean (*Vicia faba*) in Bulgaria

by Siyka ANGELOVA*, Mariya SABEVA* and Yana GUTEVA

Abstract: The grain legume collection at Institute of Plant Genetic Resources "K. Malkov" (IPGR) consists over 5,000 accessions. The main task within the project SEELEGUMES was identifying faba bean local landraces, still grown and maintained in the home gardens and small farms, with about 100 accessions collected. The collected accessions were classified by duration of the growing season, plant habit and height, mass and color of the seeds and content of crude protein. The *ex situ* conservation of faba bean in the gene bank is necessary because they are endangered and are an important part of the rural cultural heritage of the country.

Key words: Bulgaria, *ex sittu* conservation, faba bean, *in situ* expeditions

The organization of ten expeditions was motivated by a single reason - depopulating numerous villages and losing valuable genetic resources.

In our country, the traditional grain legume crops that are still grown are mostly faba bean and common beans (*Phaseouls* spp.) and, rather rarely, pea (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.). The results of the characterization and evaluation show that the local faba bean landarces are very diverse in phenotype and genotype. The collected accessions were classified by duration of the growing season, plant habit and height, mass and color of the seeds and content of crude protein.

The plant height of 2/3 of the studied accessions ranges from 81 cm to 100 cm. The rest of them are lower than 80 cm or higher than 100 cm. Most of the samples are of a medium and large seed size, with 3-4 seeds per pod and a size of the pod of 80-90 mm /13-15 mm. The content of crude protein varies widely, from 26% to 38% in grain dry matter (Fig. 3). The old traditional plant materials are grown, conserved and adapted to the conditions of the environment with specific regional characteristics. The next stage of the evaluation will include testing the tolerance to various biotic and abiotic stress factors.

Faba bean (*Vicia faba* L.) is an old traditional crop. In Bulgaria, it is grown on small areas and primarily for human consumption. Faba bean is used to a smaller extent as forage and green manure.

The grain legume collection at Institute of Plant Genetic Resources "Konstantin Malkov" (IPGR) consists over 5,000 accessions. The faba bean is represented by 770 accessions, with only 10-12% of Bulgarian origin.

The main task within the project SEELEGUMES was collecting faba bean local landraces, because they are still grown and maintained in the home gardens and small farms almost in the whole country (Fig. 1). They were visited and taken an inventory of over 20 regions, with collecting about 100 accessions of local populations, varieties and forms (Fig. 2).

Figure 1. Gathering valuable information on the locally maintained faba bean landraces in one village in Bulgaria

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Figure 2. Some of the local faba bean landraces from Bulgaria maintained at IPGR, Sadovo, Bulgaria

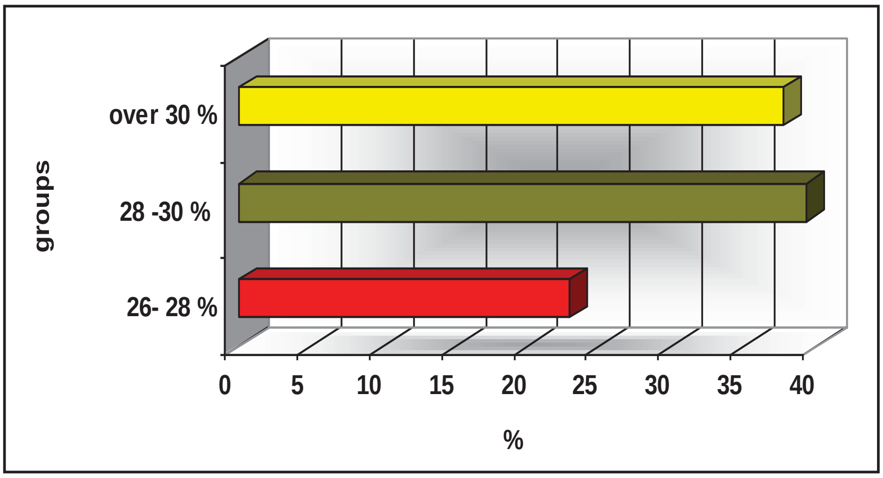

Figure 3. Local faba bean landraces maintained at IPGR, Sadovo, Bulgaria, grouped after the average grain crude protein content (%)

The project implementation allowed us to enrich the existing collection with local forms, populations and varieties of faba bean that are still grown in our country. The collected database provides the information on origin, farmer's name and utilization.

The *ex situ* conservation of seeds in the gene bank is a necessary preventive measure for small crops because they are endangered and are an important part of the rural cultural heritage of the country. ■

Cicer reticulatum Ladizinsky, progenitor of the cultivated chickpea (*C. arietinum* L.)

by Cengiz TOKER[1]*, Jens BERGER[2], Abdullah KAHRAMAN[3], Abdulkadir AYDOGAN[4], Canan CAN[5], Bekir BUKUN[6], R. Varma PENMETSA[7], Eric J. von WETTBERG[6] and Douglas R. COOK[7]

Abstract: *Cicer reticulatum* is a member of the genus *Cicer* and is considered to be the progenitor of cultivated chickpea (*C. arietinum*). During the latest expedition in Turkey, 763 new accessions were collected on a single plant basis from 41 collection sites, namely 513 of *C. reticulatum* and 169 of *C. echinospermum*. Both *C. reticulatum* and *C. echinospermum* possess many characteristics that are potentially useful in breeding, such as variable phenology and morphology, extreme temperature tolerance, and resistance to biotic and abiotic stresses.

Key words: *Cicer arietinum*, *Cicer reticulatum*, crop wild relatives, genetic resources

Cicer reticulatum Ladizinsky (Fig. 1) is a member of the genus *Cicer* L., the tribe Cicereae Alef., subfamily Faboideae or Papilionoideae, family Fabaceae or Leguminosae (9), and is considered to be the progenitor of cultivated chickpea (*C. arietinum* L.) (4, 11). Although the oldest archeological seed samples of *C. reticulatum* were dated to 7,260 BC from Tell-el-Kerkh, Syria (10), it is an annual species endemic to southeastern Turkey (1, 3, 14). Berger et al. (2) reported only 18 unique accessions of *C. reticulatum* in national and international genebanks that have been widely replicated, subsampled and renamed to create the number of accessions currently available.

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Therefore, a new mission to collect an expanded number of accessions of *C. reticulatum* and *C. echinospermum* P.H. Davis, both of which can be crossed with domestic chickpea, was initiated in 2013 in SE Turkey. The species' natural habitat is in fallow fields, vineyards, rocky lands and oak savannas from 600 to 1,630 m. The modus operandi of the collection mission was to visit previously known areas of *C. reticulatum* and *C. echinospermum* distribution and expand from there, paying close attention to the habitats outlined above.

On many occasions *C. reticulatum* could not found in locations where it had previously been collected (5, 6) indicating that it is declining species of conservation concern. Threats include road and dam constructions and the attendant increase in grazing pressure associated with easy access, conversion of pastures and savannas to tilled agricultural land, and habitat degradation from mining, and are particularly evident currently as Turkey enjoys decade-long economic growth. 763 new accessions were collected on a single plant basis from 41 collection sites, largely in *C. reticulatum* (*n* = 513) and *C. echinospermum* (*n* = 169), representing a substantial increase over the 18 and 10 original accessions in the current world collection (2).

Both *C. reticulatum* and *C. echinospermum* possess many characteristics that are potentially useful in breeding, such as variable phenology and morphology (6), extreme temperature tolerance, and resistance to biotic and abiotic stresses (Table 1). Moreover the species harbour significant genetic variation (8) representing an under exploited resource for base-broadening cultivated chickpea in breeding programs (7, 14) and in the development of genome sequences and mapping (12).

Acknowledgments

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Figure 1. ***Cicer reticulatum*** **Ladiz. in its natural habitat with leaves, flower and immature pod (left) and mature pod and seed (right)**

Table 1. Sources of *Cicer reticulatum* Ladiz. for resistance to biotic and abiotic stresses

Stresses	Accessions	References*
Cold	ILWC 81, ILWC 112, ILWC 113, ILWC 117, ILWC 139, ILWC 140, ILWC 141	Singh et al. 1990, 1994, 1995, 1998
	ILWC 81, ILWC 104, ILWC 105, ILWC 106, ILWC 108, ILWC 109, ILWC 110, ILWC 111, ILWC 112, ILWC 113, ILWC 114, ILWC 115, ILWC 116, ILWC 117, ILWC 118, ILWC 120, ILWC 122, ILWC 123, ILWC 124, ILWC 125, ILWC 126, ILWC 127, ILWC 128, ILWC 129, ILWC 130, ILWC 131, ILWC 134, ILWC 135, ILWC 136, ILWC 137, ILWC 139, ILWC 140, ILWC 141, ILWC 142, ILWC 182, ILWC 183, ILWC 184, ILWC 216, ILWC 218, ILWC 219, ILWC 229, ILWC 231, ILWC 233, ILWC 242	Robertson et al. 1995
	AWC 600, AWC 601, AWC 602, AWC 603, AWC 604, AWC 605, AWC 606, AWC 607, AWC 608, AWC 609, AWC 610, AWC 611, AWC 612, AWC 613, AWC 614	Toker 2005
	ILWC 81, ILWC 106, ILWC 139	Saeed et al. 2010
Drought and heat	AWC 605, AWC 616, AWC 620, AWC 625	Canci and Toker 2009
Drought	ILWC 36, ILWC 116	Imtiaz et al. 2011
Ascochyta blight	ILWC 113	Singh et al. 1998
	ILWC 26, ILWC 130	Infantino et l., 1996
	ILWC 104, ILWC 118, ILWC 119, ILWC 139,	Collard et al. 2001
	ILWC 17+21	Shah et al. 2005
Fusarium wilt	ILWC 26, ILWC 130	Infantino et l., 1996
	ILWC 81, ILWC 112, ILWC 117, ILWC 139, ILWC 140, ILWC 141	Singh et al. 1998
Botrytis gray mold	IG 72959, IG 72933, IG 72941	Pande et al., 2006
Crenate broomrape	C 553	Rubiales et al. 2004
Cyst nematode	ILWC 119	Di Vito et al. 1996
	ILWC 81, ILWC 112, ILWC 117, ILWC 139, ILWC 140, ILWC 141	Singh et al. 1998

South East European (SEE) autochtonous *Phaseolus* bean germplasm genetic diversity and reintroduction of traditional landraces

by Vladimir MEGLIČ*, Jelka ŠUŠTAR-VOZLIČ and Marko MARAS

Abstract: Genetic diversity of 25 accessions of common bean (*Phaseolus vulgaris* L.) from Bosnia and Herzegovina, 18 from Croatia, 28 from Macedonia, 30 from Serbia and 18 from Slovenia was assessed with 13 Simple Sequence Repeats (SSR) markers. The results showed that selected set of SSR markers are highly informative and applicable for studies of common bean genetic diversity. The gene bank at the Agricultural institute of Slovenia (AIS) holds a collection of 1,035 bean accessions collected from various parts of Slovenia in the last decades. With published information and description of old varieties and growing technologies, their traditional use, the ways of preservation and/or processing as well as recipes of traditional dishes, we will promote preservation of traditional knowledge.

Key words: common bean, genetic resources, landraces, *Phaseolus*, Slovenia

The selected regions of Slovenia and other regions of Southeast Europe (SEE) are still rich in biodiversity and genetic resources of grain legumes constituting a great potential for their utilization in breeding programs or for direct reintroduction back to the farm. Due to changes in climate conditions and agricultural practices there is an impending risk of losing valuable genetic diversity. The SEELEGUMES project helped to ensure a long-term conservation of legume plant genetic resources and to promote their sustainable utilisation in the SEE region and strengthened the national efforts.

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Microsatellite markers, also called Simple Sequence Repeats (SSR), are user-friendly highly polymorphic markers suitable for genetic diversity studies, purity control, differentiation of plant cultivars, elimination of duplicates and selection of appropriate parents based on genetic distances in breeding programs (1). The genetic diversity of 119 common bean (*Phaseolus vulgaris* L.) accessions originating from five SEE countries was performed using 13 SSR markers (2). Analysis included 25 accessions from Bosnia and Herzegovina, 18 accessions from Croatia, 28 accessions from Macedonia, 30 accessions from Serbia and 18 accessions from Slovenia. The calculated mean number of alleles per locus was 9.08. The average polymorphic information content over all loci reached value of 0.72. These results showed that the selected set of SSR markers is highly informative and applicable for studies of common bean genetic diversity (2). In the Principal Component Analysis (PCoA) plot (Fig. 1), the majority of accessions were grouped into two distinct clusters, which resemble to Mesoamerican and Andean gene pools. Both clusters comprised accessions from each of the five countries. The accessions from Macedonia and Serbia were distributed evenly between the two gene pools, while the accessions from Croatia, Bosnia and Slovenia clustered predominantly within the Andean gene pool. Additionally, PCoA and Bayesian cluster analysis indicated the existence of potential hybrids between the two gene pools. The analysis of molecular variance revealed that significant part of accessions from different countries is closely related. This study show that common bean might be introduced into the territory of former Yugoslavia several centuries ago by a common route (2).

Common bean has been cultivated in Slovenia for centuries, resulting in the development of numerous landraces, some of which are still grown today. The gene bank at the Agricultural Institute of Slovenia (AIS) holds a collection of 1,035 bean accessions collected from various parts of Slovenia in the last decades (3, 4). The results revealed that during the centuries of common bean cultivation in Slovenia a diverse collection was formed that should be preserved for the future (4). With the aim to reintroduce traditional landraces back to cultivation, 16 Češnjevec accessions and 23 accessions of landrace Lišček (another traditionally grown common bean type) were selected from the AIS gene bank. Field studies were conducted in three successive years; plant growth, resistance to diseases and pests were evaluated and the yield was determined. The chemical composition of raw seeds was analysed, which included determination of macro- and microelements, dry matter and starch content, crude proteins, crude fiber and polyphenols, and the sensory characteristics were evaluated. Based on the results obtained, three landraces, one Češnjevec and two Lišček accessions, were selected for the inscription in the national list of varieties of Slovenia as conservation varieties (5).

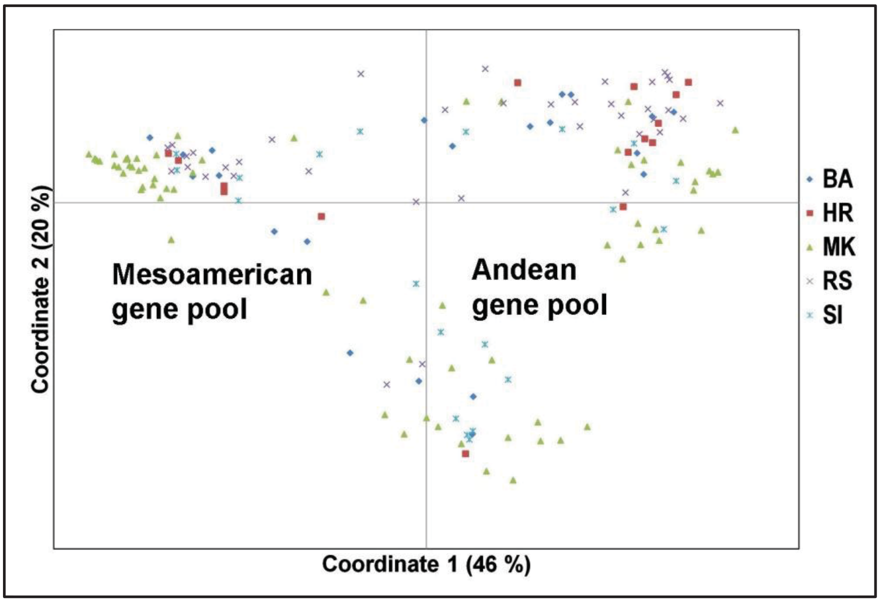

Figure 1. A Principal Component Analysis (PCoA) plot showing genetic relationships among 167 common bean accessions from five South East European countries

Both data and material of the newly collected and evaluated accessions will be added in the database and SEE gene banks that will serve scientific community, plant breeders, farmers and hobby gardeners. This will present the information that will enable the insight in morphological and molecular characteristics of the collected/stored material. With published information and description of old varieties and growing technologies, their traditional use, the ways of preservation and/or processing as well as recipes of traditional dishes, we will promote preservation of traditional knowledge.

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Lignified tissues in vegetative organs of wild-growing *Trifolium* L. forage species

by Lana ZORIĆ[1], Jadranka LUKOVIĆ[1], Dunja KARANOVIĆ[1] and Aleksandar MIKIĆ[2]

Abstract: Wild-growing *Trifolium* forage species are often grazed or cut for hay or fresh forage in their natural habitats. The proportion of lignified tissues in vegetative organs is important parameter that affects digestibility, since lignified tissues resist rumen degradation. The examined species had the highest proportion of lignified tissues in stems and peduncles and the lowest in lamina. They proved to have preferable proportion of lignified, thick-walled tissues and thin-walled, parenchymatic tissues, and were assigned as forages of potentially high digestibility and good quality.
Key words: anatomy, clovers, forage quality, *Trifolium* spp.

The *Trifolium* L. forage species are among those forages that are used in planted pastures, but also utilized by grazing animals in their natural habitats or cut for hay or fresh forage, without being deliberately sown (4). Plant breeding programs improve agronomic characteristics of legume forages, but also their adaptation to the environment. Wild relatives of the few commercialized *Trifolium* species occur naturally and are frequently grazed. Moreover, local populations, which are likely to be better adapted to local environmental conditions, can serve as sources of genetic variation.

Lignified tissues have important role in providing plant's resistance, but negatively affect their digestibility (2). Tissues with a high proportion of thick-walled cells are incompletely digested or resist degradation, and the reduction of their proportion is one of the objectives of forage breeding programs (1). In *Trifolium* species such tissues are sclerenchymatous parenchyma (present only in stem and peduncle), vascular tissue and sclerenchyma, the proportion of which should be minimized, without negatively affecting plant's resistance and strength (3). Parenchyma tissues are thin-walled, whilst epidermis and collenchyma have cells with thickened, non-lignified cell walls.

Using anatomical method, light microscopy observations and measurements of cross-sections, the proportions of lignified and non-lignified thin-walled tissues were determined in plant organs of wild-growing *Trifolium* forage species (Fig. 1). Stems and peduncles had the highest proportion of lignified tissues (15.5% - 29.6% and 14.9% - 26.3%, respectively), which made these organs the most responsible for digestibility limitations. Parenchyma tissue was best developed in lamina (87.7% - 92.4%), which could contribute to high leaf digestibility, which is characteristic of legume forages. The examined species showed high level of similarity in examined parameters, which generally had low variability. The most desirable ratio of lignified and non-lignified tissues was recorded in *T. subterraneum* L.

The proportion of lignified tissues in examined *Trifolium* species was significantly lower compared to the one obtained for wild-growing *Trifolium* species that were not recognized as forages (3). The examined species also had preferable percentage of lignified tissues compared to the percentage recorded in vegetative organs of cultivated *Medicago truncatula* Gaertn. (6), or in stems of wild-growing *Lathyrus* L. species (5). According to the results of anatomical analysis, the examined *Trifolium* species represent wild-growing forages of potentially high digestibility and good quality.

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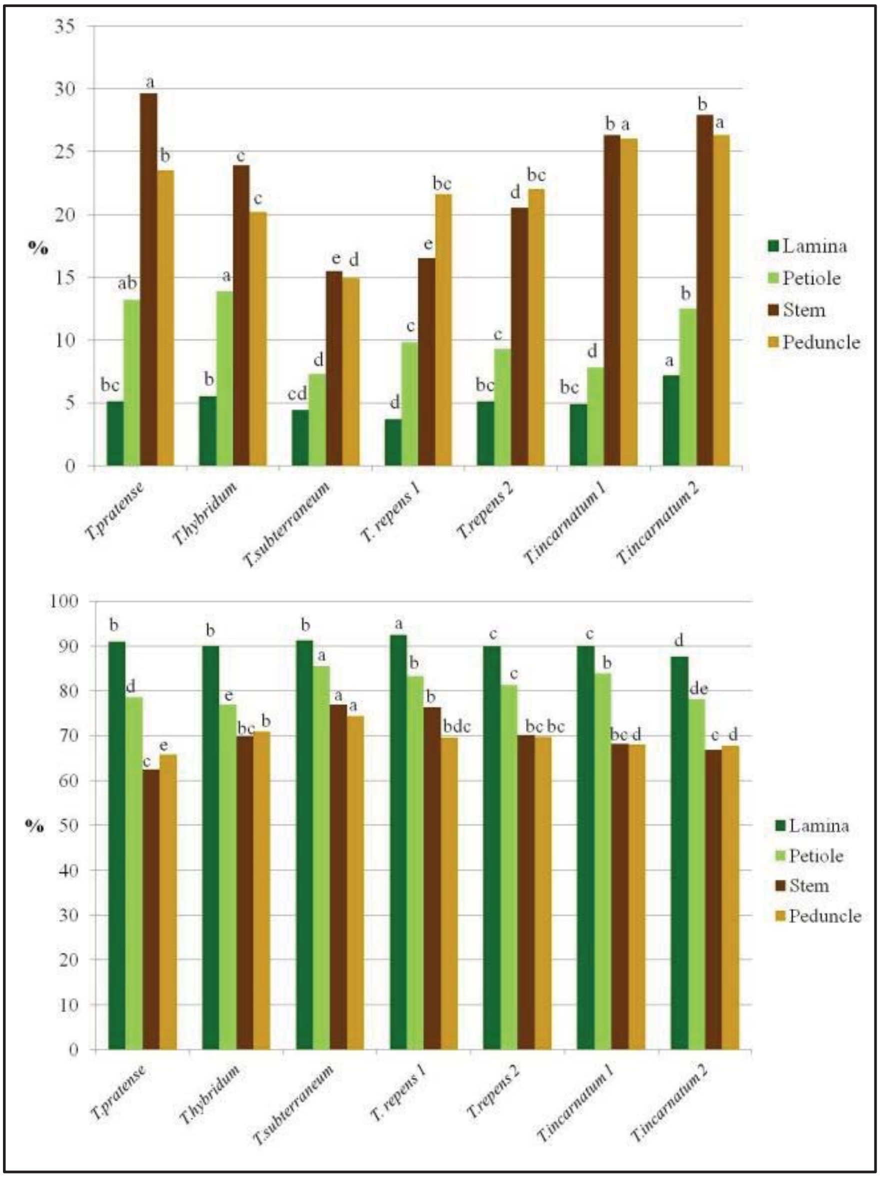

Figure 1. The percentages of tissues composed of lignified (above) and non-lignified thin-walled cells (below) in different organs of *Trifolium* forage species

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Economic value of perennial vetchlings (*Lathyrus* L.) species from the Russian Far East

by Margarita VISHNYAKOVA*, Alla SOLOVYOVA, Andrey SABITOV, Pavel CHEBUKIN and Marina BURLYAEVA

Abstract: This paper presents the results of the estimation of protein amount in green mass of several *Lathyrus* species, introduced at VIR collection in 2010-2011 from the territory of the Russian Far East. The accessions have been collected from the vast territory during the expedition surveys. The interpopulation variability of the traits have been revealed. Preliminary conclusions about the economic value of these species as forage plants are highlighted.

Key words: crude protein content, green mass, *Lathyrus*, Russian Far East, vetchlings

The collection of the genus *Lathyrus* representatives in the N.I. Vavilov All-Russian Research Institute of Plant Industry (VIR) in St. Petersburg, Russia, contains 1,835 accessions belonging to 56 species. In Russia, only five species are involved in breeding, namely *L. sativus* L., *L. sylvestris* L., *L. tingitanus* L., *L. chloranthus* L. and *L. odoratus* L.. The last three species are used as ornamental plants.

The increase in the species composition of the collection expands the possibility of domestication of new plants and their transformation into crops. Especially interesting is the potential of perennial vetchling (*Lathyrus* spp.) species, renown for a high protein content, longevity, early spring regrowth, ability to regenerate after several mowing during one season. These species, along with perennial vetches (*Vicia* spp.) could be the components of intercropping, designed for many years of use, especially in the areas with unfavourable and complex surface terrain, where they must be mowed by hand. They also could enrich the diversity and increase the quality of natural pastures (1).

The flora of Siberia and the Far East of the Russian Federation contains at least 8 perennial *Lathyrus* species, including 4 endemic (3). During 2010-2011, VIR organized the expeditions to the regions of Primorsky and Khabarovsk in Russia and the Heilongjiang Province in Northeast China. The plant samples of 8 species were collected during late August and September, when mature pods were formed, enriching the VIR collection with 7 new *Lathyrus* species.

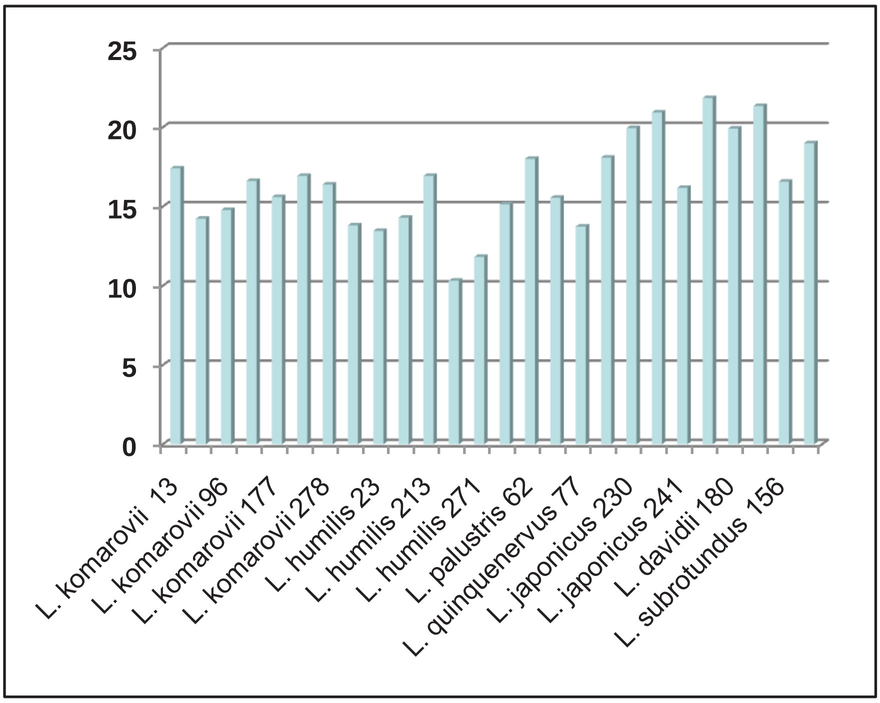

Figure 1. Protein content in forage dry mass (%) of the perennial accessions of *Lathyrus* species collected in the Russian Far East during 2010-2011

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The protein content in forage dry mass of the collected accessions

Since the collected accessions are promising, especially as potential forage crops, the protein content in green mass was determined by the standard Kjeldahl method. The protein content was expressed as a percentage in forage dry weight. The results showed interspecific and intraspecific (inter-population) variability of the trait. The samples collected in different habitats had the following protein content in green mass: *L. komarovii* Ohwi, from 13.81% to 17.41%; *L.humilis* (Ser.) Sprengel, from 10.32% to 16.93%; *L. palustris* L., from 15.15% to 18.01%; *L. japonicus* Willd., from 16.18% to 20.94%; *L. davidii* Hance, from 19.92% to 21.85%; *L. subrotundus* Maxim., from 16.57% to 19.00% (Fig. 1).

Previously, we had analyzed this character in the representatives of some perennial species from the collection grown in the experimental fields in Central Russia (the township of Ekaterinino, the region of Tambov). The highest value of the trait (20.7%) was observed in the cultivated species *L.sylvestris* L. (2).

The data for other species grown with an advanced level of agronomy in Ekaterinino are comparable with those obtained for the samples from the Russian Far East, with the advantage of the latter. The highest protein content was observed in two samples of *L. davidii*, collected in Primorye, with 21.34% and 21.85%.

The next step in assessing the nutritional value of these species should be aimed at determining the content of anti-nutritional substances, such as ODAP, as well as of sugars, chlorophyll A and carotenoids, beta-carotene and the proportion of organic substances.

Prospects

At this stage, the study of biological characters and economic assessment has not been carried out for the all *Lathyrus* species collected and introduced in the VIR collection. However, it is known that some of them, such as *L. japonicus, L. davidii, L. palustris* and *L. subrotundus*, in their natural habitats may grow up to between 1 m and 1.5m in stem length (Fig. 2), have abundant foliage and are considered as good forage species. It is also known that certain species, along with feeding value, have good soil-fixing value and could be used as weed-cleaning crops (4). Most of the collected species are mezo-xerophytes, but *L. palustris* grows on the moist and wet soils and usually well endures long flooding. So, the intraspecific variability of characters of the examined *Lathyrus* species allows a selection improvement, pre-breeding and developing a novel protein-rich forage resource.

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Figure 2. Some *Lathyrus* species in their natural habitats in the Far East of Russian Federation: *L. japonicus* (above, left), *L. davidii* (above, right), *L. subrotundus* (below, left), *L. palustris* (below, right)

On some biomorphological peculiarities of seedlings of *Vavilovia formosa* (Stev.) Fed. (Fabaceae)

by Janna A. AKOPIAN[1], Andrey A. SINJUSHIN[2], Ivan G. GABRIELYAN[1] and Gayane G. SHABOYAN[1]

Abstract: Wild perennial pea, *Vavilovia formosa* is a rare representative of the alpine flora. and its growing in *ex situ* conditions is very complicated. The assumption that the border between cataphylls and true leaves of perennial Fabeae coincides with the border between wintering and annual plant parts is confirmed by observations on *V. formosa* samples grown from seeds in laboratory conditions and in natural habitats.
Key words: biomorphology, *ex situ* conservation, ontogeny, *Vavilovia formosa*

Wild perennial pea, *Vavilovia formosa* (Stev.) Fed., is a rare representative of the alpine upper-mountain flora with distribution confined to small areas of moving detritus and screes. That is why its growing in *ex situ* conditions is very complicated. Moderate air temperature, even lower ground temperature, continuous hydration (from melting glaciers), together with sufficient aeration of the substrate, dryness of the upper layers of the scree and a low concentration of mineral salts in the soil with pH 6.5-7.0 are important for the successful growing of this plant (1, 2). Nevertheless, attempts to grow and study *V. formosa* in other conditions continue.

The aim of this work was to study the early stages of *V. formosa* ontogeny on samples grown from seeds in laboratory conditions. The seedling morphology was also studied in natural habitats (Geghama ridge, Mt. Sevsar, 2014). Seeds were collected in the territory of Armenia from two populations: from the slopes of Geghama ridge (Mt. Sevsar, 3200-3300 m asl., 2002) and Syunik ridge (Mt. Ukhtasar, 3300-3350 m asl., 2012).

The *V. formosa* seeds from the slopes of Mt. Sev Sar (Fig. 1a) are mostly elongated or roundish-oval, flattened (5.0 mm - 4.8 mm × 2.8 mm - 3 mm × 2.4 mm -2.5 mm), while the seeds from the vicinity of Mt. Ukhtasar (Fig. 1b) are almost spherical, less flattened (3.8 mm × 3.7 mm - 3.8 mm × 2.8 mm - 2.9 mm).

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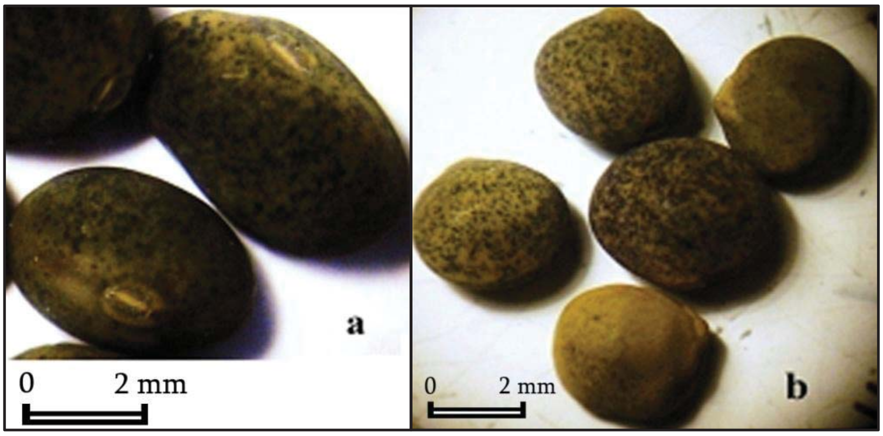

Figure 1. *Vavilovia* **seeds from two Armenian populations: Geghama ridge, Mt. Sevsar (a), and Syunik ridge, Mt. Ukhtasar (b)**

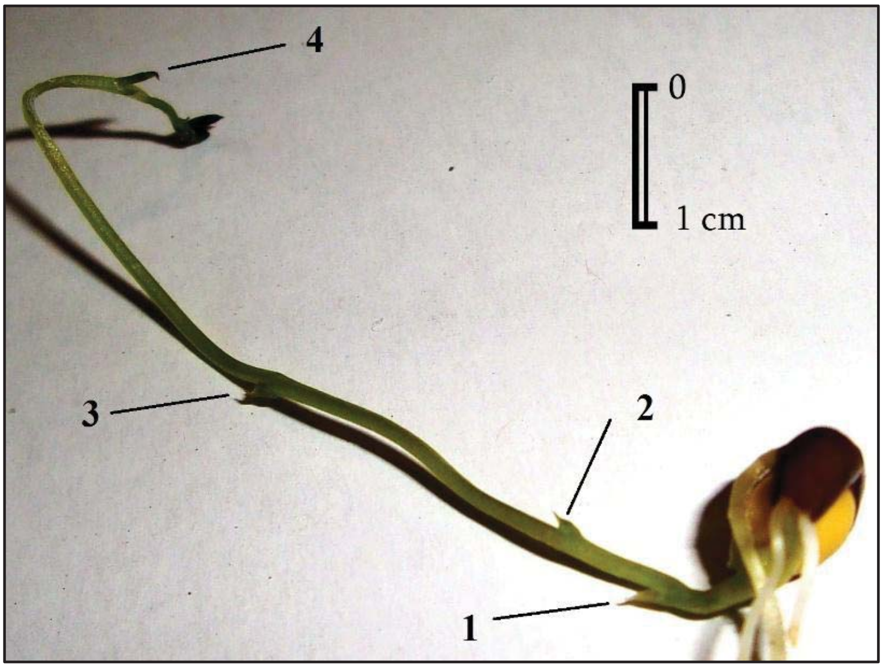

Figure 2. A twelve-day old seedling of ***V. formosa*** **with basal scale leaves (1-4)**

Seed surface is smooth, the color is dark olive with black dots or spots. Immature seeds are bright brown. Seed hilum is broadly-ovate (0.7 mm - 0.8 mm × 0.5 mm) with thin white coating and a white line on the border of the cotyledons. Chalaza trail is dark brown or black, inconspicuous.

Seeds were germinated in laboratory conditions in Petri dishes on humid filter paper. After 5 days, the swollen seeds were scarified, necessary for their germination 10-12 days after sowing. The seedlings were transplanted into small containers with soil and gravel, with holes on the bottom and on the entire surface to ensure proper aeration of the root system. They were watered sparingly, mainly from the pallet.

The root system of *V. formosa* seedlings usually has a taproot and numerous lateral roots. However, in the early stages of germination, in some seedlings, the taproot ceases to grow and whitish adventitious roots form near the cotyledonary node (Fig. 2). In other plants, reducing the taproot and lateral root formation to the fourth order was observed (Fig. 3). The seedling hypocotyl had a length of 6 (7) mm, epicotyl 4 (5) mm, 2nd internode length 4 (6) mm, 3rd 17 mm and 4th 20 (23) mm.

Four cataphylls (scaly leaves) are observed at the base of the seedling (Fig. 2). The first cataphyll is on the outer side of the epicotyls bend, 1.8 mm - 1.9 mm × 1.0 (1.2) mm, acutely lanceolate, entire, with a prominent main vein, with membranous edges narrowly at the base and widely at the top. The next two cataphylls are alternate, tridentate, with acutely lanceolate middle tooth, 1.9 mm × 1.8 mm (the second), 1.8 mm × 1.2 mm (the third). The fourth cataphyll is greenish, 3.0 mm x 1.0 (0.9) mm, lateral teeth are 0.8 mm. It should be mentioned that earlier three cataphylls were reported for the *Vavilovia* seedling (4). Leaflets of first true leaves are obovate, about 5mm × 4 mm, sparsely pubescent with white, soft trichomes from abaxial side and by edges and almost glabrous from adaxial side. Stem and leaf petioles are covered with sparse, minute (0.03 mm - 0.05 mm), narrowly clavate, reddish glandular hairs.

Seed germination of *V. formosa* is hypogeal. Naturally epicotyl, three next internodes and 3(4) cataphylls remain under moving scree layer. They lack chlorophyll (Fig. 4), unlike seedlings obtained in laboratory conditions (Fig. 2, 5a). In all cataphyll axils, buds are formed, from which in laboratory conditions the shoots of the following orders are developed (Fig. 5A and 5B).

The assumption that the border between cataphylls and true leaves of perennial Fabeae coincides with the border between wintering and annual plant parts (3), is confirmed by observations on *V. formosa*. In autumn in natural habitats, under a layer of scree, wintering hypogeal shoots of renewal develop from the buds in axils of cataphylls (Fig. 5C). Annual aerial part of the plant during the first year of development represents unbranched orthotropic shoot up to 5-6 cm in height. By the end of the growing season, up to 10-13 leaves with elongated internodes are formed on a plant in the laboratory, the same number of leaves and short internodes are observed under natural conditions.

Acknowledgements

The authors are grateful to Dr. M.E. Oganesian and Dr. A.G. Ghukasyan (Institute of Botany of National Academy of Sciences of Armenia) for kindly providing the seeds of *Vavilovia formosa*.

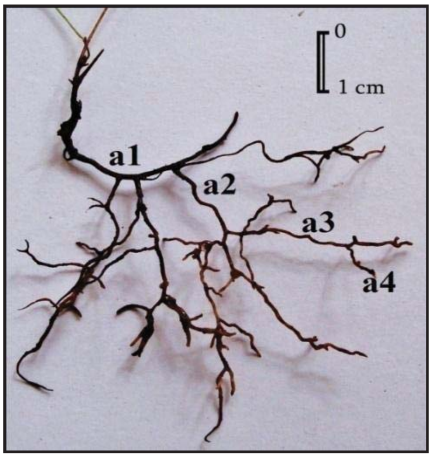

Figure 3. Root system of *V. formosa* in the first year of development under laboratory conditions: reduced main taproot (a1) and lateral roots (a2, a3 and a4)

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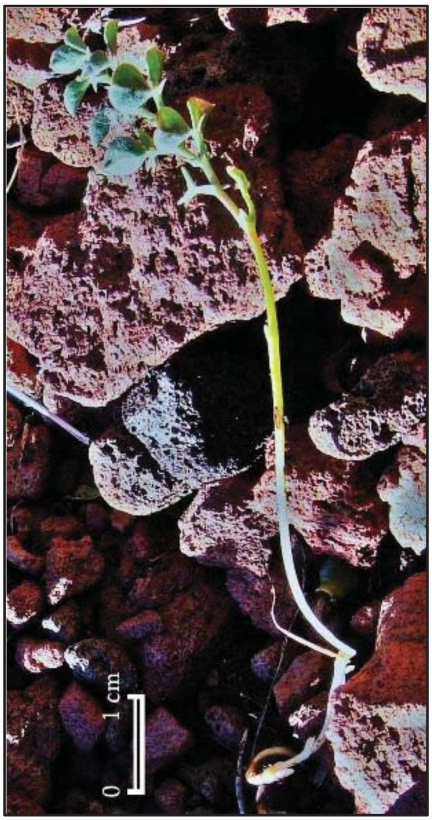

Figure 4. A *V. formosa* plant in the first year of development on the moving scree slope of Mt. Sevsar, at the beginning of September

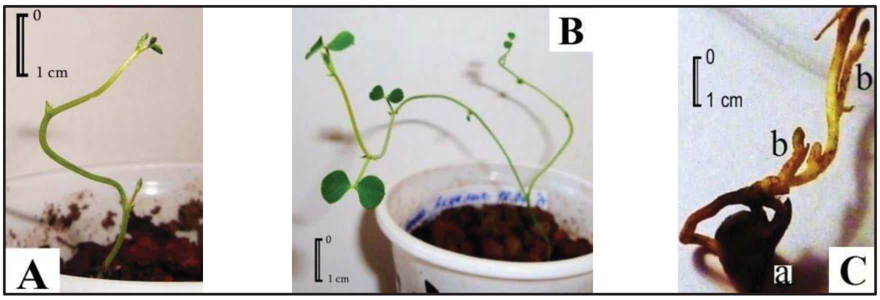

Figure 5. The *V. formosa* development features in 18 (A) and 33 days (B) after germinating in laboratory conditions and the basal part of the *V. formosa* plant in the first year of development in natural conditions at the beginning of September (C): seed (a) and hypogeal shoots of renewal (b)

In vitro biotechnology approaches now available for 'beautiful' vavilovia (*Vavilovia formosa*)

by Sergio J. OCHATT* and Catherine CONREUX

Abstract: Efficient *in vitro* propagation of *Vavilovia formosa*, plant regeneration from callus, protoplast isolation and culture to differentiated callus of *V. formosa* were developed and its relative nuclear DNA content by flow cytometry was established. The summation of biotechnology tools now available should foster evolutionary studies on the tribe Fabeae and, in time, *V. formosa* could become a potential source of novel agronomic adaptive traits.

Key words: callus culture, evolutionary studies, *in vitro* propagation, *Vavilovia formosa*

Vavilovia (*Vavilovia formosa* (Stev.) Fed.) material is as sparse as reports on it and a collaborative work started recently in several European laboratories (2, 3). Among other topics, development of biotechnology tools for this perennial herbaceous, endangered and protected plant was undertaken. A few mature seeds were collected in the Caucasus, its main area of distribution, and *in vitro* cultures were initiated at Agritec Plant Research Ltd in Šumperk, Czech Republic, and the Institute of Botany in Yerevan, Armenia. We now developed efficient *in vitro* propagation, plant regeneration from callus, protoplast isolation and culture to differentiated callus of *V. formosa*, and we also established its relative nuclear DNA content by flow cytometry (1, 5).

Rapidly propagating, high quality shoots of *V. formosa* were produced after testing a range of media with varying hormonal concentrations (6). The main problem was that shoots were stunted, enfeebled and incapable of rooting. This was resolved by applying a sequence of recurrent passages on medium with low NAA and high BAP first, then to a similar BAP content with increased NAA where multiplication rate was reduced but shoot quality was increased. Thereafter, shoots were kept by cycling 4 successive passages on medium with a balanced NAA/BAP content followed by 4 passages on medium with the stronger cytokinin TDZ as sole hormone. Shoots with with elongated internodes were thus consistently produced and 35% - 40% of them could be rooted (Fig. 1a) on media containing full or half-strength Murashige and Skoog's (4) formula with or without auxin (IBA or NAA).

Callus cultures were obtained from the internodes and leaves of propagated shoots on medium with 0.5 mg l^{-1} BAP and 1.0 mg l^{-1} NAA, and shoots were also regenerated *de novo* (Fig. 1b), then rooted and transferred *ex vitro*. Finally, experiments were undertaken on protoplast culture and regeneration from the leaves and stems of propagated shoots and also from the rapidly proliferating calluses above. To date, callus could be proliferated from the isolated protoplasts of all three tissue sources but only callus-derived protoplasts were capable of differentiation but, to date, *de novo* regeneration of protoplast-derived plants has remained elusive.

Regenerated and propagated plants were true-to-type in terms of phenotype and relative nuclear 2C DNA content, which we determined to be of 4.74 pg DNA.

The summation of biotechnology tools now available should foster evolutionary studies on the tribe Fabeae and, in time, *V. formosa* could become a potential source of novel agronomic adaptive traits.

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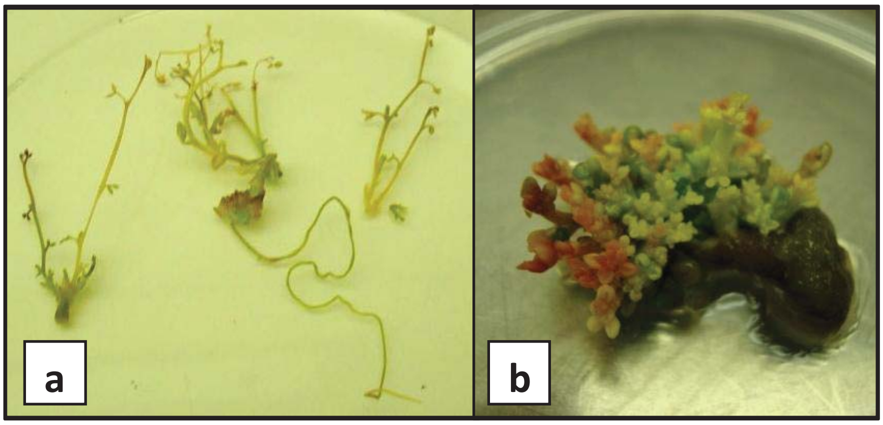

Figure 1. Some in vitro responses obtained with *Vavilovia formosa*: shoots with elongated internodes, the middle one shows rooting (a), and shoot regeneration from internode culture (b)

Nutritive value of grass pea (*Lathyrus sativus*)

by Tihomir ČUPIĆ[1]*, Marijana TUCAK[1] and Aleksandar MIKIĆ[2]

Abstract: Grain legumes are a rich source of high quality food for human and animal consumption. Their cultivation has been constrained by water deficit, high or low acidity of soil and flowering sensitivity to temperature. Landraces of grass pea have a large genetic potential for important production traits including tolerance to drought and flood, winter-hardiness and tolerance to diseases and insects. They are a rich source of protein, carbohydrates as well as isoflavones. Our future research on grass pea will focus on further increasing yield and creating lines without the ODAP toxin which could be of increasing importance as the climate changes.

Key words: grass pea, landraces, *Lathyrus sativus*, nutritive value, ODAP

Figure 1. One of the grass pea local landraces from Dalmatia: in bloom (above) and seeds (below)

The genus *Lathyrus* has 187 species and subspecies (1). Of these, only a small number are used in food and feed. Only grass pea (*Lathyrus sativus* L.) is cultivated and used as human food, forage for livestock or as a suitable crop for spreading green manure in extreme environmental conditions. Across the world it has different names, but the most common is grass pea. In different parts of the world people use different names or synonyms like (5) *chickling pea* (UK and North America), *almora* (Spain), *khesari* or *batura* (India), *alverjas* (Venezuela), *gilban* (Sudan), *guaya* (Ethiopia), *matri* (Pakistan), *gessette* (France), *pisello bretonne* (Italy), *sikirica* or *tijarica* (Croatia), *grah poljak* (Bosnia and Herzegovina) and *sastrica* (Serbia). Botanists have identified a close connection between *L. sativus* (grass pea) and *L. cicer* (dwarf chickling) and a possibility of common origin. The archaeobotanical evidence has shown that *L. sativus* and *L. cicer* have been grown on the Iberian Peninsula during the Neolithic (6) from where they spread throughout the Mediterranean. The evidence shows the *L. sativus* is probably the oldest cultivated crop in Europe (3).

The interest for production of grass pea has varied during human history and was influenced by wars or other natural disasters like drought, floods, high temperatures, etc. The last great exploitation of grass pea in Europe has happened during the Second World War. Inhabitants in dry Mediterranean areas sowed undemanding grass pea landraces in order to feed themselves and keep from hunger. Because of this, many researchers have begun to explore this highly adaptable legume. Its small demands on the ground, combined with tolerance to drought and other stress conditions (stagnant water, tolerance to high soil acidity and alkalinity) makes it a plant species that will become more and more important as the climate changes.

The main ingredients in grass pea grains are carbohydrates with a share of about 55% and about 28% of protein of grain dry matter (DM). The rest of the material are mineral components (4%) and oil (about 2%). The differences in the composition of grain are influenced by weather conditions, soil composition and the types of landraces that are used in specific geographic regions. The biological value of grass pea protein is estimated at 70% of the biological value of soy protein, while the digestibility is approximately equal. The most common protein in the structure of the grass pea seed is globulin (50% - 66%). Albumin values vary widely depending on the environmental conditions (14% - 26%), while glutenin (about 15 %) and prolamin (5% - 6%) have the lowest percentage. Of all the amino acids, grass pea is the richest in lysine (4.5% - 9.6%) and threonine (2.6% - 5.1 %), while, like other legumes, it is poor with cysteine (about 1%) and methionine (about 0.8%). The predominant carbohydrate in grass pea is starch, with 33.0% - 43.9%, followed by fiber which comprises about 7%. Total sugars vary depending on location and year (2.7% - 5.2%), which is reflected in the water-soluble oligosaccharides (1.9% - 3.3%). The most common oligosaccharid in the seed is stachyose, accounting for 1.1 g 100 g^{-1} to 1.7 g 100 g^{-1}, while the shares of verbascose and raffinose are lower with an average of 0.77 g 100 g^{-1} and 0.39 g 100 g^{-1}, respectively. Oligosaccharides belong to the prebiotic group and as such promote the growth and proliferation of bifidobacteria in the colon and have a beneficial effect on health and the prevention of cancer.

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Grasspea, like most species of the genus *Lathyrus*, contains anti-nutritional components (4) such as trypsin inhibitors, lectins, tannins, phytic acid and the water soluble non-protein amino acid ODAP (oxalyl di-amino propanoic acid). Prolonged and excessive intake of ODAP (neurotoxin) causes lathyrism that can cause uncomfortable muscle spasms and certain neurological problems because of the inhibition of lysyl oxidase that directly affects the spinal cord. Lathyrism seems to occur when the grass pea ratio in food is at least 25% continously over 1.5 to 6 months. The traditional varieties of grass pea contain 0.5% - 2.5% of β-ODAP. Our present findings suggest that European genotypes have significantly less neurotoxins as compared to African and Asian. However, the level of neurotoxins is not only a genetic trait but is also influenced by environmental factors. These have a significant effect on the level of a ODAP. It has been established that the stress caused by drought increases the level of neurotoxins and soil salinity reduces it. However, there is evidence in the landraces of grass pea that people, selecting by color of flower and seeds, unconsciously chose plants with less ODAP and tannins and created local populations in Dalmatia and Herzegovina, which have been used as food for many years. Laboratory tests are currently conducted in order to find genotypes with a low concentration of neurotoxins and determine agronomic factors that will reduce the level of ODAP below 0.10%. Similarly, studies are conducted on other anti-nutritional compounds to obtain lines that would be suitable for the creation of new more cultivated genotypes suitable for consumption. Thermal treatment (high temperature) can also inactivate most anti-nutritional compounds which enables the grass pea to be used for human consumption.

Apart from the above mentioned anti-nutritional substances, grass pea is abundant in phytoestrogens that appear in more than 300 plant species of which legumes constitute a major part. The total phenol content represents the potential value of antioxidant activity of each population. Isoflavones are part of the isoflavonoides group that has a characteristic chemical structure on which the physiological activity of individual isoflavones depends. The antioxidant activity of isoflavones highlights possible potential for lowering cholesterol levels, prevention of prostate cancer and breast cancer, osteoporosis, cardiovascular disease. It also relieves the symptoms of menopause. Two of the most important and most researched sources of isoflavones are soybean (from grain) and red clover (from the whole plant), but other legumes such as grass pea can also be a good source of isoflavones. Grass pea is a legume that is consumed the most in Ethiopia, India and Bangladesh. The amount of isoflavones in the grain varies depending on the genotype, geographic area of growth and production conditions. In the trials conducted with grass pea accessions from Dalmatia the share of isoflavones was around 20 mg 100 g^{-1} DM. It was found that some populations with multi-colored seed had significantly more total isofalvones, even over 100 mg 100 g^{-1} DM. In most populations an absence of genistein was determined. The presence of genistein was found in one population, and is a valuable source of potential inhibitory activity of protein-tyrosine kinases, which can reduce the possibility of cancer cell growth. Formononetin has the largest isoflavone share in the grass pea. The conducted analysis has shown the presence formonetin in all populations (9.5 mg 100 g^{-1} DM on average). One local population contained a ten times higher proportion of daidzein and is very promising material for further study, if the results replicate and confirm it as a stable quantitative trait. Bioactive compounds in most legumes vary depending on the genotype, the weather and other environmental conditions. This opens new opportunities for further research related to individual isoflavone content in grass pea.

Apart from good nutritional values, the grasspea should potentially achieve high and stable grain yield in order to expand production. The current yield potential in large scale production ranges from 900 kg ha^{-1} to 1500 kg ha^{-1}, while yields in the United States in the production of inoculants achieve quantities of 2000 kg ha^{-1}. In the area of Bosnia and Herzegovina, from 2008 to 2009, experiments that were performed produced the highest average yield of 3422 kg ha^{-1} of grass pea grain with intensive use of agronomy (2). The results of this study show that the genetic potential for the yield of grass pea is great and that using appropriate agricultural technology needs to be maximized. Identification of high-yield and low concentrated deliverance grasspea lines, should be increase the production in the future. ■

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The effect of cooking and enzymatic digestion on phenolic content of grass pea seeds flour extracts

by Nemanja STANISAVLJEVIĆ[1]*, Živko JOVANOVIĆ[1], Tihomir ČUPIĆ[2], Jovanka LUKIĆ[3], Jovanka MILJUŠ ĐUKIĆ[1], Svetlana RADOVIĆ[4] and Aleksandar MIKIĆ[5]

Abstract: Legume seeds are a rich source of a wide variety of bioactive non-nutrient compounds, including phenolic antioxidants, dominantly located in the seed coat. Total phenolic content of methanolic and water extracts of non-processed, cooked and in vitro enzymatically digested seed flour of five grass pea (*Lathyrus sativus*) landraces was studied. There was no significant difference in phenolics content in non-processed grass pea seed flour. However, our results shown that cooking and enzymatic digestion strongly enhanced the release of phenolic compounds in methanolic extracts of all analyzed cultivars.

Key words: bioactive compounds, cooking quality, grass pea, landraces, phenols

Introduction

Legume seeds are a rich source of minerals and many nutrient compounds, including sugars, starch, certain fatty acids and proteins. These seeds also contain a wide variety of bioactive non-nutrient compounds, including phenolic antioxidants, dominantly located in the seed coat.

Phenolic substances have an important role in the defense system of the seeds, which are exposed to oxidative damage by many environmental factors, such as light, oxygen, free radicals, and metal ions (6). These compounds are widely distributed in the plant kingdom and have been reported to possess a considerable range of biological effects-antioxidant, anti-inflammatory and vasodilatory action. It is known that plant phenolics might have the role in the prevention of coronary disease, cancer and age-related degenerative brain disorders.

Legume seeds are usually cooked in order to increase palatability and improve edibility. All this process may bring about a number of changes in physical and chemical characteristics. There are many contradictory data about the effect of cooking and enzymatic digestion on phenolic content (1, 5). There is also data that extracting solvent significantly affects phenolic content and antioxidant activity.

The aim of this study was to determine the extent to which conventional cooking methods combined with gastrointestinal-simulated extraction of phenols, differ from the extraction of unprocessed grass pea seed flour with pure methanol.

Materials and methods

Legume samples. The grass pea (*Lathyrus sativus* L.) seed samples were taken from several local landraces from southern Croatia: L1-Dalmatinska zagora "Pakovo Selo", L2- Dalmatinska zagora "Drniš", L3-Ravni kotari "Škrabinja", L4-Dalmatinska zagora "Stankovci" and L5-Cetinska krajina "Tijarica". Voucher specimens of the population studied are deposited in the Herbarium of the Department of Forage Crops, Agricultural Institute Osijek, Croatia.

Chemicals and reagents. Folin-Ciocalteu reagent (FC), gallic acid (GA), sodium carbonate, pancreatin and trypsin were obtained from Sigma Chemical. Co. (St. Louis, MO, USA). All other chemicals were of analytical grade.

Preparation of methanolic extracts from dry seeds. Dry grass pea seeds were ground to a fine powder in a basic mill to pass through a 60-mesh sieve. Pure methanol (2 ml) was added in aliquot of the powder (0.2 g) and tubes were shaken at 300 rpm at 4 ºC in the dark for 24h. The extracts were centrifuged at 5000 g for 5 min and the supernatants were collected.

Preparation of methanolic extracts from cooked seed flour. Seed flour (0.2 g) was mixed with 2 ml of water in tube, shaken and the samples were boiled for 45 min. The cooking water was evaporated under vacuum at 60 °C and the samples were freeze-dried. The methanol extraction was previously described.

Preparation of methanolic extracts from cooked and in vitro enzymatically digested seed flour. The *in vitro* enzymatic digestion was performed according (2), with some modifications. Seed flour samples (0.2g) were cooked as prevoisuly described and mixed with saline solution (pH = 2.0) containing NaCl, KCl, $NaHCO_3$ and pepsin. The mixture digested at 37 °C for 90 min. The distilled water containing 2% bile was added and the mixture adjusted to pH = 8 with NaOH. After incubation for 10 min at 37 ºC in anaerobic condition, pancreatin was added to final concentration of 1%, followed by incubation for 12 min at 37 °C in anaerobic condition. The extracts were then centrifuged at 5000 g for 5 min, the liquid was evaporated at 60 ºC under vacuum, and the precipitate was freeze-dried. One group of precipitate was resuspended in pure methanol and extracted as previously described. Another group was resuspended in water and extracted to obtain aqueous extracts.

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Phenolic content/FC reducing capacity assay. Total phenol content was determined by a Folin-Ciocalteu (FC) assay (3), using gallic acid (GA) as a standard. Total phenolic content was expressed as GA equivalents (mg of GA g^{-1} samples).

Statistics. All the experiments were carried out in triplicate and data were expressed as means ± standard deviations. The data were subjected to ANOVA and Duncan's multiple range tests using SPSS 19 software to determine significant differences at $p < 0.995$.

Table 1. Phenolic content/FC reducing capacity of seed flour extracts from grass pea landraces (mg of gallic acid g^{-1} sample); values are means of three replications ($N = 3 \pm SD$); values marked by the same small case letter are not significantly different at $p < 0.05$ (Duncan test)

Landrace	Methanolic extracts	Cooked	Cooked and digested
L1	0.27 ± 0.03a	0.72 ± 0.04b	0.89 ± 0.01c
L2	0.30 ± 0.02a	0.79 ± 0.02b	0.84 ± 0.04b
L3	0.37 ± 0.02a	0.91 ± 0.06b	1.04 ± 0.13b
L4	0.33 ± 0.01a	0.69 ± 0.02b	0.86 ± 0.05c
L5	0.25 ± 0.01a	0.58 ± 0.01b	0.76 ± 0.05c

Results and discussion

Although the FC assay was not supposed to characterize antioxidant activity, it seems to be the one of the best for a rough determination of antioxidant activity of food samples. It was non-specific for phenols, as the reagent can be reduced by many non-phenolic compounds. This assay was recently proposed for the measurement of total reducing capacity. The phenolic content in methanolic extracts of grass pea seeds flour ranged from 0.25 mg GA g^{-1} for L1 to 0.37 mg GA g^{-1} for L3 (Table 1), but with no significant difference. In general, the landraces with colored seed coat have higher phenolic contents (6). In our case, the seed coat color and phenolic content were correlated - the most pigmented L3 landrace exhibited the highest phenolic conent. There is a report on a negative correlation between seed size and total phenolic content in grass pea (4), but not in our case ($r^2 = -0.14$).

Regarding the fact that legumes are usually cooked for human nutrition, there is an interest in determining the influence of cooking on phenolic content. although there are many contradictory data. Some experiments showed that boiling significantly alters phenolics content, but the changes depended on the type of legume and processing conditions. The results obtained for our cooked extracts showed a consistent increase in phenolic content after cooking. compared to the methanolic extracts (Table 1). The reason for such increase is incomplete extraction of phenolic compounds by the single extraction step that we applied.

From these results we can conclude that cooking enhance the liberation of phenols. Methanolic extracts of cooked and *in vitro* digested seed flour contained in some landraces three times more phenols than methanolic extracts. Statistically significant differences ($p < 0.05$) were observed in all investigated landraces. The increase in phenolic content in cooked and *in vitro* digested extracts over only cooked extracts could be attributed not only to liberation of phenols, but also to liberation of small peptides soluble in methanol. These peptides can interact with FC reagent, resulting in non-specific absorption at 765 nm. Similar results were obtained on soybean, where gastrointestinal extracts showed higher values for total phenols than methanolic extracts.

Conclusion

This study showed that processing legume seed flour by cooking and enzymatic digestion strongly enhanced the release of phenolic compounds. The effects of processing were consistent in majority of investigated grass pea landraces. Methanolic extraction of cooked and digested seeds yielded the highest phenolic content.

Acknowledgments

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Screening for dietary phenolics and antioxidant capacity of faba bean (*Vicia faba*) and vetches (*Vicia* spp.)

by Đorđe MALENČIĆ[1]*, Biljana KIPROVSKI[1], Branko ĆUPINA[1] and Aleksandar MIKIĆ[2]

Abstract: The phenolics in faba bean and vetches may act as antioxidants, thereby reducing the risk of atherosclerosis and coronary heart disease, which can be caused by oxidation of low-density lipoproteins. The results of our study showed that the examined *Vicia* species differed greatly in their phenolics levels, as well as that the phenolics content should be considered as an important feature of *Vicia* species since some of its nutritive and pharmacological effects.

Key words: bioactive compounds, faba bean, phenolics, vetches

Faba bean (*Vicia faba* L.) and vetches (*Vicia* spp.) belong to the family Fabaceae and represent indigenous species and traditional food in some parts of the Balkans. Although primarily grown for their protein and vitamin content and diuretic and lithontriptic properties, they also may be considered a potential biological source of dietary phenolics. The phenolics in faba bean and vetches may act as antioxidants, thereby reducing the risk of atherosclerosis and coronary heart disease, which can be caused by oxidation of low-density lipoproteins (2, 3). For the reasons of alimentary and pharmaceutical purposes, the aim of this study was to select the *Vicia* populations with higher phenolics content and increased antioxidant activity.

The 70% aqueous acetone extracts of ten *Vicia* species, grown as autochthonous populations in different regions of Serbia, were used for determination of total polyphenols, tannins and proanthocyanidins amounts (1). Amounts of total flavonoids were estimated from MeOH : H_2O : CH_3COOH extracts (140 : 50 : 10) (4). Antioxidant activity was evaluated by DPPH-radical scavenging activity assay (5). Correlation between phenolic classes contents and antioxidant activity was established by regression analysis.

The results showed that the examined *Vicia* species differed greatly in their phenolics levels. The content of total polyphenols ranged from 160.2 mg catechin 100 g^{-1} to 608.7 mg catechin 100 g^{-1} , while tannins varied between 26.2 mg catechin 100 g^{-1} and 297.9 mg catechin 100 g^{-1} of dry matter (DM). Flavonoids levels were much lower, up to 0.15 mg rutine 100 g^{-1} DM, while the content of proanthocyanidins ranged from 5.5 mg leucoanthocyanidin 100 g^{-1} DM to 92.4 mg leucoanthocyanidin 100 g^{-1} DM. The DPPH values varied widely between 21.1% and 89.6% of neutralized radicals, which mainly correlated with total polyphenols and tannins contents, with *r* ranging from 0.74 to 1.00 (Fig. 1 and Fig. 2).

The results obtained suggest that the phenolics content should be considered as an important feature of *Vicia* species, as some of its nutritive and pharmacological effects could be attributed to their presence.

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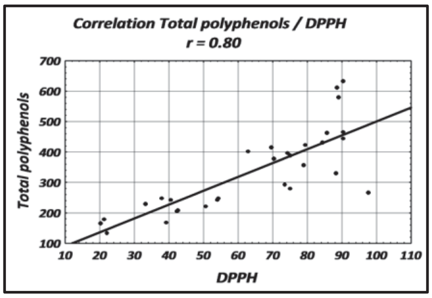

Figure 1. Correlation between DPPH and total polyphenols in *Vicia* species

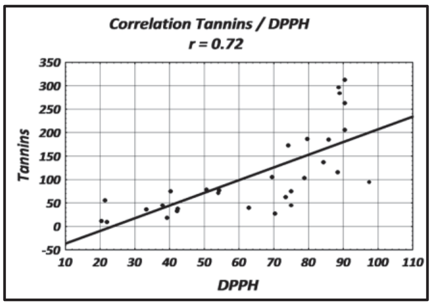

Figure 2. Correlation between DPPH and total tannins in *Vicia* species

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SEELEGUMES: One genuine piece in the jigsaw puzzle of the European legume biodiversity

by Branko ĆUPINA[1]* and Aleksandar MIKIĆ[2]

The project ERA-168 *Sustainable Preservation of Indigenous South East European Legumes and Their Traditional Food and Feed Products* (SEELEGUMES) was carried out within the SEE-ERA.NET Plus programme and under the auspices of the Seventh Framework Programme (FP7) of the European Union during 2011 and 2012.

Coordinated by Dr. Branko Ćupina from the Faculty of Agriculture of the University of Novi Sad, Serbia, SEELEGUMES gathered together 15 full and 4 associate partners from 13 countries, namely Albania, Armenia, Bulgaria, Croatia, Finland, France, FYR of Macedonia, Germany, Greece, Romania, Serbia, Slovenia and Srpska (Bosnia and Herzegovina).

The wild and agricultural floras in all West Balkan countries and other South East European (SEE) regions are rather rich in legume species. Many of these have became and nearly forgotten, remained a part of wild flora despite its great agronomic potential. The strategic goal of this project was to establish an efficient, functional and sustainable network on the genetic resources of annual legume species in the SEE countries and enable it to be fit into the similar existing EU and other international networks.

The SEELEGUMES project consisted of two workpackages (WP):

WP1 *Gathering SEE legumes*, focusing on expeditions, mapping, collecting, *ex situ* and *in situ* preservation and establishing a passport database of the collected accessions of diverse status, such as crop wild relatives, weedy species and local landraces;

WP2 *Describing SEE legumes*, emphasizing a conventional and molecular characterisation of the collected species and an analysis of their nutritional and other values.

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Figure 1. Participants of the First SEELEGUMES Workshop, Banja Luka, Republic of Srpska, Bosnia and Herzegovina, 19-21 October 2011

Figure 2. Participants of the Second SEELEGUMES Workshop, Thessaloniki, Greece, 23-26 September 2012

Along from numerous deliverables, such as two workshops (Fig. 1 and Fig. 2) and numerous scientific and popular publications, the project induced collaborating with eminent individuals from many other institutions, whose precious contributions are also presented in this issue. To rearrange a Beatles' song: *Oh, we've got by with a great help from our friends!*

Please visit the SEELEGUMES project web site at http://polj.uns.ac.rs/~seelegumes/index.html.

Second International Legume Society Conference (ILS2) *2016: Legumes for a Sustainable World*

Tróia, Portugal, 12-14 October 2016

The International Legume Society and the Instituto de Tecnologia Química e Biológica of the Universidade Nova de Lisboa cordially invite you to join us at the **Second International Legume Society Conference, scheduled from 12-14 October, 2016 at Tróia resort, in the vicinity of Lisbon, Portugal.**

In a world urgently requiring more sustainable agriculture, food security and healthier diets the demand for legume crops is on the rise. This growth is fostered by the increasing need for plant protein and for sound agricultural practices that are more adaptable and environmentally sensitive. Food, feed, fiber and even fuel are all products that come from legumes – plants that grow with low nitrogen inputs and in harsh environmental conditions. The Second Legume Society Conference will be held during **2016 - the United Nations' International Year of Pulses. The goals of this UN International Year include: the encouragement of connections throughout the food chain that would better utilize pulse based proteins; increase global production of pulses; better utilization of crop rotations; and to address challenges in the trade of pulses.**

The conference will address the following themes: **Legume Quality and Nutrition; Farming Systems/Agronomy; Abiotic and Biotic Stress Responses and Breeding; Legume Genetic Resources; and New "Omics" Resources for Legumes. The health and environment benefits, as well as, the marketing of legumes will be transversal topics throughout the conference. Special attention will be given to foster the interaction of researchers and research programs with different stakeholders including farmers and farmer associations, seed/feed and food industries, and consumers. For this, the conference will also be the site of the Final Meeting of the EU-FP7 ABSTRESS project, the Annual Meeting of EU-FP7 LEGATO project; and final dissemination events of EU-FP7-ERANets MEDILEG and REFORMA. The results and conclusions from these four important research programs will be shared with conference attendees.**

Please join us in beautiful Tróia, Portugal from 12-14 October, 2016! Plan now to include the Second ILS Conference in your busy agenda. Kindly share this information with any colleagues dealing with legumes.

Diego Rubiales, on behalf of the Scientific Committee
Pedro Fevereiro, Carlota Vaz Patto and Susana Araújo, on behalf of the Organizing Committee

Local Organizers
The Instituto de Tecnologia Química e Biológica /
Universidade Nova de Lisboa (ITQB/UNL)
will be responsible for the organization of the Conference,
in cooperation with the International Legume Society.
The official language of the Conference will be the English.

Conveners
Pedro Fevereiro – psalema@itqb.unl.pt
Carlota Vaz Patto - cpatto@itqb.unl.pt
Susana Araújo - saraujo@itqb.unl.pt

Scientific Coordinator
Diego Rubiales

Local Organizer Committee
Ana Barradas – Fertiprado
Carlota Vaz Patto - ITQB/UNL
Eduardo Rosa – Universidade de Trás-os-Montes e Alto Douro
Isabel Duarte – Instituto Nacional de Investigação Agrária e Veterinaria (INIAV)
Manuela Costa – Universidade do Minho
Manuela Veloso – INIAV
Marta Vasconcellos – Escola Superior de Biotecnologia, Universidade Católica
Nuno Almeida - ITQB/UNL
Pedro Fevereiro –ITQB/UNL
Sofia Duque – ITQB/UNL
Susana Araújo - ITQB/UNL
Susana Leitão - ITQB/UNL

Scientific Committee
Adrian Charlton: FERA, York, UK
Aleksandar Mikić: Institute of Field and Vegetable Crops, Novi Sad, Serbia
Charles Brummer: University of California, Davis, USA
Diego Rubiales: Institute for Sustainable Agriculture, CSIC, Spain
Fred Stoddard: University of Helsinki, Finland
Gerard Duc: INRA-Dijon, France
Judith Lichtenzveig: Curtin University, Australia
Kevin McPhee: USDA-ARS, Washington State University, USA
Michael Abberton: IITA, Nigeria
Noel Ellis: ICRISAT, India
Paolo Annichiarico: CRA-FLC, Lodi, Italy
Pedro Fevereiro: ITQB/UNL, Portugal
Stephen E. Beebe: CIAT, Colombia
Shiv Kumar Agrawal: ICARDA, Syria
Tom Warkentin: University of Saskatchewan, Canada

Venue

The conference will be held at Troia Design Hotel (http://www.troiadesignhotel.com) in Tróia in the vicinity of Lisbon, Portugal. Tróia is a beautiful sand peninsula dividing the Sado River from the Atlantic Ocean.

The nearest airport is the Lisbon International Airport, about 50 Km away. Shuttles will be made available from and to Lisbon International Airport.

During the period of Roman occupation, date from the 1st century to the 6th century AD, Tróia was an island of Sado delta, called Ácala Island.

Sado Estuary Nature Reserve, where dolphins swim, and the Serra da Arrábida Natural Park, where a full developed Mediterranean forest can be seen, are two of the main natural attractions nearby Tróia peninsula.

The Tróia Golf Championship Course is considered the best course in Portugal in the categories of difficulty and variety. It also stands in 20th place in the list of the best golf courses in Europe drawn up by the Golf World magazine.

Tentative Programme

October 11th, 2016
Morning-Afternoon: Satellite projects meetings
Evening: Conference Registration

October 12th, 2016
08:00 Registration; 09:00 Welcome addresses;
09:45 Session 1 (Opening plenary)
11:15 Coffee break
11:45 Sessions 2 & 3
12:45 Lunch
14:30 Sessions 2 & 3
16:30 - 19:00 Sessions 4 & 5
20:45 Third International Legume Football Cup

October 13th, 2016
9:00 Session 6
11:15 Coffee break
11:45 Sessions 7 & 8
12:45 Lunch
14:30 Sessions 7 & 8
16:00 Coffee break
16:30 International Legume Society Assembly
20:45 Third International Legume Football Cup

October 14th, 2016
09:00 Session 9
11:15 Coffee break
11:45 Sessions 10 & 11
12:45 Lunch
14:30 Sessions 10 & 11
16:00 Coffee break
16:30 Session 12 (Closing plenary)
20:00 Farewell Dinner

October 15th, 2016
Satellite projects meetings

Bem-vindo ao Tróia, amigos dos leguminosas!

18th Symposium of the European Grassland Federation
Grassland and Forages in High Output Dairy Farming Systems
Wageningen, the Netherlands, 15-18 June 2015
http://www.europeangrassland.org/events.html

EUCARPIA European Association for Research on Plant Breeding
Europäische Gesellschaft für Züchtungsforschung
Association Européenne pour l'Amélioration des Plantes

Symposium of the Protein Crops Working Group, Protein Crops Section, and Spanish Association for Legumes
Plant Proteins for the Future
Pontevedra, Spain, 4-7 May 2015
http://www.symposiumproteincrops.org/

Symposium of the Fodder Crops and Amenity Grasses Section
Breeding in a World of Scarcity
Ghent, Belgium, 14-17 September 2015
http://www.eucarpia-fcag2015.be/

UN International Year of Pulses - 2016
http://www.iyop.net/en/

IN MEMORIAM

Professor Reid G. Palmer

Dr. Reid Palmer, born on June 21, 1941, in Pemberville, Ohio, a USDA Research Geneticist at the Iowa State University and affiliate Professor of Agronomy after retiring in 2012, passed away in Ames, Iowa, on August 17, 2014.

Reid has been giving his precious support to the idea of global legume science networking, conceived within the Grain Legume Technology Transfer Platform (GL-TTP), in which he actively participated as one of the most remarkable representatives of the international soybean research community.

The International Legume Society and the *Legume Perspectives* journal editors and readers most sincerely commiserate with Reid's widow Nuray and his numerous cousins, friends and colleagues.

May our dear Reid tend his beloved soybean forever and in peace on the fields of heaven.

Professor Reid G. Palmer giving his presentation on hybrid soybean at the Second GL-TTP Workshop, Novi Sad, Serbia, November 28, 2008

LEGUME PERSPECTIVES

Sponsorship list

LEGUME SOCIETY
www.ils.nsseme.com

INSTITUTE OF FIELD AND VEGETABLE CROPS, NOVI SAD, SERBIA
www.nsseme.com

REGIONAL PROGRAMME FOR COOPERATION WITH SOUTHEAST EUROPE
http://www.see-era.net/

UNIVERSITY OF NOVI SAD
FACULTY OF AGRICULTURE, NOVI SAD, SERBIA
http://polj.uns.ac.rs/

SPANISH MINISTRY OF SCIENCE AND INNOVATION
SPANISH NATIONAL RESEARCH COUNCIL
www.csic.es

Want to help the legume research network in Europe and worldwide?

Support Legume Society and become its sponsor today!

legume.society@gmail.com