POSSIBILITIES FOR IDENTIFICATION OF THE GENOTYPE BY PHENOTYPE IN WHITE LUPINE (*LUPINE ALBUS* L.) CULTIVARS

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Abstract

The application of traditional analyses and methods in breeding-genetic researches is necessary to be combined with the study of the ecological-genetic model for organization of the quantitative trait revealing a new perspective in the plant breeding. The present study was conducted during the period 2015-2016 at the Institute of Forage Crops (Pleven) with 23 white lupine cultivars with different origin. The issue of breeding identification of the best white lupine cultivars from the available collection in their phenotype was examined. A rationale of the principle "background trait" was represented with purpose for effective breeding activity. Characteristic of the possibility of using the mean values of plant seed weight and crude protein content in seeds in the selection of parental forms for the needs of the combinative breeding was made.

The developed method of orthogonal regressions to identify phenotype by genotype has characterized BGR 6305, Tel Keram and Pflugs Ultra as cultivars having "strong" genetic systems regarding attraction and adaptability of seed productivity and crude protein content of the seeds.

The assessment of ecological stability showed that the cultivars Tel Keram, Bezimenii 2, Pflugs Ultra and Solnechnii were stable, highly productive and suitable for growing in a wide range of environmental conditions. Cultivars BGR 6305, Tel Keram, Pflugs Ultra, Horizont and Solnechnii exhibited stability of their genetic systems under changing the environmental conditions and can be used as donors in regard to attraction and adaptiveness to develop hybrid forms with high seed productivity and crude protein content in the seeds.

Key words: Selection, Lupine, Physiological systems, Ecological-genetic model.

Introduction

The fundamental question of the interaction between the share of heredity and influence of the environment on the formation and expression of a given quantitative trait remains a question of present interest in the general genetics and breeding. In the scientific researches, demand for new approaches for resolving the question of the efficiency of selection of the quantitative trait does not stop. The methodology of each study is determined by the scientific paradigm or by a set of initial postulates (Notov, 2008; Kocherina, 2009).

In the present case, it is related to the definition of two key concepts - "genotype" and "phenotype". Modern wider treatment defines the genotype as a unified system of interacting hereditary genetic elements that determine the pathway of development of the organism. In fact, the initial significance of genotype is a complex of all hereditary potentials, and the phenotype is a combination of different properties defined by the interaction between the hereditary factors and environment (Inge-Vechtomov, 1989; Ismoilov, 2005; Kocherina & Dragavtsev, 2008).

Until now, genetic investigations have been related to an analysis of the characteristics based on the popular postulate of unequivocal compliance "gene-trait" and respectively of the conception for the genotype as a complex of potential signs. The biometric approach in the genetic analysis of the quantitative trait does not ensure all requirements of the theory and breeding practice because it uses different methods and models (Dragavtsev, 2005; Petrova, 2005; Solovov, 2006).

The limited use of the coefficient of heritability in the breeding is due to the fact that the calculation of inheritance in broad sense (H2) is only applicable to mass selection, and in narrow sense (n2) it requires full knowledge of the genetics of trait formation, which makes unnecessarily the calculation of this coefficient. The division of the phenotypic variance of constituent components has not been confirmed in the practical breeding. The phenomenological approach, on the contrary, uses precise mathematical-statistical methods for analysis of the quantitative trait, but really does not take into account the specific mechanism of inheritance of the trait (Komarov & Druzhinina, 2008; Dragavtsev *et al.,* 2012).

These approaches are not mutually exclusive, each of them has its merits and demerits. The theoretical study of existing approaches and methods of quantitative chracteristics has already been done (Chesnokov *et al.,* 2008; Dragavtsev, 2010).

The present research was aimed for an ecologo-genetic evaluation of white lupine cultivars with regard to seed productivity and crude protein content through application of the method of orthogonal regressions.

Material and Methods

The study was carried out at the Institute of Forage Crops (Pleven, Bulgaria) during the period 2015-2016. The collection of white lupine (*Lupine albus* L.) cultivars included 23 accessions with different origin as follows: Russian cultivars – Tel Keram, Pflugs Ultra, Solnechnii, Bezimenii 1, Bezimenii 2, Manovitskii, Dega, Barde, Desnyanskii, Horizont, Termis Mestnii, Pink Mutant; Ukrainian cultivars – Garant; Polish cultivars – Amiga, Astra, Ascar, BGR 6305, Nahrquell, Shienfield Gard, Kijewskij Mutant, Hetman, Start, WAT. The experiment was conducted in a field for organic production after a

two-year conversion period. A randomized block method was used. The seeds were sown in the third decade of March with a sowing rate of 50 seeds per m^2.

The parameters of seed productivity (g/plant) and crude protein content of the seeds (g/kg DM) were analyzed over two years with contrasting air temperatures and rainfall. The first experimental year (2015) was characterized by a unique combination of average daily air temperature with 3 °C higher and a sum of rainfall with 35% lower than the average values in 2016. These variations, quite contrasted by years and in comparison to long-term data, were a prerequisite for receiving different results under evaluation of one and the same cultivar.

Meteorological conditions during the experimental period were characterized by great variety. The precipitation in April (73.1 mm) and May (76.3 mm) in 2016 was about twice more than the same period in 2015, while in June 2016, the amount of fallen rainfall was less (45.8 mm) than the previous two months. The monthly temperature for the same period of the first and second year of the survey fluctuated in relatively narrow limits without considerable differences in months. The average monthly temperature for 2015-2016 was 16.8-17.6°C.

The crude protein content of lupine seeds was determined by Kjeldal method (Anon., 1990). The stability of the studied traits was determined according to the method of Francis & Kannenberg, (1978). The coefficient of variability was calculated (VC, %) (Dimova & Marinkov, 1999). The method of orthogonal regression described by Kramer (Dragavtsev, 2002) was applied.

For statistical processing of the experimental data, including regression, variation and rank analysis, was used the MS Excel software (2003).

Results and Discussion

In the first case, the theory of identification was regarded as a theory of the line of regression, expressed by the limitations of the two parabolas. The dots outside the parabola are considered as reliable from a genetic point of view regarding one of the physiological systems (attraction, microdistribution, adaptability, polygenic immunity, tolerance to limiting factors of environment, stand density, genetic variability of the ontogenesis stages duration), depending on selection trait (ST) and background (foil) trait (FT) and chosen when building the graph in a two-dimensional coordinate system.

In the second case, when the mean values of the traits of the cultivars were situated on the graph. The well-known formula regarding the significance in differences of the mean values was applied successfully. If in the plant population there was a quantitative trait with a genetic dispersion close to zero, then the variation of this trait would be only ecological. Then the individual which was distinguished by plus deviation over the mean for the population (in this trait) would had been fallen in a better micro-ecological niche (this trait is called background trait).

If some selection trait of the plant individual is located in the "plus-side" (I quadrant) of the coordinate system of the given background, this indicates that there is modification variability. This plant will be excluded and should not be selected. If another individual with respect

to "background trait" showed an average level of the trait for the population, but with respect to "breeding trait" was located in the "plus" area, this suggested a presence of recombination (or mutation) which was necessary for the success of the breeding work.

The graph for "divergence" described in details by Dragavtsev (2005), can be used to solve two basic tasks. The first one was in regard to identifying the genotype of individual plants in disintegrating populations (mostly hybrid populations) by their phenotype, as the indicators "breeding trait" and "background trait" of the individual plants were put on the graph. The second was related to a choice of suitable parents for hybridization in the combined breeding, and on the graph were situated the mean values of above mentioned indicators from the working collection of the breeder.

The cultivars, which were subject of this study, differed in the stability of their genetic systems upon a change of the limits of the environment (Fig. 1). Cultivars Tel Keram and Solnechnii were characterized by maximum displacement regarding adaptiveness and respectively with tolerance to unfavorable environmental conditions, suggesting that they had "good" genes for adaptiveness and tolerance. Cultivar Horizont exhibited a maximum positive deviation in attraction, from which follows that it was a carrier of "strong" genes for attraction, and varieties BGR 6305, Pflugs Ultra, Tel Keram and Solnechnii) were ecologically stable donors in attraction.

If in the figure, there is a genotype that occupies a maximum position in attraction, and another genotype is with a maximum displacement along the line of adaptability, these genotypes are the most suitable as parents for hybridization in the combinative breeding. In F2 generation of these genotypes, transgressive forms can be obtained, which combine strong genes both in attraction and adaptability.

The position of varieties Pflugs Ultra, BGR 6305 and Tel Keram which occupied the quadrant, limited by the positive part in attraction and adaptability, was retained at both limits of the environment. These cultivars showed stability independently of deterioration of growing conditions (unfavorable year). Stable but with negative adaptability were varieties Start, Amiga, Manovitskii and Barde.

The position of Termis Mestnii, whose plants in 2016 formed the highest seed weight and showed a level of crude protein around the average for the studied population, was substantially altered. A stable position in the quadrant formed by the negative part of adaptability and attraction, occupied varieties Nahrquell, WAT and Kijewskij Mutant.

In regard to the genetic change in attraction of the white lupine varieties, can be established a presence of a strong polymorphism. Some varieties (such as Ascar, Shienfield Gard and Garant) changed their position in the coordinate system under change the growing conditions either in the positive or negative part of the regression line, which defined a good perspective for breeding improvement in this crop.

In Figure 1, the positive part of the orthogonal regression identified the changes in regard to adaptability. The graphical analysis showed that the magnitude of variability in adaptability considerably exceeded the magnitude in attraction.

It should be noted that if only the two best parents crossed (one for attraction and the other for adaptability) it was very important to know the way of interaction of their gene complexes and the extent to which this interaction had additive character (Dragavtsev & Dyakov, 1998). Thus, through graphical representation, the donor characteristic of each genotype can be determined in terms of adaptability and attraction (Ismoilov, 2006).

Ecological stability of white lupine cultivars: For breeding of white lupine in limiting environmental conditions, it was important to combine high productivity with high environmental stability. The years of growing were used as different environments in which varieties realized their phenotypic dispersion.

The distribution of the accessions, by seed yield and stability, expressed by the coefficient of variation (according to Francis & Kannenberg, 1978) was presented in (Fig. 2) The average values of seed productivity and the coefficient of variation divided the corresponding coordinate system into four quadrants. In the second quadrant cultivars Tel Keram, Bezimenii 2, Pflugs Ultra and Solnechnii fell which had a high ecological stability (low variability) and high productivity. They kept their location in the same quadrant during both years of study. From a breeding point of view, they were the most valuable. The cultivars in the first quadrant were characterized by high average productivity but with low stability. These varieties were responsive only under favorable conditions.
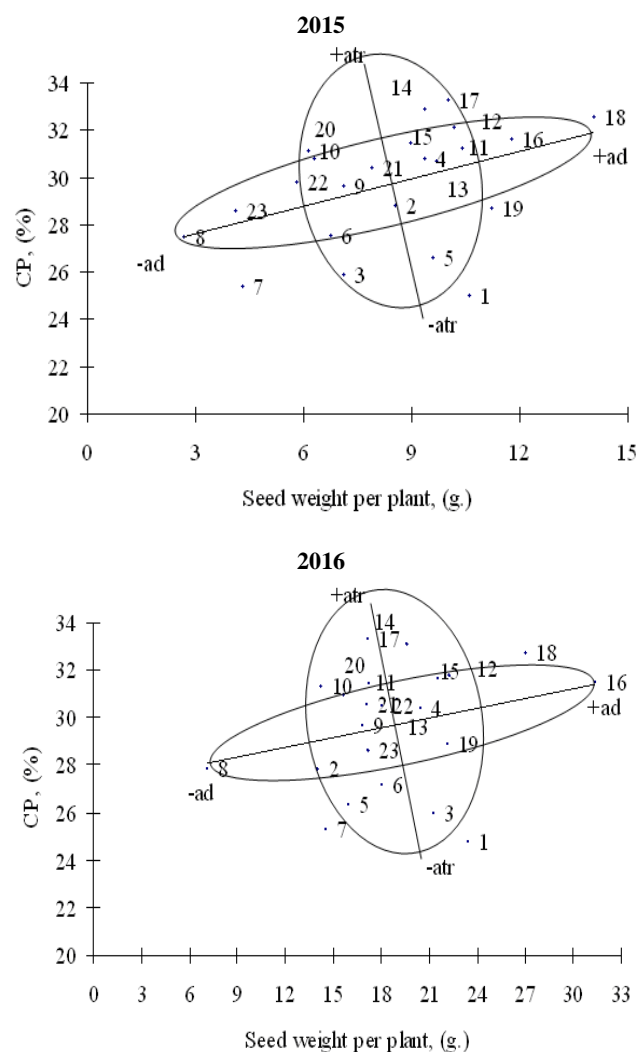
1-Astra, 2- Nahrquell, 3-Ascar, 4-BGR 6305, 5-Shienfield Gard, 6-WAT, 7-Kijewskij Mutant, 8-Hetman, 9-Start, 10-Amiga, 11-Garant, 12-Tel Keram, 13-Bezimenii 1, 14-Bezimenii 2, 15-Pflugs Ultra, 16-Termis Mestnii, 17-Horizont, 18-Solnechnii, 19-Pink Mutant, 20-Manovitskii, 21-Barde, 22-Dega, 23-Desnyanskii
+atr –atr - positive/ negative genes for attraction; +ad/ -ad - positive/negative genes for adaptability; CP – crude protein

Fig. 1. Method of orthogonal regressions for identification of the genotype by phenotype in white lupine.
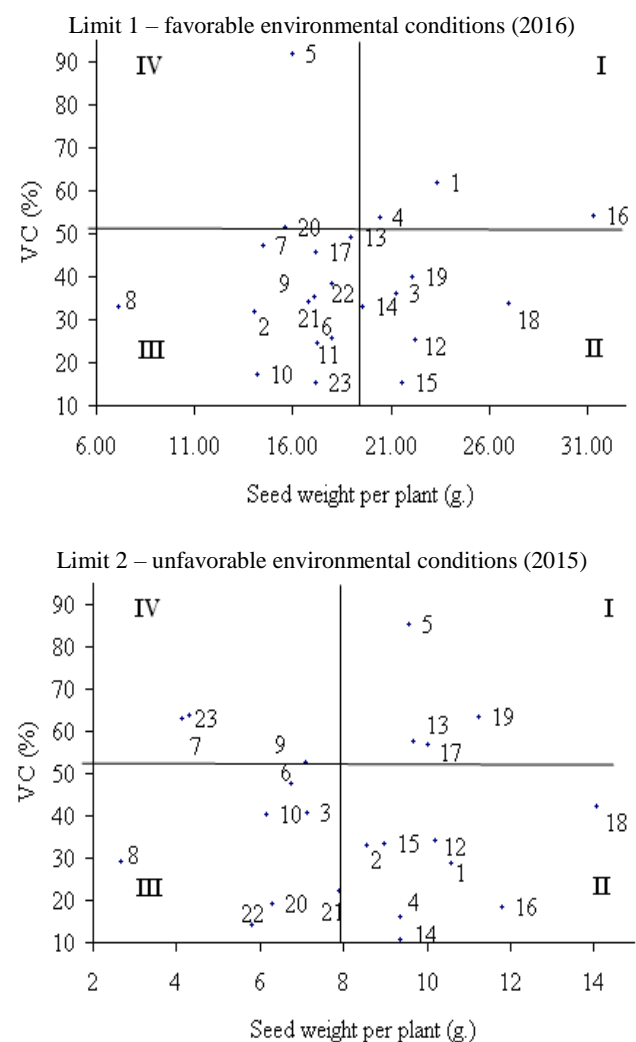
1-Astra, 2- Nahrquell, 3-Ascar, 4-BGR 6305, 5-Shienfield Gard, 6-WAT, 7-Kijewskij Mutant, 8-Hetman, 9-Start, 10-Amiga, 11-Garant, 12-Tel Keram, 13-Bezimenii 1, 14-Bezimenii 2, 15-Pflugs Ultra, 16-Termis Mestnii, 17-Horizont, 18-Solnechnii, 19-Pink Mutant, 20-Manovitskii, 21-Barde, 22-Dega, 23-Desnyanskii

Fig. 2. Ecological stability according to Francis and Kannenberg (1978) in white lupine cultivars regarding seed productivity.

Table 1. Rank analysis of white lupine cultivars regarding crude protein content in the seeds and seed weight per plant, 2015-2016.

Cultivars	Crude protein content		Seed weight per plant	
	Rank 2015	Rank 2016	Rank 2015	Rank 2016
Astra	23	23	4	3
Nahrquell	15	17	13	22
Ascar	21	21	15	7
BGR 6305	9	13	10	8
Shienfield Gard	20	20	9	18
WAT	18	19	17	11
Kijewskij Mutant	22	22	21	20
Hetman	19	18	23	23
Start	14	14	16	17
Amiga	8	8	19	21
Garant	7	7	5	13
Tel Keram	4	4	6	4
Bezimenii 1	11	10	8	10
Bezimenii 2	2	2	11	9
Pflugs Ultra	6	5	12	6
Termis Mestnii	5	6	**2**	**1**
Horizont	1	1	7	15
Solnechnii	3	3	**1**	**2**
Pink Mutant	16	15	3	5
Manovitskii	10	9	18	19
Barde	12	11	14	16
Dega	13	12	20	12
Desnyanskii	17	16	22	14

A significant part of varieties fell into the third quadrant, which determined them as stable (with low variability) and low-productive. To this group may be added cultivars Nahrquell, WAT, Amiga, Manovitskii, Barde and Dega, for which the change in growing conditions had an impact to a less extent and the displacements were within the same quadrant. With low productivity and high variability were characterized cultivars Kijewskij Mutant, Start and Desnyanskii (during 2015) and Shienfield Gard (during 2016), located in the fourth quadrant.

Rank analysis of seed productivity and crude protein content: As a result of the rank analysis conducted (Table 1), it was found that, according to the quality trait of crude protein content (CP) in the seed, most of the white lupine cultivars retained their position or fell into the adjacent rank. Cultivars Horizont, Bezimenii 2, Solnechnii, Tel Keram, Pflugs Ultra and Termis Mestnii occupying the first positions (from 1 to 6) in the order, had higher protein content.

By the complex quantitative trait "seed weight per plant" was observed a phenomenon known as a redefinition of the genetic formula of the trait when changing the limits of the environment i.e. cultivars changed their rank by productivity. Cultivars Termis Mestnii and Solnechnii occupied stably 1 and 2 positions in the ranked list during both years, followed by cultivars Pink Mutant and Astra with ranks 3-5 and 4-3, respectively. For the study period, Cultivars Hetman (23), Kijewskij Mutant (20-21), Amiga (19-21) and Manovitskii (18-19) showed productivity below the average for the group and a high rank.

According to Skuridin & Baginskaya (2001), the concept of "hereditary elements" was broad enough within the meaning and included not only the combination of the potential traits of the organism but also a combination of all interconnections determining by the hereditary constitution. In this case, the base postulate can be formulated as "genotype - the whole set of potential traits of the organism and the interrelationships between them determined by the heredity," and "paratip - the not-herited in the organism." These postulates defined another common methodology for analyzing the influence of heredity and environment on a given phenotype, as well as identifying the relationships determined by the factors of biological variability, heredity, environment, etc.

Dragavtsev (2002) and Ismoilov (2006) considered that in the hybrid populations the paratypical (ecological) variability of the quantitative parameters significantly exceeded the genotypic variability. For this reason, the selection of hybrids on phenotype, as well as the subsequent evaluation when creating a candidate variety, was extremely difficult.

Skuridin & Kova (2002) was of the opinion that methods for standardization of conditions or use of background indices and corrections were necessary to reduce the paratypic dispersion in the total phenotypic dispersion. The task is complicated because the subjects of the selection are usually separate traits, while the overall yield is generally a result of the interaction of these parameters.

In her studies, Kocherina (2007) reported that the traditional principle of identifying the genotype of the individual organism by its phenotype was being applied now - by testing the generations. However, the frequency of occurrence of unique genotypes in the real plant population is so low, and the population is so large, that the breeder is forced into the early stages of the selection process to scrap by phenotype to 80-90% of the individuals in the crumbling (on separate traits) population.

Conclusions

The application of traditional analyzes and methods in selective genetic research should be combined with the studying the ecological and genetic model for the organization of the quantitative trait which discloses a new perspective for the selection of plants. It was presented an opportunity for measuring the phenotypic and genotypic dispersions in a two-dimensional system of trait coordinates without changing the generation.

The issue of breeding identification of the best white lupine cultivars from the available collection in their phenotype was examined. A rationale of the principle "background trait" for more effective breeding activity was presented.

The assessment of ecological stability determined cultivars Tel Keram, Bezimenii 2, Pflugs Ultra and Solnechnii as stable, highly productive and suitable for growing in a wide range of environmental conditions. Cultivars Nahrquell, WAT, Amiga, Manovitskii, Barde and Dega. can be characterized as low-variable and low-productive.

Cultivars BGR 6305, Tel Keram, Pflugs Ultra, Horizont and Solnechnii showed stability of their genetic systems with environmental changing limits and can be used as donors in regard to attraction and adaptiveness to develop hybrid forms with high seed productivity and crude protein content in the seeds.

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