J. Glob. Innov. Agric. Soc. Sci., 2018, 6(1):23-27
ISSN (Online): 2311-3839; ISSN (Print): 2312-5225
http://www.jgiass.com

BIOCHEMICAL ASSESSMENT OF GRASS PEA (*Lathyrus sativus* L.) VARIETIES

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Biochemical assessment of 6 grass pea (*Lathyrus sativus* L.) varieties with different origins was performed with a view to their use in the selection process. In the beginning of flowering stage with high crude protein content were distinguished BGE015741 (10.85%) and LA5108 (10.96%) varieties. In the technical maturity stage of the seeds, BGE015741 variety is of interest with maximum values of crude protein (28.44%), calcium (1.90%) and phosphorus content (0.29%). At the beginning of flowering, a positive correlation between green mass yield and crude ash content (r = 0.839), phosphorus (r = 0.761) and calcium (r = 0.361) was found and negative with crude fiber content (r = 0.863). In the technical maturity stage crude protein content in the seeds is negatively correlated with crude fiber (r= - 0.798) and with phosphorus content (r= - 0.208) and positive correlated with calcium (r=0.822) and crude ash (r=0.557). The indicators tested were found low variable in both phenological stages, especially in technical maturity, where coefficients of variation for crude fiber were 2.45%, for calcium - 2.50% and for phosphorus - 7.36%.
Keywords: Grass pea, chemical composition, correlation, green mass, grain..

INTRODUCTION

The main reason for the reduced production of products of animal origin is the poor feed base. The development of livestock is related to the deficiency of plant protein. The low quality of the feed is compensated by the use of additional quantities of grain (Kosolapov and Trofimov, 2011).
The problem of producing high-quality feed can be solved by expanding the species composition of plants. One of the promising crops for moderate climate conditions is the grass pea (*Lathyrus sativus* L.). In the areas with frequent droughts, grass pea has advantages over other legumes in terms of green mass yield, grain yield and protein content (Vishnyakova and Burlyaeva, 2006).
Lathyrus sativus L. is the most important and widely grown species of the genus *Lathyrus* belonging to the *Fabaceae* family. Grass pea is an excellent protein crop, providing protein and carbohydrates for human diet and animal feed in drier areas. It is a component of crop rotation and is of particular interest to the countries of the Mediterranean region (Vaz Patta *et al*., 2006; Başaran *et al.*, 2011).
Grass pea is an ancient culture that has been widely used for consumption in the past. Other economically important species of grass pea are *Lathyrus cicera* and *Lathyrus tingitanus*, which are cultivated for grain, and *Lathyrus orus*, *Lathyrus latifolius* and *Lathyrus sylvestris*, which are grown as fodder species. Most *Lathyrus* species are grown because of their nutritional value (Kumar *et al.*, 2011; Yang *et al.*, 2014).
Seeds of grass pea are used in feeding ruminants and monogastric animals and birds. Grass pea is one of the first among the grain legumes with essential nutrients (essential amino acids and carotene, lysine, arginine, tryptophan and flavonoids, as well as potassium, calcium, magnesium, iron, copper, zinc etc.) (Zaichikova *et al.*, 2001).
The aim of the study is a comparative biochemical characterization of introduced grass pea specimens to be done with a view to their use in the selection process.

MATERIALS AND METHODS

The study was conducted in 2014-2016 in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria. (43.41ºN, 24.61ºE). Aboveground and root biomass plant material of 6 grass pea varieties, originating in Spain, vz BGE027129, BGE015741, BGE025277, and from Portugal, vz LAT4362, LA5108, LAT5038 was analyzed.
Sowing was carried out manually in optimal time, according to the technology of cultivation of grass pea in 3 replications, between row spacing of 50 cm and depth of sowing of 5 cm. Green mass yield (kg/da), grain yield (kg/da) and duration of vegetation period (days) were measured.
Samples for biochemical analysis were taken over two phenological stages of plant development - beginning of flowering and technological maturity of the seeds. They were dried at 60 ºC to constant weight. All dried samples were milled to pass through a 1 mm screen and analysed. Crude protein content (CP) as percentage of absolute dry matter (%, DM) was determined according to Kjeldahl method (CP = N × 6.25), crude fiber (CF) (%, DM) and crude ash (Ash) (%, DM) by Weende methods (AOAC, 1990), macro elements

calcium (Ca) (%, DM) complex metrically and phosphorus (P) (%, DM) by hydroquinone (AOAC, 1990).

The dependencies between the indicators were determined by correlation analysis and the variability by the coefficient of variation (VC, %) (Dimova and Marinkov, 1999). Statistical processing (dispersion analysis) of experimental data was performed through the Microsoft Excel 2002 and Statgraphics Plus 2.1 for Windows Excel programs.

RESULTS

Grass peas genotypes varied between the analyzed quality parameters, both in the beginning of flowering and in technical maturity stage. The exception is the crude protein content in the beginning of flowering (Table 1) as well as the calcium content in the fresh biomass of the plants, on which the conditions of the environment (the year) did not have a significant influence. The factor environment (year) has a significantly greater part of influence in the overall variation in crude protein, crude fiber and phosphorus content indices in both phenological stages of plant growth. The effect of the genotype (variety) factor in the formation of crude ash content at the end of the vegetation period is less than the influence of the environment.

The values of the variation coefficient indicate that the qualitative indicators tested are low variable in general. As the most unstable the content of phosphorus (10.96%) and crude ash (10.01%) in the beginning of flowering stage can be characterized. With the exception of crude protein content, the other parameters show an increase in stability (crude fiber - 2.45%, calcium - 2.50%, phosphorus - 7.36%).

The results of the chemical analysis are presented in Table 2. The crude protein content in the fresh aboveground mass varies very narrowly from 10.12% to 10.96% (LAT5038 and LA5108). There are no significant differences in crude protein content and in other varieties as well.

Greater differences in the crude fiber content are found. Particularly impressive is the LA5108 with crude fiber above 25.00% and significantly exceeds other varieties.

Table 1. Analysis of variance (ANOVA) of the quality parameters of grass pea varieties.

Source	df	MS					
		CP	CF	Ca	P	Ash	Ca:P
		beginning of flowering					
Year	2	17.8227**	90.0046**	0.3828^{ns}	0.0253**	0.0008**	0.4637*
Genotype	5	0.3788^{ns}	13.2813*	9.4397**	0.0058**	0.0064**	0.9570**
CV (%)		2.45	7.45	6.23	10.96	10.01	6.5
		technical maturity					
Year	2	90.0046**	17.8227**	93.0392**	0.1604**	0.0090**	8.5502**
Genotype	5	13.2813*	0.3788**	16.0913**	0.0376*	0.0052**	5.3342**
CV (%)		7.45	2.45	2.5	5.73	7.36	5.48
Error	10						

CP - Crude protein, CF - Crude fiber, CV (%) - coefficient of variation

*; ** significant at $P < 0.05$; $P < 0.01$

Table 2. Biochemical assessment of grass pea varieties (2014-2016).

Varieties	CP	CF	Ca	P	Ash	Ca:P
			beginning of flowering			
BGE027129	10.20a	21.68a	1.12a	0.52ab	10.31b	2.19ab
BGE025277	10.24a	23.33ab	1.06a	0.50a	10.36b	2.10ab
LAT4362	10.51ab	23.39ab	1.01a	0.50a	9.82ab	2.04ab
LA5108	10.96b	25.74b	1.05a	0.46a	9.07a	2.28b
LAT5038	10.12a	25.80b	1.04a	0.50a	9.97ab	2.09ab
BGE015741	10.85b	22.01a	1.12a	0.60b	10.69b	1.88a
			technical maturity			
BGE027129	25.72bc	43.81c	1.72ab	0.24b	13.85bc	7.47ab
BGE025277	24.91ab	43.35c	1.61a	0.19a	12.82ab	8.75bc
LAT4362	25.99bc	41.33ab	1.67a	0.28c	14.82c	6.03a
LA5108	22.09a	42.69bc	1.60a	0.24b	12.55ab	6.94ab
LAT5038	24.21ab	46.75d	1.63a	0.19a	10.87a	9.80c
BGE015741	28.44c	39.99a	1.90b	0.29c	12.63ab	6.97ab

CP - Crude protein, CF - Crude fiber

a, b, c, d - statistically proven differences in P=0.05

BGE027129 and BGE015741 varieties have a lower crude fiber content (21.68% - 22.01%). These values indicate that grass pea is an important source of energy.

The calcium and phosphorus content of the plants has an impact on the nutritional value of the feed and hence on the productivity and health of the animals. The results obtained (Table 2) show that the calcium content in the seeds varies very slightly from 1.04% at LAT5038 to 1.12% at BGE027129 and BGE015741. With a wider range the phosphorus content was found differs. The highest is the phosphorus content of BGE015741 (0.60%), followed by BGE027129 (0.52%). LA5108 (0.46%) ranked last.

Crude ash content ranges from 9.07% to 9.97% (LA5108, LAT4362 and LAT5038) to 10.69% - 10.31% (BGE015741, BGE025277 and BGE027129).

From Table 2, it appears that in the technical maturity stage higher crude protein content was recorded in BGE015741 (28.44%). For varieties LAT5038, BGE025277, LAT4362 and BGE027129 this indicator ranges from 24.21% to 25.99%. Of the sample group grass pea tested with the lowest crude protein content was characterized by LAT5038 (22.09%).

By crude fiber content in grains it was found LAT5038 significant exceeds all other varieties. LAT5038 (46.75%) was followed by BGE027129 (43.35%), BGE025277 (43.81%) and LA5108 (42.69%).

On average, the phosphorus content was the highest in BGE01574 (0.29%) and LAT4362 (0.28%) varieties. The calcium level in the seeds is higher than the corresponding phosphorus level. Data shows that BGE025277, LAT4362, LA5108 and LAT5038 varieties have a lower calcium content (1.61% - 1.67%) in the seeds versus BGE015741 (1.902%). Generally, the seeds have a higher calcium concentration than the aboveground mass. The content of crude ash indicator in maturity stage varies considerably compared to the beginning of flowering stage.

***Correlation analysis*:** Correlation schemes illustrate and explain some of the specifics of the varieties studied. At the beginning of flowering stage (Table 3), green mass yield as one of the most important indicators summarizing the influence of all factors on plant growth and development strongly correlated with crude ash content (r = 0.839). Positive correlations with phosphorus content (r = 0.761) and calcium (r = 0.361) were also found, although they were not statistically significant. Very strong but negative is the dependence of green mass yield with crude fiber content (r = -0.863). Negative correlation coefficient between crude protein content and green mass yield (r = - 0.171) was found. There is a strong negative relationship between crude fiber content and calcium (r = - 0.627), phosphorus (r = - 0.570) and crude ash content (r = - 0.590). Medium to strong correlation is characterized by crude ash content with calcium content (r = 0.547) and phosphorus (r = 0.856).

In selection point of view a particular importance is given of the presence of certain dependencies among the individual indicators because of the possibility of more efficient and simultaneous selection of two or more attributes.

The crude protein content in the seeds (Table 4) is in negative correlation with the crude fiber (r = - 0.798) and with phosphorus (r = - 0.208) and in the positive with the calcium content (r = 0.822) and ash (r = 0.557). Negative values of the correlation coefficients between the crude fiber and phosphorus content with all other parameters were reported, with a weak statistically insignificant relationship found between them (r = 0.180).

Table 3. Correlations between the investigated parameters in the beginning of flowering stage.

	CP	CF	Ca	P	Ash	Ca:P
CF	-0.061					
Ca	0.445	-0.627**				
P	0.232	-0.570*	0.530*			
Ash	0.271	-0.590**	0.547*	0.856**		
Ca:P	0.183	-0.013	0.441	-0.522*	-0.371	
Green mass yield	-0.171	-0.863*	0.361	0.761	0.839*	-0.779

CP - Crude protein, CF - Crude fiber

* p≤ 0.05; ** p≤ 0.01

Table 4. Correlations between the investigated parameters in maturity stage.

	CP	CF	Ca	P	Ash
CF	-0.798**				
Ca	0.822**	-0.830**			
P	-0.208	0.180	-0.149		
Ash	0.557*	-0.777**	0.547*	-0.088	
Ca:P	0.425	-0.377	0.441	-0.909**	0.165

CP - Crude protein, CF - Crude fiber

* p≤ 0.05; ** p≤ 0.01

In the sample group tested seed yields (Fig.1) tend to be mainly influenced by two parameters: calcium content (g = 0.84) and crude protein (g = 0.75), and to a lesser extent by phosphorus (r = 0.38) and crude ash content (r = 0.75).
The statistically unreliable value of the correlation coefficient between crude protein content (in the maturity stage) and grain yield does not imply a successful selection of genotypes combining high productivity and seed quality. A similar finding can be done by the duration of the vegetation period despite the positive correlation with crude protein content (r = 0.69), calcium (r = 0.53) and crude fiber (r = 0.18).

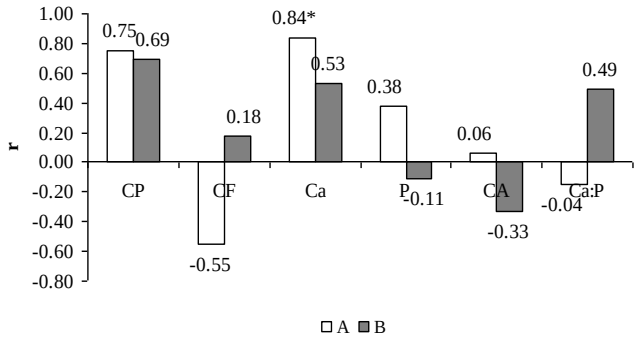

Figure 1. Correlations of the studied parameters with the grain yield and duration of the vegetation period.

CP - Crude protein, CF - Crude fiber, A - grain yield (kg/da), B - vegetation period (days); * p≤ 0.05

DISCUSSION

Donskoy (2013) reported that a set of grass pea specimens examined the crude protein content ranged from 27.42% to 3.14% and depended on the genotypic and soil-climatic conditions.
The data obtained in this study confirms the results reported by Urga *et al*. (2005) and Grela *et al*. (2012) according to which the mineral content of seeds of *Lathyrus cicera* and *Lathyrus sativus* is similar and the content of phosphorus is slightly lower than that of calcium.
Tuna *et al*. (2004) and Vahdani *et al*. (2014) reported that crude protein and crude fiber parameters for grass pea are relatively variable. According to the authors, the different cultivation conditions, the peculiarities of the individual genotypes used in the research, the specific phenological stage of the sample taking for analysis can partly explain this variability.
According to Pinheiro *et al.* (2010) and Talukder *et al*. (2010) their positive correlations between certain minerals in bean genotypes (*Phaseolus vulgaris* L.) suggest that the selection to increase the content of one mineral will increase the other.
Beyer *et al*. (2015) reported that in *Lathyrus angustifolius* the yield correlated positively and statistically significant with the 1000-seeds weight and with the crude protein content in the seeds.
The analysis of the correlations between quantitative and qualitative parameters in bean samples in the studies of Zilio *et al*. (2017) has shown that grain yield is in negative correlation with the crude protein content, phosphorus, potassium, phytic acid, zinc, suggesting the tendency for high-yielding genotypes to have lower concentrations of these nutrients. Similar models were studied by Nakova and Christova in maize.

***Conclusions*:** The chemical composition of the green mass and grain of the grass pea varieties studied determines this species as promising for use as a source of protein in livestock. As a result of the research, the following conclusions can be drawn.
In the beginning of flowering stage with high crude protein content were distinguished BGE015741 (10.85%) and LA5108 (10.96%) varieties, with high crude fiber content LAT5038 (25.74%) and LA5108 (25.80%) and with high phosphorus content BGE015741 (0.60%) variety.
In the technical maturity stage of the seeds, BGE015741 variety is of interest with maximum values of crude protein content (28.44%), calcium (1.90%) and phosphorus (0.29%) and LAT5038 by crude fiber content (46.75%).
At the beginning of flowering, a positive correlation between green mass yield and crude ash (r = 0.839), phosphorus (r = 0.761) and calcium content (r = 0.361) was found and negative with crude fiber (r = 0.863).
In the technical maturity stage crude protein content in the seeds is negatively correlated with crude fiber (r= - 0.798) and with phosphorus (r= - 0.208), and positive correlated with calcium (r=0.822) and crude ash (r=0.557). The tested qualitative indicators are low variable in both phenological stages, especially in technical maturity (crude fiber - 2.45%, calcium - 2.50%, phosphorus - 7.36%).

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