

Morphological characterization of Grass pea (*Lathyrus sativus* L.) Varieties

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ABSTRACT

Purpose: The maintenance of grass pea varieties and a comparative assessment of their biological characteristics, which will serve as a basis for future breeding work is required. The purpose of the study is to assess the biological potential of grass pea varieties in productivity and productivity components.

Research Method: Both, above ground and root biomass of plants was analyzed at the beginning of flowering stage and at the technical maturity of seeds. The coefficient of earliness was determined. The statistical methods viz hierarchical cluster analysis was applied to group the genotypes by similarity. Relationships between the characteristics tested and their variability were established by correlation analysis.

Findings: According to the coefficient of earliness the varieties studied were grouped as follows: LA5108 - early variety; LAT4362 and BGE027129 - middle-early varieties, and LAT5038 and BGE015741 - late varieties. Variety BGE027129 showed a high level of biological abilities on most of the characteristics. BGE025277 is distinguished with high productivity of seed weight (4.26 g/plant). BGE015741 was characterized by high both, number of nodules (25.27 nodules per plant) and weight of nodules (0.59 g per plant), the best specific nodulating ability (0.62) as well the highest seed weight productivity (5.52 g/plant). The nodule weight was positive correlated with the plant weight ($r = 0.517$). High coefficient of inheritance in a broad sense was found for the root weight (99.24%) and root length (92.65%) at the technical maturity stage, for the seed weight (77.94%) and number of branches per plant (72.32%), root weight (59.67%) and root length (48.09%) in the flowering stage.

Original Value: Grass pea varieties of breeding interest in regard to the both nodulating ability and seed productivity were shown.

Keywords: correlation, genetic diversity, grass pea, inheritance

INTRODUCTION

Grass pea (*Lathyrus sativus* L.) is one of the oldest crops grown in Europe Millennia before the new era. The seeds were mostly used for food. It is a multifunctional culture intended for grain, fodder, biomass and green fertilization (Campbell, 1997; Mikić *et al.*, 2010; Basaran *et al.*, 2011) like other relatives in the systematic field of crops such as peas (*Pisum sativum* L.) and vetch (*Vicia spp.*).

The qualities of leguminous crops, including the grass pea in terms of high protein content are undeniable. Due to their high biological

potential, they have the ability to develop well in a variety of environmental conditions and capacity to improve soil fertility (Wani *et al.*, 2012).

Grass pea has many qualities that make it an attractive crop during drought or in wet areas, with poor quality soil and prevailing extreme environmental conditions. Despite the tolerance

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of grass pea to drought, it is not affected by excessive rainfall and can be cultivated on areas subject to flooding. The grass pea is characterized by a penetrating root system and can be cultivated on many soil types, including very poor soils (Sidorova *et al.*, 2013). There is a growing interest in cultivation of this culture, dictated by the need to restore particularly eroded soils (Polignano *et al.*, 2009).

In Bulgaria, the grass pea is not that familiar and is almost forgotten, with no official data for its distribution and use. It could definitely be incorporated into the structure of protein crops and to occupy areas which are not suitable for traditionally grown leguminous peas, soybeans, chickpeas, etc. This necessitates the creation of a collection of samples and a comparative assessment of their biological characteristics, which will serve as a basis for future breeding work (Petrova and Angelova, 2013).

The assessment of the genetic diversity and characterization of primary genetic resource specimens is one of the most important conditions for the proper and rational use of working collections in future breeding programmes. Particular interest to breeders are genotypes with high yield and protein content, and low content of neurotoxin (Vaz Patto *et al.*, 2006).

One of the most important requirement for the production efficiency of typical spring grain legumes is the search for and introduction of drought resistant species with short vegetation period. The inclusion of the diversity of species, varieties and forms of grain legumes in production is an important alternative for the future of agricultural production (Mihailovic *et al.*, 2013).

The purpose of the study was to assess the biological potential of grass pea varieties in productivity and productivity components.

MATERIALS AND METHODS

The study was conducted in 2014-2016 in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria. Sowing was carried out manually in optimal time, according to the technology of cultivation of grass pea. Above ground and root biomass plant material of 6 grass pea varieties, originating in Spain, vz BGE027129, BGE015741, BGE025277, and from Portugal, vz LAT4362, LA5108, LAT5038 were used.

The following characteristics have been assessed: i) in the beginning of flowering stage: root mass length (cm), root mass fresh weight (g), nodule number per plant, nodule weight per plant (g), specific nodulating ability (g nodules/g roots); ii) in the technical maturity of seeds stage: plant height (cm), above ground mass weight (g), number of branches per plant, number of pods per plant, number of seeds per plant, seed weight per plant (g), root mass length (cm), root mass fresh weight (g). For root characteristics measurement soil monoliths were taken.

Biometric measurements were made to 10 plants of each variety. During the vegetation all observations were done for phenological date periods of sowing - beginning of flowering and sowing-technical maturity and the coefficient of earliness by Kuzmova (2002) was assessed. Criteria for assessing the coefficients of earliness were adopted the date of the beginning of flowering, and for the quantitative assessment the coefficient of earliness. For ultra early varieties the value of this coefficient was from 1.00 to 1.17, for the early varieties from 1.17 to 1.33, for middle-early ones from 1.34 to 1.66 and for the late varieties was greater than 1.66.

For all traits broad sense heritability ($H^2_{bs}, \%$) was calculated using the formula proposed by Mahmud and Kramer (1951). Hierarchical cluster analysis of Ward (1963) - for the grouping of genotypes by similarity as a measure for the difference (the genetic distance), the Euclidean distance between them was used, having previously standardization of the data carried

out. Relationships between the characteristics and their variability were established by correlation analysis (Dimova and Marinkov, 1999). For statistical data processing a variance analysis has been applied to the individual characteristic.

All experimental data were processed statistically with using MS Excel (2003) for Windows XP and the computer software STATGRAPHICS Plus for Windows Version 2.1.

RESULTS AND DISCUSSION

Productivity of grass pea depends largely on climatic conditions and on the individual characteristics of different varieties (Tamkoc *et al.*, 2009). The study period covers three consecutive years differing in climatic terms. Table 01 presents the data on average monthly temperatures and the amount of precipitated rainfall by months during vegetation. The vegetation 2014 is the most favorable for the study period with average monthly air temperatures (April 12.3 °C, May 16.7 °C, June 20.6 °C) and rainfall 139.8 l/m², 83.0 l/m² and 54.3 l/m², respectively. The balanced combination of air temperature and optimum rainfall has been favorable for plant development. The second year (2015) had relatively higher temperatures in May of 18.8 °C and uneven precipitation distribution, characterized by a certain drought

in April (43.6 l/m²) and May (30.6 l/m²), and a larger quantity in June (95.7 l/m²). The third year (2016) occupied an intermediate position over the other two years with temperatures in the months of April and May, close to normal (15.3-16.4 °C) and rainfall between 73.1 and 76.5 l/m².

The plants height was highly variable and was significantly affected by environmental conditions (Grela *et al.*, 2010). According to the experimental data received (Table 02), the specimens tested were characterized by non-essential differences in relation to this attribute. The variation range was within 44.13 cm in BGE015741 to 52.53 cm in LAT5038, followed by a variety of LAT4362 (50.73 cm), the differences in values are not statistically significant.

The study of the main elements of productivity is an important step in establishing the best variety for the particular growing conditions. It is clear from the analysis that the varieties BGE027129 and LA5108 are distinguished by the highest values in number of branches, pods and seeds per plant, 6.00, 15.80, 26.20 and 5.53, 15.53, 28.27, respectively. Basaran *et al.* (2013) considers that the number of branches of the plant is a very important character that has a strong impact on productivity. In a study of 51 grass pea specimens the authors found that this attribute fluctuates within the range of 4.00 to 6.46 number of branches per plant.

Table 01: Climatic conditions of the experimental period (2014-2016, Pleven)

Month	2014			2015			2016		
	t °C	rainfall l/m ²	humidity %	t °C	rainfall l/m ²	humidity %	t °C	rainfall l/m ²	humidity %
January	0.8	41.8	82.0	1.9	12.4	80.0	-0.5	98.0	78.0
February	2.3	3.4	82.0	2.3	39.2	80.0	8.7	46.0	75.0
March	9.7	76.9	68.0	6.7	68.4	71.0	8.5	76.6	73.0
April	12.3	139.8	76.0	12.2	43.6	54.0	15.3	73.1	66.0
May	16.7	83.0	70.0	18.8	30.6	66.0	16.4	76.5	71.0
June	20.6	54.3	67.0	20.7	95.7	64.0	23.0	45.8	67.0
July	23.1	71.8	67.0	25.8	21.5	54.0	24.6	7.8	57.0

Table 02: Distinct features of the investigated cultivars

Variety	BGE 027129	BGE 025277	LAT 4362	LA 5108	LAT 5038	BGE 015741	LSD 0.05	LSD 0.01
Beginning of flowering								
Root length (cm)	13.41	11.73	11.59	9.95	11.55	11.09	2.21	3.15
Root fresh weight (g)	0.89	1.04	0.82	0.47	0.66	0.95	0.24	0.35
Nodule number	41.13	28.28	34.32	10.41	12.67	25.27	21.93	31.20
Nodule weight (g)	0.35	0.23	0.27	0.17	0.15	0.59	0.45	0.64
SNA*	0.40	0.22	0.32	0.36	0.23	0.62	0.46	0.66
Technical maturity								
Plant height (cm)	49.21	50.67	50.73	45.60	52.53	44.13	19.23	27.36
Number of branches	6.00	4.92	4.60	5.53	3.73	4.87	2.52	3.58
Pods per plant	15.80	10.00	12.27	15.53	9.53	14.93	5.85	8.33
Seeds per plant	26.20	16.00	22.80	28.27	23.27	28.53	15.27	21.72
Seed weight per plant (g)	4.65	4.26	3.17	2.98	2.80	5.52	2.53	3.60
Root length (cm)	6.86	9.33	7.87	9.93	8.97	8.83	4.22	6.09
Root fresh weight (g)	0.67	0.75	0.72	0.88	0.73	0.92	0.25	0.36
Above ground mass weight (g)	18.07	17.62	19.49	9.28	13.47	21.01	8.17	11.63

*SNA – specific nodulating ability

From the tested grass pea specimens LA5108 although it manages to form a large number of seeds per plant, they are relatively small and their weight does not exceed 3.00 g. The highest seed weight distinguishes the plants from BGE015741 (5.52 g). A good combination between the number and weight of seeds per plant was seen in BGE027129 and BGE025277 varieties. Donskoy (2013) found that seed productivity in grass pea samples to the highest degree was determined by three elements: the weight of the plant ($r = 0.87$), the number of pods per plant ($r = 0.64$) and the weight of the pods per plant ($r = 0.97$). According to Polignano *et al.* (2005) the grass pea is suitable for growing mainly in the direction for grain and the efforts of researchers should be directed in such a direction. In the study of 106 grass pea lines Ribinski *et al.* (2008) reported that average grain productivity was from 7.20 g to 21.40 g.

There was also a difference in the characteristics concerning the root mass of plants. In terms of the length and weight of the roots at the technical maturity stage LA5108 and BGE015741 are of interest and their values were above the average. In the flowering stage the same trend prevail over the varieties BGE025277 (11.73 cm, 1.04 g) and BGE027129 (13.41 cm, 0.89 g).

With respect to the weight of the above ground mass, BGE015741, LAT4362 and BGE027129 significantly exceeded the other varieties, particularly LA5108 (9.28 g). These varieties are defined as desirable and with reference to the number of nodules (25-41), the nodule weight per plant (0.27-0.22 g) and the specific nodulating ability (0.32-0.62). Although none of them exhibited maximum value for any of these indicators.

The varieties differ in their phenological development. Differences were found from the onset of beginning of flowering stage (Table 03). With the smallest average duration of the period sowing - the beginning of flowering (57 days) is characterized by LA5108. In BGE027129, LAT4362 and BGE025277 this period is within the range of 62-64 days. The varieties LAT5038 and BGE015741 bloomed very late (67-68 days). The differences observed in the occurrence of individual phenological periods are retained until the end of the vegetation period. The group of early varieties may be referred to LA5108 with a coefficient of earliness = 1.00 and vegetation of 91 days. As middle-early ones with coefficient of earliness = 1.5 and vegetation period of 96-98 days characterized the specimens LAT4362 and

BGE027129. Late are found to be the varieties LAT5038 and BGE015741 with vegetation period more than 100 days.

Cluster Analysis

The results are presented in Figure 01 in the form of a dendrogram. They show a different grouping of varieties. It is clear from the dendrogram that the specimens were clustered into two main groups (A and B). Group “A1” contained only BGE027129 variety. It is characterized by high biological possibilities in terms of most of the characteristics studied, except for the length and weight of roots in technical maturity. The subgroup “A2” is formed by BGE025277, LAT4362 and BGE015741. The last two varieties are genetically closer to each other by means of the values of the number of branches, number of pods per plant, as well as the plant weight in technical maturity. The data show that a certain genetic distance is observed between the BGE025277 variety and the other specimens of group “A2”, mainly in the lesser expression of the number of pods and number of seeds per plant.

The other main group „B“ includes varieties LA5108 and LAT5038, which show a similarity in the number and weight of seeds per plant, root weight and nodule weight per plant.

This shows that different specimens have differing genetic structures that determine the manifestation of each attribute. The phenomenon is known as the redefining of the genetic formula of the attribute when changing environment conditions.

Coefficient of Variation

The grass pea samples have also been explored with regard to the variability of the characteristics studied through the coefficient of variation (VC,%), which characterizes the phenotypic variety (Figure 02). It varies within different limits for each attribute. The least variable is expressed for the root length (3.01%) in the LAT4362 variety in technical maturity and in BGE025277 in the flowering stage (4.84%). The length of the roots is distinguished by comparatively greater stability compared to other indicators, the highest level being at LAT4362 (16.36%).

Table 03: Phenological development of grass pea varieties

Variety	BGE 027129	BGE 025277	LAT 4362	LA 5108	LAT 5038	BGE 015741
Sowing-beginning of flowering, days	62	64	62	57	67	67
Sowing- maturity, days	98	98	96	91	101	101
Earliness coefficient	1.5	1.7	1.5	1	2	2

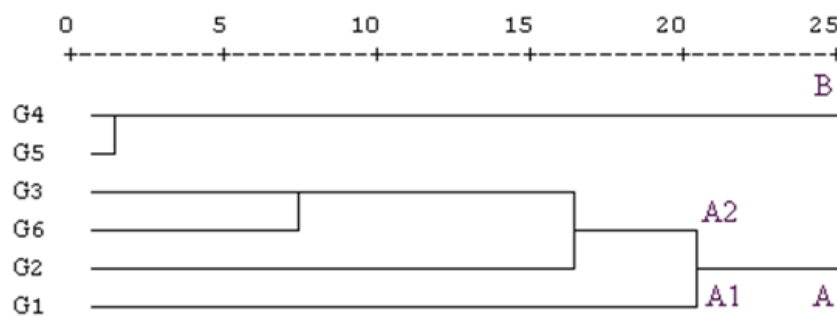


Figure 01: Dendrogram of the varieties for the investigated characteristics

G1 - BGE027129, G2 - BGE025277, G3 - LAT4362, G4- LA5108, G5 - LAT5038, G6 - BGE015741

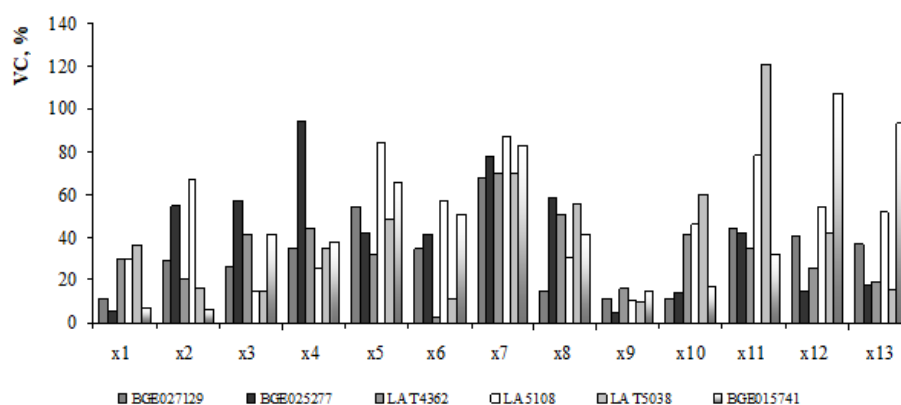


Figure 02: Variation coefficients for the quantitative signs studied

x1 - plant height (cm), *x2* - number of branches, *x3* - pods per plant, *x4* - seeds per plant, *x5* - seed weight per plant (g), *x6* - root length (cm), *x7* - root fresh weight (g), *x8* - above ground mass fresh weight (g), *x9* - root length (cm), *x10* - root fresh weight (g), *x11* - nodule number per plant, *x12* - nodule weight per plant (g), *x13* - specific nodulating ability

The variation was low for plant height (5.09%) in BGE027129 as well in BGE015741 variety by plant height (7.08%) and number of branches per plant (6.27%). By number of pods per plant with lower variation (lower than 15%) was detected in LA5108 and LAT5038 varieties, and by plant weight the BGE027129. Kumar and Dubey (2001) reported that the number of pods per plant ranged from 6.52 to 46.80 in the study of 60 endemic Spanish grass pea specimens.

The variability in the root weight in the beginning of flowering stage was found from medium to high in BGE027129 (11.27%) and BGE025277 (13.91%). By the values of the coefficient of variation of the specific nodulating ability BGE025277 (17.55%), LAT4362 (18.74%) and LAT5038 (15.63%) varieties can be characterised as medium stable and the others as highly variable.

Strong wingspan of coefficient of variation (VC,% > 20%) was established for all varieties of the number of seeds per plants, seed weight, roots weight in technical maturity stage and number of nodules per plant.

Coefficient of Inheritance

The inheritance is a characteristic of the relative share of genetic differences and differences that are dictated by the external environment of phenotypic variability. If the genotype

or environmental conditions change, the assessment of inheritance should be changed. The part of the total variability determined by the genetic differences between the varieties of the quantitative characteristics investigated is determined by the coefficient of inheritance in a broad sense ($H^2_{bs}, \%$).

With a relatively high coefficient of inheritance in a broad sense was the root weight (99.24%) and root length (92.65%) in technical maturity stage, the seed weight (77.94%) and number of branches per plant (72.32%) followed by the root weight (59.67%) and root length (48.09%) in flowering stage and plant weight in technical maturity stage (56.39%) (Figure 03). Analysing grass pea specimens Mevlut *et al.* (2007) obtained significant variation in the values of the coefficient of inheritance in a broad sense on the quantitative characteristics they examine.

In the studied group of specimens the inheritance was average in height of the plant (22.22%), number of pods (27.63%) and number of seeds (25.81%) per plant and weight of the nodules per plant (20.91%). The inheritance was found low in the nodule weight per plant (0.01%) in flowering stage. Similarly, the inheritance of the specific nodulating ability was found (0.01%) in the same stage. The low coefficient of inheritance on these characteristics implies a large influence of climatic conditions in the inheritance of the attribute.

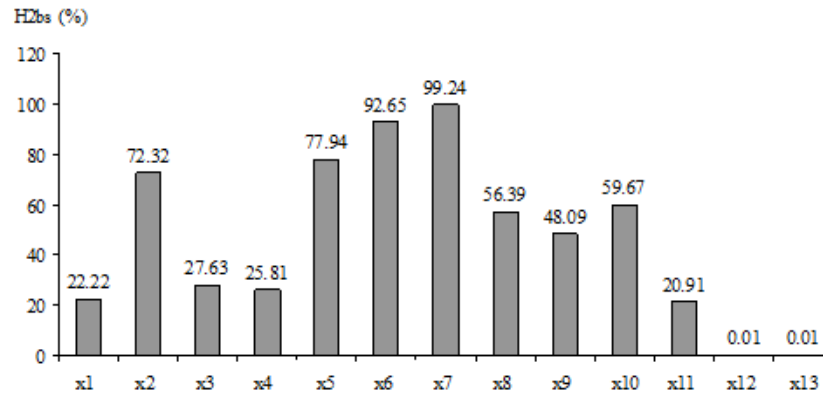


Figure 03: Broad sense H2 of the varieties of the investigated characteristics

x1 - plant height (cm), *x2* - number of branches, *x3* - pods per plant, *x4* - seeds per plant, *x5* - seed weight per plant (g), *x6* - root length (cm), *x7* - root fresh weight (g), *x8* - above ground mass fresh weight (g), *x9* - root length (cm), *x10* - root fresh weight (g), *x11* - nodule number per plant, *x12* - nodule weight per plant (g), *x13* - specific nodulating ability

Correlation Analysis

Correlations have been established between the quantitative parameters studied and the seed productivity, expressed by weight of seeds, as well as the weight of the nodules per plant (Table 04).

From the structural elements determining the seed productivity, the most significant positive influence on it were the characteristics of the plants weight in technical maturity ($r = 0.639$) and the fresh root weight in the flowering stage (0.539), with the correlation coefficients being very good statistical significance. It has been found that the root length (-0.503) and root weight (-0.695) in technical maturity stage negatively interact with the seed weight. Talukdar (2009) received a positive and statistically significant correlation between seed yield with components plant height, number of primary branches, number of pods and 100 seeds weight, and considered that the number of pods has maximum contribution to getting higher productivity. The remaining characteristics (without the root length in beginning of flowering

stage) correlated from low to medium positive, although statistically not significant. The results of the correlation analysis, reported by Levko (2009) showed significant correlations between the number of branches of first order and the number of peduncles ($r = 0.89$), between the plant height with a length of peduncle ($r = 0.84$) and the number of nodes in the main stem ($r = 0.80$), which determine the increased number of plant flowers.

The nodule weight per plant was at the medium level of a positive correlation with only the plant weight (0.517) and in the negative with the root length (-0.479). The number of branches per plant (0.147) and root length (0.317) in the flowering stage have a slight positive effect on the nodule formation of plants. Basaran *et al.* (2013) reported a high positive statistically significant correlation between the number of branches and the leaf length, between the seed weight and the number of pods per plant, as well as between grain yields and most of quantified characteristics studied.

Table 04: Correlations between investigating characteristics

	Plant height (cm)	Number branches	Pods per plant	Seeds per plant	Seed weight per plant (g)	Root length (cm)	Root weight (g)	Above ground mass weight (g)	Root length (cm)	Root fresh weight, g	Nodule number per plant	Nodule weight, g
Number branches	0.373											
Pods per plant	-0.289	-0.086										
Seeds per plant	-0.267	-0.015	0.764**									
Seed weight per plant (g)	0.166	0.418	-0.142	-0.316								
Root length (cm)	-0.323	-0.717**	0.377	0.289	-0.503*							
Root weight (g)	-0.065	-0.208	0.429	0.508*	-0.695**	0.591**						
Above ground mass weight (g)	-0.008	-0.059	-0.393	-0.609**	0.639**	-0.341	-0.703**					
Root length (cm)	0.188	0.107	-0.168	-0.145	-0.024	-0.168	0.073	0.177				
Root fresh weight (g)	0.395	0.345	-0.47*	-0.534*	0.539*	-0.486*	-0.440	0.678**	0.375			
Nodule number per plant	0.099	0.283	-0.027	-0.124	0.463	-0.360	-0.333	0.491*	0.409	0.633**		
Nodule weight(g)	-0.186	0.147	-0.187	-0.197	0.396	-0.479*	-0.291	0.517*	0.317	0.436	0.370	
SNA	-0.410	-0.013	0.045	0.040	0.187	-0.274	-0.145	0.258	0.168	0.038	0.169	0.899**

** Correlation is significant at the 0.01, * at the 0.05 level

CONCLUSIONS

The results show that the main factor for higher productivity of the varieties was not the maximum display of certain characteristics, but their optimal combination.

According to the coefficient of earliness the studied varieties can be grouped as follows: to the group of early varieties - LA5108; middle-early were LAT4362 and BGE027129; the late varieties with vegetation period over 100 days - LAT5038 and BGE015741.

BGE027129 variety showed a high level of biological abilities on most of the characteristics, both, above ground and root mass of plants. BGE025277 are distinguished with high productivity of seed weight per plant (4.26 g). BGE015741 was characterized by an increased both, number of nodules (25.27 nodules per plant) and weight of the nodules (0.59 g per plant) the best specific nodulating ability (0.62) as well the highest seed weight productivity per plant (5.52 g).

The nodule weight was in a positive correlation with the plant weight (0.517). High coefficient of inheritance in a broad sense was established for the root weight (99.24%) and root length

(92.65%) in the technical maturity stage, the seed weight (77.94%) and number of branches per plant (72.32%), root weight (59.67%) and root length (48.09%) in the flowering stage and plant weight in technical maturity stage (56.39%).

For the purposes of combinatorial selection for obtaining varieties combining high productivity of seeds and good nodulating ability, the selection interest represent the BGE027129, BGE025277 and BGE015741 varieties.

Authors have an equal contribution to the work.

Data Availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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