

## Genetic diversity of *Lathyrus sativus* L. collection and characteristics of seeds produced in Slovenia and Serbia

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### Abstract

Grass pea (*Lathyrus sativus* L.) is a less common and nearly forgotten crop in many countries, including Slovenia and Serbia. In the present study, genetic analysis and seed characteristics of the collection of 21 grass pea accessions from Southeast Europe were examined. The collection was produced in an open-field experiment in two countries/ locations, i.e. Slovenia/Ljubljana – Jablje and Serbia/ Novi Sad – Rimski Šančevi, during the 2019 growing season. To analyse genetic diversity, specific high polymorphic SSR markers were applied. Initial screening at 12 species-specific SSR loci using binary data revealed great genetic diversity among the grass pea accessions within a collection and three genetic groups were formed. Differences in seed size were measured using descriptors for seed length, width and thickness, and 100-seed weight. Furthermore, several seed colour characters using IBPGR descriptors were visually assessed. The average seed length, width, and thickness of the seeds in the collection were 9.0 mm, 8.3 mm, and 5.4 mm, respectively, while 100-seed weight ranged considerably from 11.7 g to 38.9 g. The highest differences between growing location were seen for 100-seed weight where the coefficient of variability reached 23.5%. The examined grass pea accessions had less monochromatic (one colour) and more colourful seeds, with two or three colours. Based on the seed coat colour accessions were classified into several groups, the most numerous being yellow-white and yellow-green seeds. The data obtained will be the basis for describing *Lathyrus sativus* genetic resources in databases. Further genetic studies such as next-generation sequencing are suggested.

*Key words:* descriptor; genetic diversity; grass pea; seed size; SSR marker

## Introduction

Grass pea (*Lathyrus sativus* L.) belongs to Fabaceae (Leguminosae) family and is the only species from the genus *Lathyrus* that is widely cultivated as a food crop (Shiferaw et al., 2012). It is a robust self-pollinated legume that can survive in harsh climate conditions and be survival food during drought-triggered famines. Grass pea is a typical orphan or underutilised crop although it plays a significant role in nutrition security in many developing countries (Lambein et al, 2019). The domestication of grass pea began in the Balkan peninsula as the result of its expansion from the Near East that is the probable origin of domestication (Asadova et al., 2020; Hobdari and Gixhari, 2017; Chowdhury and Slinkard, 2000). Using the pyrosequencing techniques, a large-scale microsatellite approach was developed for *Lathyrus sativus* (Yang et al., 2014). SSR (Simple Sequence Repeats) markers can potentially make a significant contribution to the genomics enabled improvement of grass pea. Compared to other agronomically important plant species, SSR markers are the most frequently employed to assess intra- and inter-specific genetic diversity and its genetic structure (Pipan and Meglič, 2019; Pipan et al., 2018; Sinkovič et al., 2017).

Plants of grass pea are herbaceous annuals with upright, 20-90 cm high stems, that often lie down. The flowers are solitary and axillary, usually reddish-purple, pink, blue, or white coloured. A few centimetres long oblong pods typically contain from two to five seeds that are white, greyish-brown, or yellowish, and usually marbled or dotted (Girma and Korbu, 2012). Detailed descriptors for *Lathyrus* spp. were developed by the International Plant Genetic Resources Institute (IBPGR) with the purpose of conservation and use for the benefit of present and future generations (Pandey, 2000). The *Lathyrus sativus* collection from several Southeast European gene banks was established and cultivated within research activities of the two countries, i.e. Slovenia and Serbia. A genetic diversity study was performed along with measures of seed characteristics from both production locations. Genetic association between the accessions was established according to species-specific SSR markers using binary data. Multiplication and evaluation of the unexplored *Lathyrus sativus* collection is the initial step for future utilization and breeding proposes.

## Material and Methods

The grass pea collection comprised 21 accessions obtained from six Southeast European national plant gene banks: from Portugal (3 accessions; ISOP1177, ISOP1189, ISOP1190), B&H (6 accessions; GB00954, GB00999-GB01003), Romania (2 accessions; SVGB19385, SVGB20803), Serbia (7 accessions; KL2, KL4, KL5, KL7-KL10), Bulgaria (2 accessions; BGR40415, BGR43334), and Slovenia (1 accession; SRGB5486). This collection was sown and cultivated in the experimental fields in Slovenia (Jablje; 302 m a.s.l.; 46.08°N 14.33°E) and

Serbia (Rimski Šančevi; 84 m a.s.l.; 45.20°N 19.51°E). The field trial was performed in 2019 according to the established production technique in each country. The climate data obtained from the national meteorological data service centres for two experimental sites during the growing season are presented in Fig. 1.

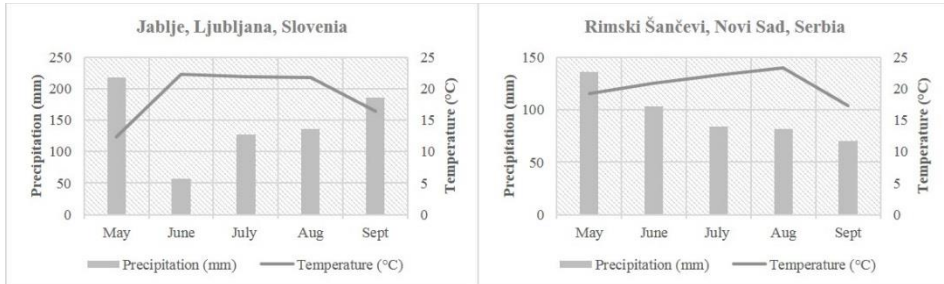


Fig. 1. Climate data for experimental sites during the growing season of 2019

The field experiment was carried out under open-field conditions with 10 plants/ accessions at each location. In the initial phase of growth young leaves from four individual plants/ accessions were sampled for the genetic analysis. DNA extraction was performed on a MagMax isolation robot using BioSprint96 Plant kit (Qiagen) under optimized manufacturer’s instructions. To distinguish *Lathyrus* spp. accessions, a genotyping procedure by Wang et al. (2015) was performed using 12 SSR markers (G5, G9, G26, G67, G157, G17922, G18078, G18109, G18308, G18549, G19207, G19337, G15771, G18200, and G17243). The optimal PCR mixtures, applied chemistry, and PCR conditions for each specific marker modifying with M13 label were used as described by Pipan and Meglič (2019); Meglič and Pipan (2018). From the agarose gel results (1,4%; EtBr staining, 100 bp DNA ladder), a binary matrix was performed for the combination of 12 analyzed loci. Genetic associations of grass pea accessions were analyzed according to the Nei’s standard genetic distance (Nei, 1972) and UPGMA (Unweighted Pair Group Method with Arithmetic mean) clustering method calculated in FreeTree software (Pavlíček et al., 1999) and visualized using TreeView (Page, 1996).

Seed characteristics were measured using air-dried grains of individual grass pea accessions produced within each country. The following quantitative characters were evaluated: seed length (mm), seed width (mm), seed thickness (mm), and 100-seed weight (g). These were measured on 10 individual representative seeds of each sample using digital Vernier calliper readings to 0.1 mm. 100-seed weight was determined in four replicates using an electronic laboratory-scale weight to 0.01 g. The qualitative characters were the number of colours, seed coat colour, seed coat pattern, and seed coat pattern colour, and were evaluated visually using IBPGR descriptors. Results of seed characteristics were analysed using the MS Excel program where the statistics included mean, minimum, maximum, standard deviation (SD), and coefficient of variation (CV).

## Results and Discussion

Genetic association results, i.e. the Nei's standard genetic distance and UPGMA clustering method, of analysed grass pea samples within the genus *Lathyrus* spp. are presented in the form of a dendrogram in Fig. 2. Three specific genetic groups were formed. The accession GB1001 stands out the most based on genetic background, followed by GB1001, KL4, GB00954, GB01000, and ISOP1190, which together form the first diverse group. The second group of genetic resources is the largest and consists of 11 genetic resources (ISOP1190, GB00999, GB01002, SVGB19385, SVGB20803, KL2, KL5, KL7, KL8, KL9 and BGR43334). The third genetic group includes five accessions, namely ISOP1177, GB01003, KL10, BGR40415, and SRGB5486. Compared with worldwide *Lathyrus* spp. germplasm, a model-based population structure analysis divided the germplasm resources into three subgroups: the relative species, the grass pea from Asia, and the grass pea from Europe and Africa. Within the cultivated species, European and African accessions were aggregated together and partially overlapped with some Asian accessions due to the possibility of flow between the two subgroups (Wang et al. 2015).

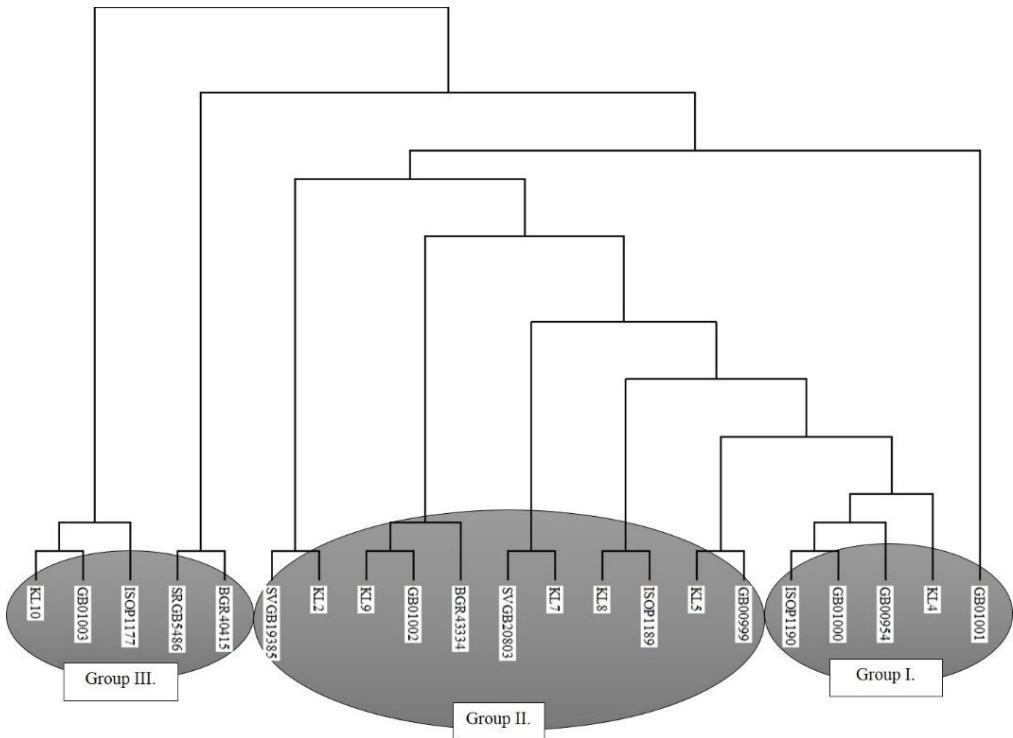


Fig. 2. Genetic association of grass pea samples within the collection.

A total of 8 quantitative and qualitative descriptors considering the seeds were evaluated for the collection of 21 grass pea accessions originating from Southeast Europe. Table 1 summarises the statistics of the 4 quantitative seed characteristics for this collection for an individual country and as the means. The means for seed size characteristics produced in Slovenia were recorded for seed length 9.4 mm, seed width 8.7 mm, seed thickness 5.4 mm, and 100-seed weight 26.0 g. For the seeds produced in Serbia, the means were as follows: seed length 8.7 mm, seed width 7.9 mm, seed thickness 5.4 mm, and 100-seed weight 22.4 g. The results showed that the seeds of grass pea accessions grown in Slovenia were rather longer and wider but not thicker, while the seed weight was to some extent higher. Regardless of the production country, the highest coefficient of variation was calculated for the 100-seed weight (23.5%), followed by seed width (12.6%), and seed length (11.1%).

Tab. 1. Summary statistics for numeric seed characteristics in the grass pea collection

<b>Numeric characteristic</b>	<b>Country of production</b>	<b>Units</b>	<b>Min - Max</b>	<b>Average ± SD</b>	<b>CV (%)</b>
Seed length	Slovenia		7.6 - 11.4	9.4 ± 1.0	10.1
	Serbia	mm	7.2 - 11.3	8.7 ± 0.9	10.3
	<b>Average</b>		<b>7.2 - 11.4</b>	<b>9.0 ± 1.0</b>	<b>11.1</b>
Seed width	Slovenia		6.7 - 10.8	8.7 ± 1.1	12.2
	Serbia	mm	6.4 - 10.4	7.9 ± 0.9	11.4
	<b>Average</b>		<b>6.4 - 10.8</b>	<b>8.3 ± 1.1</b>	<b>12.6</b>
Seed thickness	Slovenia		4.9 - 6.0	5.4 ± 0.3	5.8
	Serbia	mm	4.2 - 5.9	5.3 ± 0.5	8.7
	<b>Average</b>		<b>4.2 - 6.0</b>	<b>5.4 ± 0.4</b>	<b>7.4</b>
100-seed weight	Slovenia		18.5 - 34.4	26.0 ± 4.5	17.2
	Serbia	g	11.7 - 38.9	22.6 ± 6.3	27.8
	<b>Average</b>		<b>11.7 - 38.9</b>	<b>24.3 ± 5.7</b>	<b>23.5</b>

SD - standard deviation; CV - coefficient of variation.

The frequency distributions for four characteristics related to the seed colour are shown in Fig. 3. The observed grass pea genetic resources had a different number of colours within the individual accession: one (5 accessions), two (9 accessions), and three colours (7 accessions). The most common seed coat colours were yellow-white, yellow-green, brown, and green mottled. The seed coat pattern was absent for 11 accessions and present as a mixture for 10 accessions. The most common seed coat pattern colours were green, brown, and black.

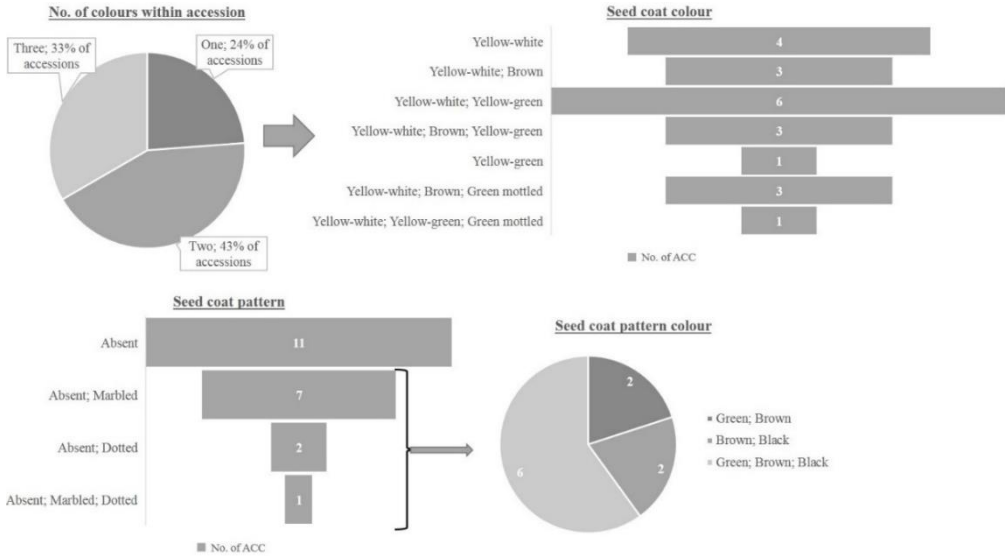


Fig. 3. Frequency distributions for descriptive characteristics related to seed colour in the grass pea collection

## Conclusion

The grass pea collection from Southeast Europe showed high genetic diversity and seed characteristics variability was not influenced by the country of genetic origin. Three specific genetic groups were formed based on the genetic analysis using 12 SSR markers. The most variable characteristics between seeds produced in Slovenia and Serbia were found for seed size, 100-seed weight, and seed coat colour. Grass pea accessions within the collection had less monochromatic and more colourful seeds, with two or three colours. The most represented seed coat colours were yellow-white and yellow-green.

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# Генетска разноликост колекције *Lathyrus sativus* L. и особине зрна произведеног у Словенији и Србији

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## Сажетак

Састрица или грах пољак (*Lathyrus sativus* L.) је рјеђа и готово заборављена култура у многим земљама, укључујући Словенију и Србију. У овом раду урађене су генетске анализе и проучене су особине сјемена у оквиру колекције од 22 узорка састрице из југоисточне Европе. Колекција је узгајана на отвореном пољу у обе земље/локације, тј. Словенији/Љубљана – Јабље и Србији/Нови Сад – Римски Шанчеви, током вегетације 2019. године. За анализу генетске разноликости примијењено је дванаест специфичних високо полиморфних SSR маркера. Почетни преглед микросателитним маркерима открио је велику генетску разноликост међу узорака састрице унутар колекције. На основу тога узорци су се раздвојили у три специфичне групе. Разлике у величини зрна приказане су преко дужине, ширине, дебљине и масе 100 зрна према дескриптору за анализу генетских ресурса састрице. Поред тога, визуелно је процијењено неколико особина боје зрна. Просјечна дужина, ширина и дебљина зрна у колекцији износила је 9,0 mm, 8,3 mm и 5,4 mm, док је маса 100 зрна била у распону од 11,7 g до 38,9 g. Највећи утицај мјеста гајења забиљежен је код масе 100 зрна, а коефицијент варијабилности достигао је 23,5%. Испитивани узорци састрице су мањим дијелом имали једнобојна зрна, а већим дијелом шарена, од двије или три боје. На основу боје сјеменог омотача сврстали су се у више група, а најбројнија су била жутобијела и жутозелена зрна. Добијени подаци биће основа за описивање генетичких ресурса *Lathyrus sativus* у базама података и предлажу се даљња генетска истраживања попут секвенцирања слједеће генерације.

*Кључне ријечи:* дескриптор, генетска разноликост, састрица, величина зрна, SSR маркер.

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