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Original scientific paper

COMPARATIVE BIOLOGICAL CHARACTERISTIC OF WHITE LUPIN (*Lupinus albus* L.) VARIETIES

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The study was conducted in 2014-2016 on the experimental field of the Institute of Forage Crops, Pleven, Bulgaria. Aboveground and root biomass plant material of seven white lupin (*Lupinus albus* L.) varieties different originated was analyzed in two phenological stages. Plants were analyzed for height, fresh weight, number of leaves, nodule number and nodule weight in the beginning of flowering stage, and for number of pods, number of seeds and seed weight in the technical maturity stage. Degree of earliness of varieties was assessing as well. The group of ultra early varieties can be defined PI533704 and Zuter varieties with coefficient of earliness 1.00, to early - PI368911, PI457938 and KALI (coefficient of earliness 1.25), and to late Lucky801 and PI457923 (coefficient of earliness >1.66). A strong positive correlation was found between the seed productivity with number of seeds per plant (r=0.943) and plant height (r=0.765); close relationship of fresh aboveground mass weight with plant height (r=0.822), number of leaves (r=0.965) and fresh root mass weight (r=0.876). The varieties of interest for breeding were selected by different signs. It was concluded that the number and weight of nodules as well fresh root mass weight can be used as selection criteria for creating varieties with a higher symbiotic nitrogen fixation potential.

Keywords: earliness,productivity, root mass, white lupin

INTRODUCTION

Lupin (*Lupinus* L.) is a species-rich legume genus and comprises a few hundred species. The most important crops in this genus are white (*Lupinus albus* L.), yellow (*Lupinus luteus* L.) and narrow-leafed (*Lupinus angustifolius* L.) that, like many other annual legumes, are multi-

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functional crops and are utilised in human consumption and animal feeding in the form of green forage, forage dry matter, forage meal, mature grain, as well as green manure (MIKIĆ *et al*., 2006; MIHAILOVIĆ *et al*., 2007; MIKIĆ *et al*., 2013).

Legumes are an important source of protein, oil, fibre and micronutrients, and play a vital role in cropping cycles due to their ability to fix atmospheric nitrogen. Biological fixation of nitrogen (N) is considered more ecofriendly than industrial nitrogen fixation because the NH3 produced in the former process is readily assimilated into organic forms by the plant. Biological nitrogen fixation (BNF) in legume nodules occurs with differentiated forms of rhizobia, termed bacteroids, within specialized structures called symbiosomes, inside the host plant cells (VALENTINE *et al*., 2011; MANTRI *et al*., 2013).

The effectiveness of selection work is determined by the availability of valuable initial material for the creation of forms that are resistant to environmental stress factors (NAUMKIN *et al*., 2012).

Lupins can be improved through conventional breeding based on natural germplasm stocks and genetic engineering may play an important role in future lupin crop improvement. The countries with significant breeding programs include Australia, Poland, Russia, Germany, Belarus and Chile. Other countries including the USA, Denmark, Spain, Portugal and Iceland have smaller breeding programs (CLEMENTS *et al*., 2012).

White lupin is an agronomically important crop because the grains are high in protein and fiber and low in starch and oil (TIAN *et al*., 2009). The most significant feature among the many desirable characteristics of leguminous plants, including lupins, is their symbiosis with bacteria allowing them to use atmospheric nitrogen for the purposes of growth and development, which also leads to soil enrichment with this extremely important for organisms macroelement, resulting in higher subsequent plant yields (FRANKOWSKI *et al*., 2014).

The attractiveness of the lupine consists in the fact that, unlike soybean, it can be cultivated in different areas practically without any restrictions on soil and climatic conditions. Three year-old species of lupine (narrow-leafed, yellow and white) are used for agricultural production. Each of them has its own biological features, it occupies a certain ecological niche and does not exclude the other. Of the three cultivated species, the white lupine has the highest productive potential of the grain, the quality of the grain is close to the soybean but is more demanding to soil conditions and temperature regime (ZAKHAROVA *et al*., 2014).

For future breeding and selection it is important to ascertain the variation available for plant structure and yield components in these species. In addition, information on the relative merits of architectural traits to seed yield is necessary (HEFNY, 2013).

The purpose of the study is to assess the biological potential of white lupine varieties by productivity and components of productivity.

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 white pea. Aboveground and root biomass plant material of 7 white lupin (*Lupinus albus* L.) varieties different originated, i.e, PI457923 (Greece), PI368911 (Czech Republic), PI533704 (Spain), PI457938 (Morocco), KALI (Poland), Zuter (France) and Lucky801 (France) was analyzed.

The following characteristics have been assessed in the beginning of flowering stage: fresh aboveground mass weight (g), number of leaves and after soil monoliths taking and washing the roots of the plants with water - fresh root mass weight (g), nodule number per plant and nodule weight per plant (g). In the technical maturity of seeds stage: plant height (cm), number of pods per plant, number of seeds per plant, seed weight per plant (g). Biometric measurements were made to 10 plants of each variety.

During the vegetation all observations were done for phenological dates periods of sowing: beginning of flowering and sowing-technical maturity and the degree of earliness by KUZMOVA (2002) was assess. Criteria for assessing the degree of earliness was adopted the date of the beginning of flowering, and for the quantitative assessment the coefficient of earliness was used. 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 taits Broad sense heritability (Hbs) was calculated using the formula proposed by MAHMUD and KRAMER (1951).

Coefficient of variation (CV%) was estimated by the formula suggested by BURTON (1952). The statistical methods were used to process the experimental data: factor analysis by the method of principal components (VANDEV, 2003), hierarchical cluster analysis by the method 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 signs 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 signs.

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

The quantitative characteristic is a suitable tool for objectively assessing the difference in productivity of a group of genotypes, even if only the underlying signs directly related to it. This assessment allows the comparison of selection materials of different origins but with the same criteria (TSENOV *et al*., 2014).

In the current selection, the height of the plant is one of the most important signs associated with the resistance to settling and thus indirectly influences the yield.

Genotypes with high plants are prone to pressure, especially in intensive farming conditions, resulting in a breakdown of the grain filling process and inaccurate information on their properties.

During the study period, the height of the plant (Table 1) ranged from 46.27 cm (PI368911) to 57.30 cm (Lucky801). Among the samples tested, the Zuter (56.33 cm), PI457923 (54.20 cm) and PI533704 (52.47 cm) varieties can be referred to the group of high-growing.

The obtaining of high-yield varieties of annual leguminous crops requires the regulation of many factors that determine high biological and economic productivity (yield). Biological yield is estimated by the amount of biological mass (aboveground or root) as a result of the life of plant.

Reproductive capacity, determined by the number of seeds is a major feature of the genotype's selective advantage. On average, during the study period the PI533704 and Zuter varieties managed to form about 10-12 pods with 28-32 seeds per plant. KALI also forms a large number of pods (10-11), but with a smaller number of seeds (24).

Table 1. Distinctive features of the investigated cultivars

Cultivar	Aboveground mass						Root mass	Nodules	
	plant height (cm)	number of pods	number of seeds	seeds weight (g)	number of leaves	fresh weight (g)	fresh weight (g)	number	weight (g)
PI457923	54.20	9.74	27.23	7.69	18.26	15.47	1.92	10.20	0.40
PI368911	46.27	8.92	24.62	5.73	21.44	18.05	2.03	8.00	0.23
PI533704	52.47	11.70	32.54	7.80	16.48	19.40	1.59	5.20	0.16
PI457938	47.60	9.33	25.67	6.42	24.97	23.20	1.97	8.00	0.42
KALI	47.57	10.54	24.64	5.92	20.43	17.67	1.12	3.40	0.11
Zuter	56.33	10.30	28.79	8.77	19.35	15.12	1.30	6.78	0.39
Lucky801	57.30	9.23	28.27	8.63	22.15	17.66	2.22	15.93	0.35
$LSD_{0.05}$	9.47	4.78	12.06	3.30	7.17	10.80	1.22	9.58	0.25
$LSD_{0.01}$	13.27	6.70	16.91	4.63	10.05	15.14	1.71	13.43	0.35
$LSD_{0.001}$	18.77	9.48	23.91	6.54	14.21	21.41	2.43	18.99	0.50

One of the most complex features determined by both the genotype of the variety, and the degree of soil-climatic and agro technical conditions of cultivation is the seed productivity. With the highest yields in seed weight are Lucky801 and Zuter distinguished, where the seed weight reaches 8.63-8.77 g. Under the particular test conditions, PI368911 (5.73 g) and KALI (5.92 g) were found to be least productive.

The variety features of PI368911, PI533704 and especially PI457938 allow them to form a large amount of fresh aboveground biomass under specific meteorological conditions compared to other samples. In our studies with the lowest fresh aboveground mass weight are the varieties Zuter (15.12 g) and PI457923 (15.47 g).

Considering the peculiarities in the level of appearance of the number of leaves it can be noted that it varied between 16.48 (PI533704) to 24.97 (PI457938). For most of the samples the differences on this trait are insignificant.

By root mass weight the Lucky801 and PI368911 varieties are of interest weighing a fresh root mass over 2 grams of plant, followed by PI457938 (1.97 g) and PI457923 (1.92 g). The lowest root weights showed KALI (1.12 g) and Zuter (1.30 g).

The number of nodules varies from 3.40 to 15.93. The Lucky801 has a great advantage over other varieties and has formed the most nodules average for the period. PI457923, PI368911 and PI457938 managed to form 8-10 nodules and the rest between 3 and 6-7. In relation to the weight of the nodules, there is a considerable variation between the varieties. Nodule weight ranges from 0.11-0.16 for KALI and PI533704 to 0.40-0.42 for PI457923 and PI457938.

The main task of modern white lupine selection is the creation of varieties combining high seed yields with optimum duration of vegetation period. The duration of the period of active vegetation influences not only the genetic properties and the biological characteristics of the variety, but also the environmental factors (NAUMKIN *et al.*, 2012; KURENSKAYA, 2016). The comparative evaluation of the white lupine varieties in the earliness was performed phenological observations, the data of which are presented in Table 2.

Table 2. Phenological development of white lupin cultivars (2014-2016)

Cultivar	PI457923	PI368911	PI533704	PI457938	KALI	Zuter	Lucky801
Sowing-beginning of flowering, days	63	60	59	60	60	59	62
Sowing- maturity, days	109	105	104	105	105	104	107
Earliness coefficient	2	1.25	1	1.25	1.25	1	1.75

The studied white lupine specimens exhibited varietal peculiarities by duration of the sowing - beginning of flowering period. Earliest have blossomed PI533704 and Zuter (59 days), followed by PI368911, PI457938 and KALI (60 days). This period is slightly longer for the Lucky801 and PI457923 varieties (62-63 days). The established phenological differences are maintained until the end of the vegetation period.

The duration of the vegetation period for most varieties varied within 104-105 days. Lucky801 and PI457923 ripen later and reach technical maturity averaged over 107-109 days. To the group of ultra-early varieties can be taken PI533704 and Zuter with a coefficient of earliness 1.00; to the early PI368911, PI457938 and KALI with a coefficient of earliness 1.25, and to the late Lucky801 and PI457923 with coefficient of earliness greater than 1.66.

Cluster analysis

Based on the differences in the structural elements of the productivity of the studied quantitative signs and parameters (duration of the vegetation period and the coefficient of earliness), the samples were grouped according to genetic similarity (Figure 1). The presence of genotype diversity in the studied variety was shown.

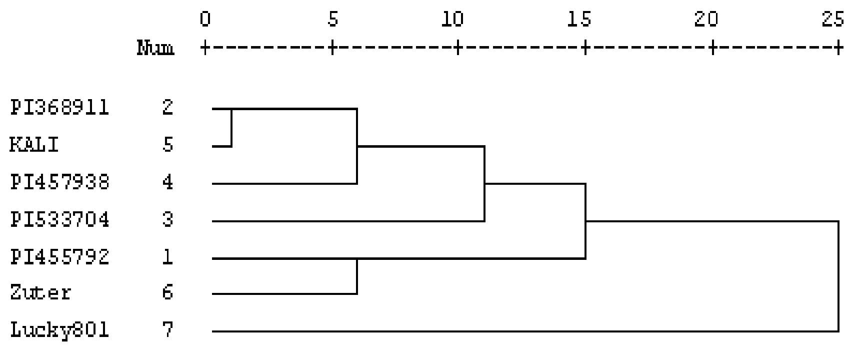

Figure 1. Dendrogram of the varieties of the investigated signs

Dendrogram sorts the varieties into two main clusters. The first one includes only the Lucky801 variety, which has the highest values of the plant height, number of leaves and number of nodules per plant, and by the other characteristics the values were above the average for the sample group tested. As a separate subgroup to the second cluster, the Zuter and PI457923 varieties are formed based on similarity in the plant height, seed number and number of leaves, aboveground mass fresh weight and nodule weight. In the second subgroup of the same cluster, the PI368911 and KALI varieties are genetically closest by the number of seeds and seed weight per plant, the number of leaves and the fresh aboveground mass weight. Although they are in a common cluster forming a second cluster, the varieties PI533704 and PI457938 are located on their own, suggesting a certain genetic distance between themselves and others in this subgroup.

Coefficient of variation

The coefficient of variation has been found to vary within a range for each attribute. The least variation was found for the plant height (10.3%). By the number of leaves per plant the variation is average (19.72%). With a wide variation of the coefficient of variation, all other signs are characterized, especially by number of nodules (66.21%) and nodule weight (97.44%), which indicates their greater genetic instability.

Coefficient of inheritance

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

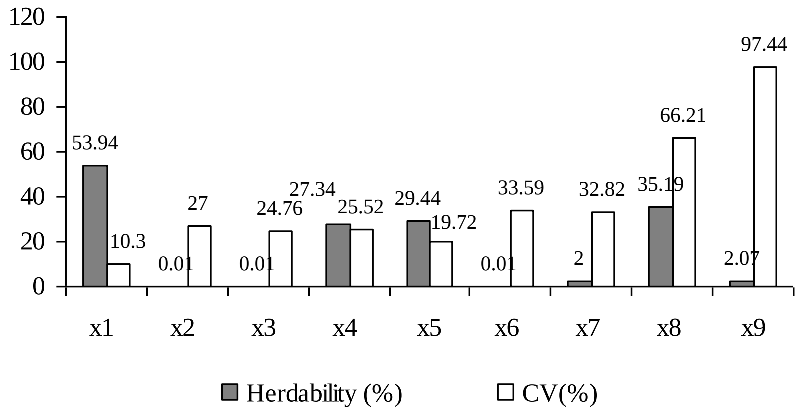

Figure 2. Coefficient of inheritance in a broad sense (H^2_{bs},%) and coefficient of variation (CV) of the varieties of the investigated signs

x1 - plant height (cm), x2 - number of pods per plant, x3 - number of seeds per plant, x4 - seeds weight per plant, x5 - number of leaves, x6 - fresh weight of aboveground mass, x7 - fresh weight of root mass (g), x8 - number of nodules per plant

With a relatively high coefficient of inheritance in a broad sense most of the studied features are characterized, the lowest being the number of pods, the number of seeds, the fresh aboveground mass weight and nodule weight (Figure 2). This shows that although genetically conditioned, the sign is strongly influenced by external conditions.

Correlation analysis

The analysis of the interrelationship between the studied characteristics (Table 3) shows a strong positive correlation of grain productivity (seed weight) with number of seeds (r = 0.943) and plant height (r = 0.765), average correlation with number of leaves (r = 0.606), fresh aboveground mass weight (r = 0.584), fresh root mass weight (r = 0.641) and number of pods per plant (r = 0.588).

Table 3. Correlations between the signs studied

	x1	x2	x3	x4	x5	x6	x7	x8
x2	0.136							
x3	0.758**	0.683**						
x4	0.765**	0.588**	0.943**					
x5	0.846**	0.082	0.647**	0.606**				
x6	0.822**	0.144	0.656**	0.584**	0.965**			
x7	0.757**	0.136	0.666**	0.641**	0.897**	0.876**		
x8	-0.486*	0.056	-0.192	-0.056	-0.380	-0.439*	-0.196	
x9	0.410	0.089	0.422	0.546*	0.545*	0.468*	0.524*	0.266

** significant at the 0.01, * at the 0.05

x1 - plant height (cm), x2 - number of pods per plant, x3 - number of seeds per plant, x4 - seeds weight per plant (g), x5 - number of leaves, x6 - fresh weight of aboveground mass (g), x7 - fresh weight of root mass (g), x8 - number of nodules per plant, x9 – nodule weight (g)

The values of the correlation coefficients of fresh aboveground mass weight reveal the close correlation of the plant height (r = 0.822), the number of leaves (r = 0.965) and the fresh root mass weight (r = 0.876), the average positive correlation of number of seeds (r = 0.656) and a weaker by nodule weight (r = 0.468). The correlation between fresh aboveground mass weight with nodule number was found negative (r= - 0.439). Nodule number correlates positively and statistically insignificant only with the number of pods per plant (r=0.056). In the nodule weight the correlation coefficients with the other signs are positive, although not all are statistically significant.

The results of this study are consistent with those found by MANGGOEL *et al*. (2012) and HEFNY (2013) reporting high values of the genotypic and phenotypic variance coefficients for all the signs they studied in lupin.

BEYER *et al*. (2015) receive results that reveal a low level of genetic diversity in the *Lupinus angustifolius* L. species tested by them, which limits the potential for species improvement through these specimens. The authors report a low coefficient of variation most signs such as grain yield, protein yield and 1000 seeds weight. Similar results obtained POPOVIC *et al*. (2012, 2015) in soybean.

Similar results are reported by DONSKOY (2013) regarding the structural elements of the yield of *Lathyrus sativus*. The author finds that the quantitative signs directly related to plant productivity have the variety specificity that is to a certain extent dependent on the soil and climatic conditions of cultivation.

By applying correlation and Path analysis, LÓPEZ-BELLIDO *et al*. (2000) obtained high correlation coefficients and a direct effect of a number of pods per plant on seed yields in white lupine varieties and a negative direct effect of plant height on grain yield.

GOLPARVAR (2012) as a result of its research reported a positive correlation between biological nitrogen fixation and other signs such as total nitrogen in the roots, number of plant nodules and grain yields of a plant of beans.

DEBELYI *et al*. (2011) using information on the dependence between individual quantitative signs reported that high-yielding narrow-leafed lupin with determinant type of growth were obtained by breeding way through selecting higher genotypes, staking a larger number of fertile nodules, pods and seeds.

According to BHARGAVA *et al*. (2003) and ATTA *et al*. (2008) alone the high value of the coefficient of inheritance does not provide a sufficiently large guarantee for quick selection success in improving the attribute. The authors consider that other parameters such as genetic advancement (progress) in the complex assessment of the attribute should be considered.

BICER and SAKAR (2010) after studying lens cultivars reported that only by the 1000 seeds weight had a high coefficient of inheritance in a broad sense with a variation in this sign between genotypes being significant.

In the studies on pea SOLOVOV (2006) found that each individual sign among the sample studied has shown a different level and type of variability, which may serve as a criterion for assessing genetic similarity or difference. When samples have shown a different type of variability, it is assumed that their signs are determined by genes found in different allele states.

CONCLUSIONS

According to the coefficient of earliness the white lupin varieties are grouped as follows: PI533704 and Zuter (coefficient of earliness 1.00) - ultra early; PI368911, PI457938 and KALI (coefficient of earliness 1.25) - early; Lucky801 and PI457923 (coefficient of earliness >1.66) - late.

A strong positive correlation was found between seed productivity with number of seeds per plant (r=0.943) and plant height (r=0.765), an average positive correlation with number of leaves r=0.606), fresh aboveground mass weight (r=0.584) and number of pods per plant (r=0.588).

A close relationship of fresh aboveground mass weight with plant height (r=0.822), number of leaves (r=0.965) and fresh root mass weight (r=0.876) was established and average positive correlation with number of seeds per plant (r=0.656).

The inheritance of the signs seeds weight and number of leaves per plant, and plant height and number of nodules was found an average to high.

The next varieties are of interest for selection: PI368911 by number of pods and seeds per plant; Lucky801 by plant height, seed weight, fresh root mass weight and nodule number per plant; PI457938 by number of leaves, fresh aboveground mass weight, fresh root mass weight and nodule weight; Zuter by number of pods, number of seeds and seed weight.

In this study, the signs number and weight of nodules and fresh root mass weight can be used as selection criteria for creating varieties with a higher symbiotic nitrogen fixation potential.

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KOMPARATIVNE BIOLOŠKE KARAKTERISTIKE VARIJETETA BELE LUPINE (*Lupinus albus* L.)

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Izvod

Istraživanje je sprovedeno 2014-2016. na eksperimentalnom polju Instituta za krmno bilje, Pleven, Bugarska. Nadzemni i korenov biljni materijal sedam različitih sorti lupine (Lupinus albus L.) različitog porekla analiziran je u dve fenološke faze. Analizirane su sledeće osobine: visina biljke, sveža masa, broj listova, broj nodusa i njihova težina na pošetku cvetanja, broj mahuna, broj i težina zrna u fazi tehničke zrelosti. Procenjen je i stepen ranostasnosti sorti. U grupu ultra ranih varijeteta svrstani su PI533704 i Zuter, sa koeficijentom ranostasnosti 1.00, ranih PI368911, PI457938 i KALI (koeficijent ranostasnosti 1.25), a kasnih Lucky801 i PI457923 (koeficijent ranostasnosti >1.66). Utvrđena je jaka pozitivna korelacija između produktivnosti zrna i broja zrna po biljci (r = 0,943) i visine biljke (r = 0,765); značajna povezanost sveže mase nadzemnog dela sa visinom biljke (r = 0,822), broja listova (r=0.965) i sveže mase korena (r=0.876). Varijeteti od interesa za oplemenjivanje odabrani su na osnovu različitih osobina. Zaključeno je da se broj i masa nodusa, kao i masa sveže korenske mase mogu koristiti kao kriterijumi u selekcije za stvaranje sorti sa višim potencijalom fiksacije simbiotskog azota.

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