Вавиловский журнал генетики и селекции. 2019;23(6):667-674
DOI 10.18699/VJ19.539

Comparative analysis of wild and cultivated *Lathyrus* L. spp. according to their primary and secondary metabolite contents

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Species of the genus *Lathyrus* L. are known as forage and medicinal plants, widely used in traditional medicine and homeopathy. The content of protein, essential amino acids and carotene in their green biomass is higher than in other annual leguminous plants traditionally cultivated in Russia. Until now, the requirements for the crop's quality were reduced to a high content of protein and dry matter in seeds and herbage. In-depth biochemical analysis of accessions from the collection of plant genetic resources will significantly improve selection of source materials for breeding. Such tasks can be solved using gas chromatography with mass spectrometry in plant diversity studies. In view of the above, our goal was to analyze organic acids, free amino acids and secondary metabolites in green biomass of *Lathyrus* to facilitate comprehensive assessment of its forage and pharmacological value. We analyzed 32 accessions of *Lathyrus sativus* L., *L. tuberosus* L., *L. sylvestris* L., *L. vernus* (L.) Bernh., *L. latifolius* L. and *L. linifolius* (Reichard) Bassler from the collection of the Vavilov Institute (VIR). The studied *Lathyrus* accessions had significant interspecific and intraspecific variability both in the composition (presence) and number of the identified compounds. The analysis of plants across different years confirmed that biochemical parameters depended on weather conditions. The colder and drier conditions of 2012 contributed to the accumulation of organic acids (mean: 890 mg/100 g), free amino acids (mean: 201.59 mg/100 g), and secondary metabolites (mean: 84.14 mg/100 g). The range of variability for organic acids ranged from 140 to 2140, for free amino acids from 11.8 to 610, and for secondary metabolites from 4.4 to 224.6 mg/100 g. Grass pea accessions with high organic acid, free amino acid and secondary metabolite contents were identified: k-900 (Colombia) for organic acids (2140, 610 and 178 mg/100 g); k-51 (Georgia) and k-959 (Afghanistan) for free amino acids (401.29 and 540.63 mg/100 g); k-893 (Eritrea) for secondary metabolites (199.39 mg/100 g), etc. They can serve as source material for the development of cultivars for different uses (forage and medicinal).
Key words: *Lathyrus* L.; wild species; varieties; green mass; gas chromatography; genetic resource; polymorphism of characters.

For citation: Solovyeva A.E., Shelenga T.V., Shavarda A.L., Burlyaeva M.O. Comparative analysis of wild and cultivated *Lathyrus* L. spp. according to their primary and secondary metabolite contents. Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding. 2019;23(6):667-674. DOI 10.18699/VJ19.539

Сравнительный анализ диких и культурных видов чины (*Lathyrus* L.) по содержанию веществ первичного и вторичного метаболизма

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Виды рода *Lathyrus* L. известны как кормовые и лекарственные растения, используемые в народной медицине и гомеопатии. Содержание белка, незаменимых аминокислот и каротина в зеленой массе чины выше, чем у других однолетних зернобобовых растений, традиционно культивируемых в России. До настоящего времени требования к качеству культуры сводились к высокому содержанию белка и сухого вещества в семенах и вегетативной массе. Углубленный биохимический анализ образцов из коллекции генетических ресурсов растений существенно улучшит отбор исходного материала для селекции. Изучение растительных ресурсов с использованием метода газовой хроматографии с масс-спектрометрией позволяет решить подобные задачи. В связи с вышесказанным нашей целью было исследование органических кислот, свободных аминокислот и соединений вторичного метаболизма в зеленой массе чины для всесторонней оценки ее кормовой и фармакологической ценности. Анализировали 32 образца *Lathyrus sativus* L., *L. tuberosus* L., *L. sylvestris* L., *L. vernus* (L.) Bernh., *L. latifolius* L., *L. linifolius* (Reichard) Bassler из коллекции Всероссийского института генетических ресурсов рас-

тений им. Н.И. Вавилова. Изученные образцы *Lathyrus* обладали значительной межвидовой и внутривидовой изменчивостью как по составу (наличию), так и по количеству идентифицированных веществ. Анализ растений в разные годы подтвердил зависимость биохимических показателей от погодных условий. Более холодные и сухие условия 2012 г. способствовали накоплению органических кислот (среднее – 890 мг/100 г), свободных аминокислот (среднее – 201.59 мг/100 г) и соединений вторичного метаболизма (среднее – 84.14 мг/100 г). Диапазон изменчивости органических кислот составил от 140 до 2140, свободных аминокислот – от 11.8 до 610, соединений вторичного метаболизма – от 4.4 до 224.6 мг/100 г. Выделены образцы чины посевной: с повышенным содержанием органических кислот, свободных аминокислот и соединений вторичного метаболизма – к-900 (Колумбия) (2140, 610 и 178 мг/100 г), свободных аминокислот – к-51 (Грузия) и к-959 (Афганистан) (401.29 и 540.63 мг/100 г), соединений вторичного метаболизма – к-893 (Эритрея) (199.39 мг/100 г) и другие, которые могут служить исходным материалом для создания сортов разного направления использования: кормового и лекарственного.
Ключевые слова: *Lathyrus* L.; дикие виды; сорта; зеленая масса; газовая хроматография; генетические ресурсы; полиморфизм признаков.

Introduction

Researching the chemical composition of cultivated plants and their wild relatives is of crucial importance in both theoretical and practical contexts. Still vital is the question of seeking new plants that may serve as sources of bioactive compounds with remedial properties, and introducing them into agricultural practice. *Lathyrus sativus* L., *L. pratensis* L. and *L. tuberosus* L. have been most comprehensively studied with regard to their chemical composition and pharmacological characteristics. Biological activity of several chemical components has been analyzed in *L. sylvestris* L., *L. vernus* (L.) Bernh. and *L. niger* (L.) Bernh. Wide pharmacological demand for peavines is induced by their macro- and microelement composition as well as the presence of flavonoids and a complex set of essential amino acids (Plant Resources of the USSR, 1987, 2011; Zaichikova, 2002a, b). *Lathyrus* spp. are also known as forage plants, outstanding for their high protein content in seed and herbage (Pavlova, 2001; Burlyaeva et al., 2012).

When peavine cultivars are developed, primary attention is given to yield, resistance to biotic and abiotic environmental factors, and the traits that secure their value as animal feed. Of late, the in-depth biochemical analysis has been used to solve numerous problems, such as the assessment of cultivar specificity of seeds (Smolikova et al., 2015; Loskutov et al., 2016) and study of the effects of domestication processes and environmental stressors (Konarev et al., 2015; Puzanskiy et al., 2015), etc. Modern gas chromatography (GC) with mass selective detection (MSD) techniques make it possible to examine plant biochemical composition varying with time and growing conditions (Konarev et al., 2015; Puzanskiy et al., 2015).

The collection of *Lathyrus* spp. at VIR contains 2055 accessions; they belong to more than 50 species. Widely represented in the collection are landraces, cultivars bred domestically and abroad, and their wild relatives from European Russia, Europe, Asia, Africa, Australia, etc. For many years, accessions from the collection have been studied mostly for protein content in seed and herbage. No in-depth biochemical research has been undertaken until now to study wild relatives and cultivated forms of *Lathyrus* spp. We have also failed to find any scientific publications where variations in the biochemical composition of peavine green biomass were analyzed under different weather conditions.

When peavine cultivars are developed for feed, food and medicinal purposes, emphasis is placed by breeders on raising the content of nutrients and secondary metabolites in green biomass. In view of this, the goal of this research was to study the biochemical composition of *Lathyrus* green biomass for comprehensive assessment of its forage and pharmacological value. Research objectives included analyzing inter- and intraspecific polymorphism of biochemical characters in *Lathyrus* spp., assessing the effect of weather conditions on the analyzed parameters, and identifying most promising accessions for breeding practice.

Materials and methods

The experiment employed 32 accessions of six *Lathyrus* species from the VIR collection: grass pea (*L. sativus*), flat pea (*L. sylvestris*), spring pea (*L. vernus*), heath pea (*L. linifolius*), everlasting pea (*L. latifolius*), and tuberous pea (*L. tuberosus*), grown in 2012 and 2013 in fields of the Pushkin Laboratories of VIR in the vicinities of St. Petersburg. Plants were grown on one-meter plots (1 m^2) in two replications (the plots were situated in one and the same place), on naturally irrigated soddy-podzolic soil. Standard agricultural practices for row crops adopted at VIR (Vishnyakova et al., 2010) were applied. Weather conditions in the growing seasons were contrasting. In 2012, the total of active temperatures was 1885.0 °C, with total precipitation of 340.7 mm. In 2013, the total of active temperatures was observed to rise to 2474.3 °C, with total precipitation going up to reach 646.4 mm.

The plants were harvested in the early pod ripening phase. Fresh green biomass of plants was analyzed (five plants from each accession: stems, leaves and pods; in three analytical replications).

A sample of 10 g was weighed, homogenized with an adequate amount of ethanol, and infused for 30 days at 5–6 °C. The extract (200 μL) was vaporized to dry residues on a CentriVapConcentrator (Labconco, USA). The solid residue was silylated with bis(trimethylsilyl)trifluoroacetamide. The silylated compounds were separated on an HP-5MS capillary column (5 % phenyl methylpolysiloxane; 30.0 m, 250.00 μm, 0.25 μm) using the Agilent 6850 chromatography system with a quadrupole mass spectrometry detector Agilent 5975B VL MSD (Agilent Technologies, USA). Conditions of chromatographic analysis: helium flow rate through the column 1.5 mL/min, column heating program +70 °C to +320 °C with the heating rate 4 °C/min, MSD temperature + 250 °C, injector temperature + 300 °C, sample volume 1 μL, internal standard: tricosane in pyridine (1 μg/μL).

The spectra were processed using UniChrom and AMDIS software, NIST 2010 mass spectra libraries, and Science parks of the St. Petersburg State University and the Komarov Botanical Institute. The results were evaluated with MS Excel 2007 and Statistica 7.0 programs. The effect of environmental conditions on the expression of biochemical characters was assessed using one-way analysis of variance (ANOVA) with Fisher's LSD-test. The effect size of the factor's influence (η^2, %) according to Fisher was calculated by Equation (1) (Ivanter, Korosov, 2003):

$$\eta^2 = \frac{SS_{factor}}{SS_{total}} \times 100\ \%,$$

where η^2, % is the effect size of the factor influence; SS_{factor} is the sum of squared deviations for the factor; SS_{total} is the total sum of squared deviations.

Results

The biochemical composition analysis of the *Lathyrus* green biomass samples revealed about 300 components. This paper discusses part of the data obtained (Table 1, Suppl. material 1[1]).

Organic acids. In 2012, the content of organic acids in the green biomass samples of *Lathyrus* averaged 844.72 mg/100 g; depending on the genotype, this parameter varied from 136.27 to 2137.37 mg/100 g. In 2013, the acid content went down to 333.77 mg/100 g, and different accessions varied within 215.37–544.24 mg/100 g (Fig. 1). For grass pea accessions, the mean content of organic acids in 2012 was 890 mg/100 g, with the range of variation from 300 to 2140 mg/100 g. In 2013, a decrease to 3120 mg/100 g was observed in the mean values, and the range narrowed to 220–430 mg/100 g. In the flat pea group, the mean content of organic acids was 590 mg/100 g in 2012, and 480 mg/100 g in 2013. For *L. sylvestris*, this parameter was relatively stable in different years, unlike *L. sativus*, which demonstrated a drop of the organic acid content to 570 mg/100 g in 2013. The organic acid contents in heath, everlasting, and spring pea accessions were somewhat higher: 610, 670, and 640 mg/100 g, respectively.

The lowest level of organic acids was recorded for tuberous pea accessions (140 mg/100 g). The highest acid content in 2012 was observed in grass pea accession k-900 (Colombia): 2140 mg/100 g; and in 2013, in flat pea accession k-591293 (Germany): 540 mg/100 g.

Organic acids were represented mostly by malic (Krebs cycle) and threonic (ascorbic acid oxidation product) acids; their respective contents were 156.22 and 120.52 mg/100 g. Glyceric and citric acids ranked next: their contents were 90.63 and 61.32 mg/100 g, respectively. Dehydroabietic, phosphoric, oxalic, lactic, and fumaric acids respectively averaged 30.42, 27.14, 25.90, 14.61, and 10.65 mg/100 g. The contents of succinic, mesoxalic, quinic, erythronic and gluconic acids did not exceed 10 mg/100 g (9.17, 6.87, 6.58, 6.34, and 5.82, respectively). Respective amounts of tartaric, ribonic and pipecolic acids were 2.77, 2.04, and 1.85. Accumulation of other acids (benzoic, nicotinic, maleic, 4-hydroxybenzoic, azelaic, saccharic, protocatechuic, shikimic, galacturonic, caffeic, sinapic, abietic and neochlorogenic) never exceeded 0.7 mg/100 g, while the respective amounts of galacturonic and saccharic acids were 0.29 and 0.06 mg/100 g.

Table 1. The contents of amino acids, organic acids, and secondary metabolites in the green biomass of some *Lathyrus* species (mg/100 g wet weight)

Species	Organic acids	Amino acids	Secondary metabolites
L. sativus	610.0 ± 64.0*	208.6 ± 16.8	59.2 ± 8.0
	220.0–2140.0**	41.0–610.0	4.4–199.4
L. sylvestris	520.0 ± 84.6	205.1 ± 45.9	43.1 ± 14.3
	340.0–830.0	67.5–340.3	15.2–79.8
L. vernus	640.0 ± 94.9	28.0 ± 2.9	28.1 ± 6.9
	540.0–730.0	25.1–31.0	21.8–35.7
L. linifolius	830.0 ± 89.5	11.8 ± 6.2	71.6 ± 29.7
	250.0–1050.0	3.1–14.8	32.2–100.1
L. latifolius	670.0 ± 75.6	136.3 ± 38.3	132.4 ± 52.1
	100.0–850.0	42.3–157.9	81.5–224.6
L. tuberosus	140.0 ± 98.9	72.9 ± 25.3	6.4 ± 9.8
	2.0–380.0	24.8–95.4	1.1–19.5

* Arithmetic mean ± standard error of the mean;
** Range (min–max).

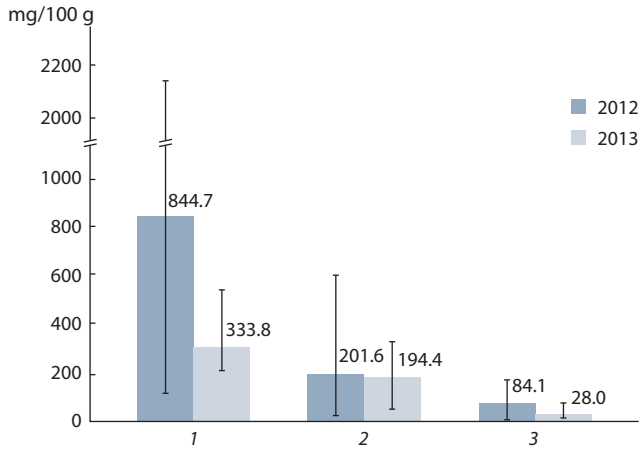

Fig. 1. The contents of organic acids, free amino acids, and phenolic compounds in the green biomass of *Lathyrus* L. in different years of cultivation (mean values, min–max, mg/100 g wet weight).

1 – organic acids; *2* – free amino acids; *3* – secondary metabolites.

Amino acids. The green biomass of *Lathyrus* accessions was found to contain 20 free amino acids, including eight essential ones (see Suppl. material 1). The mean content of free amino acids in 2012 was 201.59 mg/100 g (see Fig. 1); the variation being from 11.75 to 610.00 mg/100 g. In 2013, the amino acid content was slightly lower (194.42 mg/100 g), while the range of variability for this character in different genotypes was within the limits from 40.97 to 340.30 mg/100 g. In 2012, the highest free amino acid contents were registered in grass pea accessions (230.16 mg/100 g), and the lowest in heath pea (11.75 mg/100 g). The same parameters measured

[1] Supplementary Materials 1–2 are available in the online version of the paper: http://www.bionet.nsc.ru/vogis/download/pict-2019-23/appx12.pdf

in other species were as follows: 114.93 mg/100 g in flat pea, 136.28 in everlasting pea, 72.90 in spring pea, and 28.02 in tuberous pea. In 2013, the mean amino acid content in the green biomass of flat pea was higher (265.22 mg/100 g). Grass pea, however, showed the opposite tendency, as this parameter dropped to 185.92 mg/100 g in 2013. Other *Lathyrus* groups showed no significant variations across the two years.

Secondary metabolic compounds. One of the key indicators that reflect antioxidant activity and resistance to the impact of environmental stressors is the content of secondary metabolites, including phenol-containing compounds. The effect of secondary metabolites is directly connected with their concentrations; in this regard, their quantitative analysis is no less important than qualitative one (Spanou et al., 2010).

In the tested green biomass of *Lathyrus* accessions, secondary metabolites were represented by free phenolcarboxylic (PC) acids (mean content 52.52), quinones (0.38), flavonoids (3.49), phenylpropanoids (0.69), and iridoids (0.11), including the identified α-tocopherol (2.14 mg/100 g) (see Suppl. material 1). The mean content of secondary metabolites in the green biomass of *Lathyrus* accessions in 2012 was 84.14 mg/100 g (from 4.45 to 199.39) (see Fig. 1). In 2013, the mean decreased to 28.04 mg/100 g (from 13.23 to 53.45). In 2012, the highest contents of secondary metabolites were recorded in everlasting pea accessions (132.44 mg/100 g), and the lowest in tuberous pea (6.42 mg/100 g). In the green biomass of grass pea, flat pea, heath pea, and spring pea, the mean values of secondary metabolites (89.32, 78.01, 71.59, and 28.74 mg/100 g, respectively) were lower than in everlasting pea. In 2013, the mean secondary metabolite contents in grass pea and flat pea dropped to 29.03 and 19.80 mg/100 g, respectively.

One-way ANOVA was used to ascertain the significance of the impact of weather conditions on the content of the analyzed biochemical characters. The analysis allowed identification of 79 compounds significantly affected by growing conditions (Table 2), including total organic acids and secondary metabolites. The effect size (percentage) for the influence of environmental conditions (η^2) on the content of organic acids was 67.9 % for lactic acid, 39.0 % for glyceric acid, 37.5 % for threonic acid, 33.4 % for fumaric acid, and 34.5 % for the total organic acid content (Fig. 2). Of free amino acids, including aminoalcohols and amines, the greatest weather impact was registered for the contents of ethanolamine ($\eta^2 = 72.9$ %), leucine (51.6), GABA (48.9), methionine (47.9), putrescine (43.1), adenosine (41.2), glycine (34.6), asparaginic acid (29.9), and asparagine (29.0). For weather impact on the variability of secondary metabolites, the highest effect size was recorded for quercetin ($\eta^2 = 39.8$ %) and dehydroabietic acid (30.4). For the total content of secondary metabolites, it was 26.1 %.

Despite the large number of compounds identified in the green biomass of *Lathyrus* spp., only quantitative indicators in some of them accounted for statistically significant differences between wild species: catechin ($F(5; 54) = 10.47$, $p = 0.0000$), α-alanine ($F(5; 54) = 2.52$, $p = 0.039$), asparagine ($F(5; 54) = 3.32$, $p = 0.011$), glycine ($F(5; 54) = 3.25$, $p = 0.012$), and shikimic acid ($F(5; 54) = 31.66$, $p = 0.0000$). Asparagine, glycine, α-alanine and catechin contents were the highest in flat pea (*L. sylvestris*), and shikimic acid content, in everlasting pea (*L. latifolius*) (Suppl. material 2).

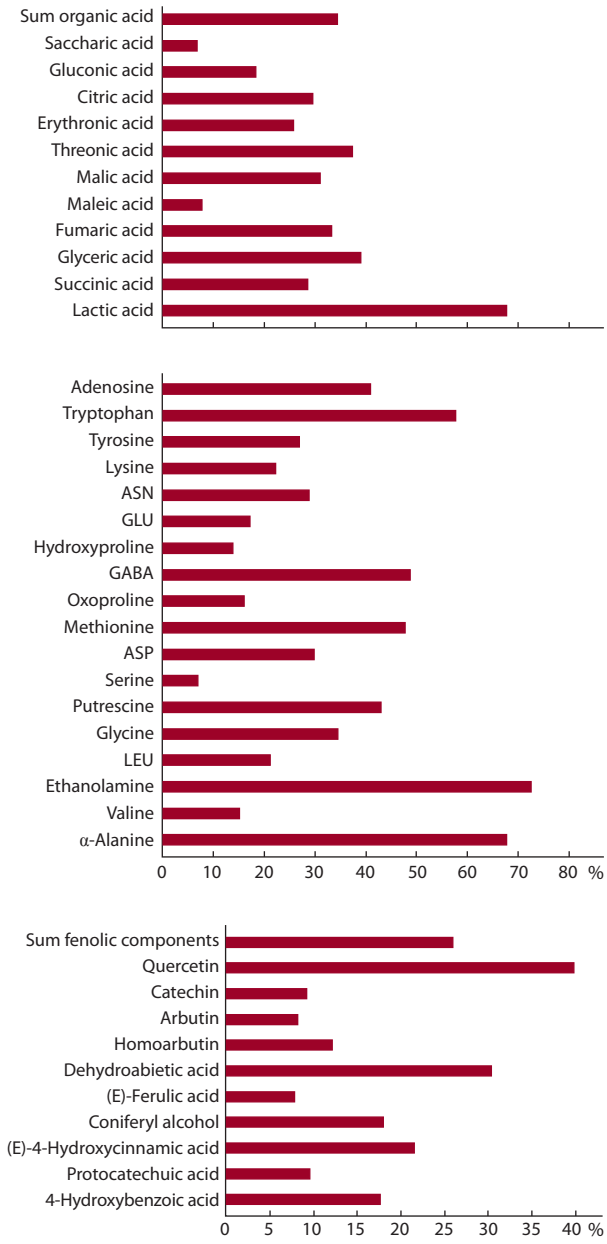

Fig. 2. The effect size (η^2, %) for the influence of weather conditions on the variability of biochemical characteristics.

Discussion

The studied *Lathyrus* accessions demonstrated broad polymorphism in the biochemical composition of green biomass. Significant interspecific and intraspecific variability was observed both in the composition (presence) and the amount of the identified compounds. The highest content of organic acids was found in *L. sativus*: 2140 mg/100 g (k-900, Colombia); free amino acids in *L. sylvestris*: 265.22 mg/100 g (k-2017, Germany); and total secondary metabolites in *L. latifolius*: 132.44 mg/100 g (i-594176, Germany). The analysis showed

Table 2. The results of one-way analysis of variance (ANOVA) to identify the association between the variability of biochemical parameters and weather conditions in the year of reproduction

Effect	df	Lactic acid			Succinic acid			Glyceric acid			Fumaric acid			Maleic acid		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	6515.0	122.9	0.00	1194.8	23.1	0.00	249259.9	37.0	0.00	5736.3	29.1	0.00	18.8	4.9	0.03
Error	58	3074.5			3004.4			390349.6			11439.2			222.2		
Total	59	9589.5			4199.2			639609.5			17175.5			241.0		
η^2		67.9			28.5			39.0			33.4			7.8		
Effect	df	Malic acid			Threonic acid			Erythronic acid			Citric acid			Saccharic acid		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	319326.9	26.2	0.00	340838.3	34.8	0.00	1664.9	20.2	0.00	92898.4	24.4	0.00	0.3	4.1	0.04
Error	58	705960.5			567394.8			4780.5			221251.6			3.9		
Total	59	1025287.3			908233.0			6445.4			314150.1			4.2		
η^2		31.1			37.5			25.8			29.6			6.7		
Effect	df	Gluconic acid			Total organic acid			4-Hydroxybenzoic acid			Protocatechuic acid			(E)-4-Hydroxycinnamic acid		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	1083.8	13.0	0.00	3624902.5	30.6	0.00	0.2	12.4	0.00	0.2	6.2	0.02	853.8	15.9	0.00
Error	58	4819.6			6880021.4			0.7			1.4			3119.8		
Total	59	5903.3			10504923.9			0.9			1.6			3973.6		
η^2		18.4			34.5			17.6			9.6			21.5		
Effect	df	Coniferol			(E)-Ferulic acid			Catechin			Arbutin			Quercetin		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	32.9	12.7	0.00	134.5	4.9	0.03	3.3	5.9	0.02	0.9	5.1	0.03	1.1	38.3	0.00
Error	58	149.9			1576.1			32.8			10.6			1.6		
Total	59	182.9			1710.6			36.1			11.5			2.7		
η^2		18.0			7.9			9.2			8.1			39.8		
Effect	df	Quinic acid			Homoarbutin			Dehydroabietic acid			Total phenolic compounds			Ethanolamine		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	2414.1	96.8	0.00	1.2	8.0	0.01	48067.3	25.3	0.00	45145.9	20.5	0.00	16.3	156.3	0.00
Error	58	1447.1			8.4			110250.0			127578.5			6.0		
Total	59	3861.1			9.6			158317.3			172724.4			22.3		
η^2		62.5			12.1			30.4			26.1			72.9		
Effect	df	α-Alanine			Valine			Serine			Leucine			Adenosine		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	937.3	9.8	0.00	146.9	10.4	0.00	2088.8	4.4	0.04	62.5	15.7	0.00	103.6	40.7	0.00
Error	58	5546.7			817.2			27385.9			231.1			147.7		
Total	59	6484.0			964.2			29474.7			293.6			251.3		
η^2		14.5			15.2			7.1			21.3			41.2		

Table 2. (End)

Effect	df	Glycine			Putrescine			Aspartic acid			GABA		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	108.2	30.7	0.00	12574.3	43.9	0.00	16051.4	24.8	0.00	763.8	55.4	0.00
Error	58	204.2			16604.5			37579.3			798.9		
Total	59	312.4			29178.8			53630.7			1562.7		
η^2		34.6			43.1			29.9			48.9		
Effect	df	Methionine			Oxoproline			Tyrosine			Hydroxyproline		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	1.4	53.4	0.00	908.1	11.3	0.00	31.1	21.6	0.00	0.1	9.4	0.00
Error	58	1.5			4662.5			83.6			0.7		
Total	59	2.9			5570.6			114.6			0.8		
η^2		47.9			16.3			27.1			13.9		
Effect	df	Glutamine			Asparagine			Lysine			Tryptophan		
		SS	F	*p*	SS	F	*p*	SS	F	*p*	SS	F	*p*
Year	1	8323.9	12.2	0.00	4368.6	23.7	0.00	149.2	16.8	0.00	168.1	79.6	0.00
Error	58	39610.6			10688.9			513.9			122.5		
Total	59	47934.4			15057.5			663.2			290.6		
η^2		17.4			29.0			22.5			57.8		

Note: SS – sum of squares; F – Fisher's criterion value; *p* – level of significance; df – degrees of freedom; η^2, % – the effect size of the factor's influence; year, weather conditions.

sizeable variation of biochemical parameters under different weather conditions. The colder and drier season in 2012 was conducive to the accumulation of organic acids, amino acids, and secondary metabolites.

There is no published information on the content of organic acids in the green biomass of *Lathyrus*. The analyzed accessions contained mostly acids participating in cell respiration, ascorbic acid oxidation products (threonic acid), natural antiseptics and antioxidants (azelaic acid), and anti-stress factors of plant cells (pipecolic and maleic acids) (Yao et al., 1998; Mahmud et al., 2017). The highest contents of individual organic acids were found in the following grass pea accessions: malic acid in k-893 (Eritrea) and k-900 (Colombia) (495 and 505 mg/100 g); threonic acid in k-900 (Colombia) (522 mg/100 g); azelaic acid in k-275 (Azerbaijan) (8.20 mg/100 g); maleic acid in k-889 (Abyssinia) and k-34 (Russia) (8.78 and 8.92 mg/100g). The everlasting pea accession k-51 (Germany) showed a high content of pipecolic acid (59.18 mg/100 g).

Our data on the free amino acid content differ from those published by S.G. Zaichikova et al. (2001), who identified 18 amino acids in *Lathyrus* herbage, including 7 essential ones, plus histidine and arginine not detected by us. In addition to the above-mentioned free amino acids, our experiment revealed tryptophan, GABA, asparagine, glycine, etc. In the said publication by S.G. Zaichikova et al., the main amino acids in peavine green biomass were glutamine and histidine: they respectively accounted for 15 and 11.6 % of the total amount of identified amino acids. E. Pastor-Cavada et al. (2010) identified 17 amino acids, including 9 essential ones, in *Lathyrus* seeds. Our research showed that the green biomass of *Lathyrus* spp. contained 20 amino acids (8 essential ones). The main amino acids in seeds were glutamic and aspartic acids, and in green biomass, according to our data, serine, glutamic and aspartic acids (16.4, 16.6, and 13.5 % of the total amount of identified amino acids, respectively). The major essential amino acids were arginine, leucine, and lysine in seeds and threonine (much more abundant than other amino acids) in green biomass. We singled out the grass pea accession k-842 (Tajikistan) for its high content of glycine (3.26 mg/100 g), an indicator of resistance to environmental stressors (Loskutov et al., 2016).

According to published data, flavonoids are the main phenol-containing compounds in the *Lathyrus* green biomass, their amount reaching 50 % of the total phenolic content. Our research has shown that the main components of phenol-containing compounds are free PC acids: they possess the highest antioxidant capacity (Shetty et al., 2002). Extracts of grass pea and flat pea in relevant concentrations are known to produce stimulating effect on the phagocytic and antibacterial activity of human neutrophils, which is associated with the presence of free PC acids in them (Zaichikova, 2002a, b).

The qualitative and quantitative compositions of secondary metabolites identified by us differed from the data published by F. Sibul et al. (2016), because different research

methods were applied. Sibul et al. used HPLC to identify a wide spectrum of hydroxybenzoic and hydroxycinnamic acids in *Lathyrus* leaves, as well as a high content of quinic acid (30.4–35.0 mg/100 g), with the total secondary metabolite content being 58.1 mg/100g dry weight (DW). In our study, the total content of secondary metabolites was much higher (246.85 mg/100 g), with ferulic and quinic acids being the most abundant (40.63 and 28.30 mg/100 g DW). We managed to identify only four hydroxybenzoic acids (pyrogallol, 4-hydroxybenzoic, protocatechuic and benzoic acids) against six identified by F. Sibul et al. (2016). The content of protocatechuic acid in our experiment was somewhat lower than those reported by other researchers (0.2 and 0.9 mg/100 g DW). Of hydroxycinnamic acids, we identified ferulic, sinapic, and caffeic acids (19.09, 0.03, and 2.09 mg/100 g DW), while our colleagues found ferulic, *p*-coumaric, and caffeic acids (1.38, 1.42, and 1.02 mg/100 g DW). The content of ferulic acid in our accessions was much higher, and that of caffeic acid slightly lower than the values reported by scientists outside Russia. The content of chlorogenic acid was low in the accessions analyzed by us and in the plants tested by F. Sibul et al. (2016): 0.8 mg/100 g DW. The content of luteolin in our genotypes was lower than the same parameter reported by other authors (1.21 and 4 mg/100 g DW). Our accessions contained more catechin and quercetin (0.95 and 4.00) than the plants tested by F. Sibul et al. (2016): 0.04 and 1.60 mg/100 g DW. The levels of kaempferol were practically identical (1.78 and 1.60 mg/100 g DW, respectively), but the content of isorhamnetin in our *Lathyrus* accessions (0.02 mg/100 g DW) was considerably lower than the value published by non-Russian researchers (0.53 mg/100 g DW). F. Sibul et al. (2016) identified a wider spectrum of flavones and glycosides. We did not identify isoflavones, coumarins, or several glycosides. However, our research efforts yielded data on other secondary metabolites (hydroquinone, shikimic acid, and coniferol).

U.D. Chavan (1998) reported the total content of secondary metabolites in sea pea (*L. maritimus* L.), which was beyond the scope of our research; those levels varied from 0.5 to 3.0 %, being roughly close to our results (0.3–0.9 %).

We selected the grass pea accessions k-893 (Eritrea) and k-900 (Colombia) for their high content of secondary metabolites (199.39 and 177.82 mg/100 g) as potential sources of resistance and pharmacological value.

Our research confirmed the impact of weather conditions (temperature and precipitation amount) on the accumulation of organic acids, free amino acids, and major secondary metabolites (Popov et al., 2016). The analysis helped us identify accessions with high contents of substances responsible for protection against adverse environmental factors (maleic and pipecolic acids, glycine, and the aggregate content of secondary metabolites) and compounds of value for pharmacology (azelaic acid), which hold promise in the development of new nutritious, resistant, or medicinal cultivars of *Lathyrus*.

Conclusion

Thus, our research has brought forth new data on the biochemical composition of peavine green biomass. Its results confirm that *Lathyrus* is a promising forage and medicinal crop with a potential for various branches of economy.

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Acknowledgements. This work was supported by State Budgeted Project 0662-2019-0002 for VIR.

Conflict of interest. The authors declare no conflict of interest.

Received March 26, 2019. Revised May 29, 2019. Accepted June 16, 2019.