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ORTHOGONAL REGRESSIONS OF PEA (*Pisum* L.) VARIETIES

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ABSTRACT

The study was conducted in 2014-2016 on the experimental field of the Institute of Forage Crops, Pleven, Bulgaria. The plant material from the aboveground and root mass of seven forage peas (*Pisum* L.) genotypes and varieties, i.e. X07P54, X06PWY, NDPO80138-B-2, CA1P, L020140, Wt6803 and Mir was analyzed. The ecological and genetic model for the organization of the quantitative trait and the method of the orthogonal regressions as a method of study and evaluation were applied. The highest and stable yield of fresh root mass was obtained at Wt6803 (12.9 kg ha^{-1}), NDPO80138-B-2 (10.9 kg ha^{-1}) and L020140 (7.24 kg ha^{-1}). X07P54, X06PWY and CA1P were low productive (6.4 to 9.1 kg ha^{-1}) and highly resistant to this signs. Stable green mass yields were Wt6803 (388 kg ha^{-1}) and Mir (376 kg ha^{-1}) ranked respectively with 1 and 2 rank. Plants from Wt6803 and Mir were shown good results on the modules productivity of fresh aboveground and root mass, and from NDPO80138-B-2 by nodule weight and fresh root mass weight per plant. The varieties Wt6803, Mir and L020140 are of interest to the selection due to the successful balance of physiological systems - attraction and adaptability to the weight of the fresh aboveground and root mass. They can be included in hybridization programs to obtain high-yield genotypes for these signs.

Key words: Adaptability, Attraction, Module, *Pisum sativum* L., Phenotype, Sign.

INTRODUCTION

In modern genetics it is assumed that quantitative signs are controlled by polygenic systems represented by multiple genes each of which has a small part in the manifestation of the sign. When analyzing the variability of the quantitative trait, the biometric approach is widely used to assess the aggregate of additive and non-additive genes, the level of inter- and intercellular interactions, and the role of random factors in the formation of an attribute. It is possible to reveal the role of genotype and environment in the formation of the phenotype (Novikova et al., 2012; Lakić et al., 2018).

Historically, pea (*Pisum sativum* L.) has been the subject of numerous genetic and physiological studies because of its ease of production, autogamous and diploid nature, relatively short generation cycle and wealth of morphological variation (McPhee and Muehlbauer, 2007; McGee, 2013; Smýkal, 2014). Furthermore, pea is cultured worldwide as an important legume crop that is able to establish a nitrogen-fixing symbiosis with *Rhizobium* bacteria (Ovchinnikova, 2012).

One of the most important directions in the selection of peas is the creation of varieties with high and stable productivity. The classical selection based on intra-hybridization methods has enabled varieties with high productive potential to be created. However, seed production still shows some instability in individual years. Contemporary varieties form a high yield only under favorable environmental conditions (Chekalin et al., 2011).

Experimental genetics has demonstrated the ability to analyze quantitative signs by the Mendel method, whose genes control a significant portion of genotypic variability (Preigel, 1989).

The study of the cenothic structure of ecosystems in different climatic areas has shown that the ratio of plants with different forms of life constituting the cenosis is dependent on environmental conditions. Although these approaches do not address the issue of the carriers of the ecological properties of plants, they contribute to the accumulation of knowledge about their ecological individuality and the understanding that the formation of adaptive responses is closely related to the physiological mechanisms in the plant organism (Shamsutdinov et al., 2014).

With regard to the observed changes in agricultural production for a shift from intensive to environmentally sustainable agriculture, one of the promising directions is to use the potential of soil micro flora more effectively. The symbiosis with nodule bacteria allows the plant to develop under nitrogen deficiency conditions (Shtark et al., 2006).

The purpose of the study is to assess the productive potential of forage pea varieties by applying the ecology-genetic model for the organization of the quantitative trait.

MATERIALS AND METHODS

The study was conducted between 2014 and 2016 years 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 the crop. Plant material from aboveground and root biomass of seven samples of forage peas - X07P54, X06PWY, NDPO80138-B-2, CA1P, L020140, Wt6803 and Mir was analyzed. The following signs and indicators have been assessing in the beginning of flowering stage: leaf fresh weight per plant (g), stem fresh weight per plant (g), aboveground mass fresh weight per plant (g), nodule number per plant, weight of one nodule (g), nodule weight per plant (g), supply of root mass of one plant in nodules, root mass fresh weight (g), root mass yield (kg ha^{-1}), green mass yield (kg ha^{-1}). Biometric measurements were made to 10 plants of each variety.

The modular organization of the quantitative trait is presented in the model of Dragavtcev (1995). According to this model, the genetic formula of the attribute consists of a multitude of discreetly displaying, functionally coherent components of a unified system. Due to the integrity of the elements of the genetic system within the whole organism, the phenotype can be presented as realization of two hierarchies - structural and temporary. The module as an elementary unit describes the organization of the quantitative trait, which consists of three interrelated attributes - one resultant and two components.

The module reflects all stages of realization of genetic formulas depending on the level of ecological factors during ontogenesis. In the modular organization of the quantitative trait, the resultant can be considered as a component in another next module.

The orthogonal regression method described by Kramer (Dragavtcev, 1995; 2002) was used. The essence of the phenomenon becomes clear when considering the divergence in a two-dimensional coordinate system with two attributes - breeding (BA) and background (BA). It should be noted that in the figures shown, so-called orthogonal regressions were used, from where the method bears the name of the orthogonal regression method. These regressions are different from those commonly used in the applied regression analysis, which are always two - A x B and B x A. Orthogonal regression is always only one - this is the major axis of the scatter ellipse or the geometric point of the points (straight line) the sum of squares of distances from which empirically the scattering point is minimal. The co-ordination system BA-BA (Breeding - Background) allows to identify the genotype of the individual organism by phenotype. In this respect, the relative share of the influence of genotype and environment is quantified in a scale of factual measurements of the attribute.

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

Climate characteristic

The study period covers three consecutive years differing in climatic terms. Figure 1 presents the data on average monthly temperatures and the amount of precipitated rainfall by months during vegetation. The vegetation 2014 is the most favorable with average monthly air temperatures for April 12.3 °C, May 16.7 °C and June 20.6 °C, and rainfall 139.8 mm, 83.0 mm and 54.3 mm, respectively. As a result of the balanced combination of air temperature and optimum rainfall it has been favorable for plant development. The second year (2015) has relatively higher temperatures in May of 18.8 °C and uneven precipitation distribution, characterized by a certain drought in April (43.6 mm) and May (30.6 mm), and a larger quantity in June (95.7 mm). The third year (2016) occupies 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 mm.

Modular organization of the quantitative trait and rank analyses

The effect of the environment on the modules determining the productivity of the fresh aboveground and root mass as well as the nodule weight of the pea specimens is presented in Table 1. The formation of the green biomass weight module is a function of the fresh weight of the leaves and stems. The conditions of the environment directly affect the expression of both component signs.

One of the most important indicators of the variability of the quantitative trait is its magnitude of variation. Within the plant population, on each of the signs, homozygous genotypes with variation can be detected and created, which does not overlap with the average amplitude of the sample. Wt6803 is ecologically more stable, ranked first, and second respectively in the rank analysis (Table 2). He has also received a median rank of 2, although in 2014 he ranks third in rank. This leniency is compensated for by the heavier leaves and stems of a plant, respectively, in the first and second year of the study of rank 1. Such compensatory reactions were found in other varieties.

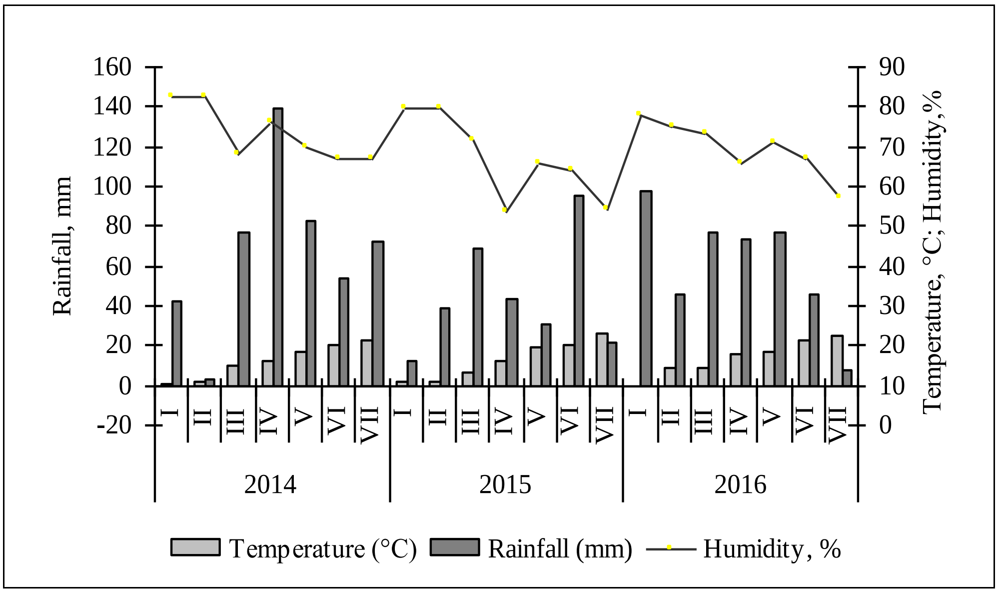


Figure 1. Climatic characterization of the experimental period

Table 1. Impact of environmental conditions on the modules fresh aboveground and fresh root mass productivity in pea varieties (at book value)

Cultivar	Year								
	2014	**2015**	**2016**	**2014**	**2015**	**2016**	**2014**	**2015**	**2016**
	Component trait 1			**Component trait 2**			**Resultant trait**		
Module I. Fresh aboveground mas weight per plant (g)									
	Leaf fresh weight per plant			Stem fresh weight per plant			Green mass weight per plant		
X07P54	2.65	8.66	2.64	6.67	24.95	9.34	11.31	31.62	11.98
X06PWY	4.19	20.95	3.60	5.94	30.46	5.60	25.14	36.40	9.11
NDPO80138B2	4.60	17.03	5.15	9.24	28.01	8.17	21.63	37.25	13.32
CA1P	5.44	6.99	3.10	14.27	21.32	8.81	12.43	35.59	11.91
L020140	6.84	13.94	5.71	12.90	20.46	15.05	20.78	33.36	19.30
Wt6803	5.79	22.45	7.88	9.70	32.41	16.22	28.24	42.11	26.65
Mir	7.27	16.05	7.69	11.23	33.41	15.95	23.32	44.64	25.99
Module II. Nodule weight per plant (g)									
	Nodule number per plant			**Weight of one nodule**			**Nodule weight per plant**		
X07P54	8.0	5.4	18.25	0.0248	0.0013	0.0023	0.198	0.007	0.043
X06PWY	7.2	8.0	29.25	0.0186	0.0039	0.0026	0.134	0.031	0.076
NDPO8013B-2	20.0	11.4	42.25	0.0093	0.0052	0.0092	0.185	0.059	0.393
CA1P	35.4	12.0	27.67	0.0067	0.0020	0.0039	0.238	0.024	0.109
L020140	16.8	56.8	30.01	0.0077	0.0082	0.0023	0.130	0.464	0.070
Wt6803	44.2	49.6	68.52	0.0031	0.0092	0.0026	0.136	0.456	0.179
Mir	12.0	35.2	90.24	0.0033	0.0083	0.0092	0.039	0.293	0.838
Module III. Fresh root mass (g)									
	Supply of root mass of one plant in nodules			**Nodule weight per plant**			**Root mass weight**		
X07P54	3.101	54.286	14.270	0.198	0.007	0.043	0.614	0.380	0.614
X06PWY	7.657	22.226	7.447	0.134	0.031	0.076	1.026	0.689	0.566
NDPO80138B2	6.005	14.610	1.935	0.185	0.059	0.393	1.111	0.862	0.761
CA1P	4.029	32.500	3.993	0.238	0.024	0.109	0.959	0.780	0.435
L020140	5.800	2.153	14.414	0.130	0.464	0.070	0.754	0.999	1.009
Wt6803	8.691	5.625	7.408	0.136	0.456	0.179	1.182	2.565	1.326
Mir	11.872	3.631	1.939	0.039	0.293	0.838	0.463	1.064	1.625

The module weight of the nodules per plant has been reassigned to the genetic control of the trait when changing the limit of the environment. The change of rank, both by the component and the resultant traits indicates that the samples have different spectra of genes that determine the final state of the module. With the highest rank in the number of nodules the Wt6803 (rank 2) variety is distinguished, while Mir and L020140 are characterized by a number of nodules above the average for the sample group tested and occupying the next rank 3.

In the second component trait - the weight of one nodule has no genotype rated at the highest rank (1). The best position (rank 3) is occupied by NDPO80138-B-2 and Mir. As a final result by the module nodule weight per plant is worth Wt6803 and NDPO80138-B-2 with a rank of 3. The low average values of X07P54 by number and weight of nodules X06PWY determine their last place of rank 5.

The two components traits - supply of root mass of one plant in nodules and nodule weight were used for the root mass module formation. Some similarity was found in the biological features of the samples tested against the green biomass yield module. The weakest variation in the supply of root mass of one plant in nodules placed in different limits of the environment is distinguished by X06PWY (rank 3). X07P54 is also rated, despite its greater instability in 2014. Its excellent performance in 2015 is the reason for it to move to a better third position. Climate conditions have had the most negative impact on plants of NDPO80138-B-2 (scale 5).

The second component trait also found differences between the pea patterns depending on the conditions of the environment. The highest nodule weight is characterized by Mir (0.838 g) and NDPO80138-B-2 (0.393 g). The applied rank analysis ranked these samples in the first position with rank 3, followed by CA1P and L020140 with rank 4, and X07P54 and X06PWY with rank 5. On average for the period of study, the highest rank (1) and corresponding mean values received the Wt6803 (1.326 g) (Table 3). The following positions were taken as follows: Mir (1.625 g) and NDPO80138-B-2 (0.761 g), L020140 (1.009 g), CA1P (0.435 g) and X07P54 (0.641 g).

In the selection of grain legumes, including peas, its productivity is used as an important criterion for the value of a genotype. It is well known that this feature, as well as other quantitative indicators, is strongly influenced by environmental factors.

Table 2. Impact of environmental conditions on the modules fresh aboveground and fresh root mass productivity in pea varieties (by rank)

Cultivar	Year											
	2014	**2015**	**2016**	**av**	**2014**	**2015**	**2016**	**av**	**2014**	**2015**	**2016**	**av**
	Component trait 1				**Component trait 2**				**Resultant trait**			
	Module I. Fresh aboveground mass weight per plant (g)											
	Leaf fresh weight per plant				**Stem fresh weight per plant**				**Green mass weight per plant**			
X07P54	7	6	7	7	6	5	4	5	7	7	5	6
X06PWY	6	2	5	4	7	3	7	6	2	4	7	4
NDPO80138B2	5	3	4	4	5	4	6	5	4	3	4	4
CA1P	4	7	6	6	1	6	5	4	6	5	6	6
L020140	2	5	3	3	2	7	3	4	5	6	3	5
Wt6803	3	1	1	2	4	2	1	2	1	2	1	1
Mir	1	4	2	2	3	1	2	2	3	1	2	2
	Module II. Nodule weight per plant (g)											
	Nodule number per plant				**Weight of one nodule**				**Nodule weight per plant**			
X07P54	6	7	7	7	1	7	6	5	2	7	7	5
X06PWY	7	6	5	6	2	5	4	4	5	5	5	5
NDPO80138B2	3	5	3	4	3	4	1	3	3	4	2	3
CA1P	2	4	6	4	5	6	3	5	1	6	4	4
L020140	4	1	4	3	4	3	6	4	6	1	6	4
Wt6803	1	2	2	2	7	1	4	4	4	2	3	3
Mir	5	3	1	3	6	2	1	3	7	3	1	4
	Module III. Fresh root mass (g)											
	Supply of root mass of one plant in nodules				**Nodule weight per plant**				**Root mass weight**			
X07P54	7	1	2	3	2	7	7	5	6	7	5	6
X06PWY	3	3	3	3	5	5	5	5	3	6	6	5
NDPO80138B2	4	4	7	5	3	4	2	3	2	4	4	3
CA1P	6	2	5	4	1	6	4	4	4	5	7	5
L020140	5	7	1	4	6	1	6	4	5	3	3	4
Wt6803	2	5	4	4	4	2	3	3	1	1	2	1
Mir	1	6	6	4	7	3	1	4	7	2	1	3

From the data presented in Table 3, it can be seen that, according to the module, the accumulation of fresh root mass, the highest and stable according to the rank analysis average yield was obtained at Wt6803 (12.9 kg ha^{-1}) (1), followed by NDPO80138-B-2 (10.9 kg ha^{-1}) (2) and L020140 (7.24 kg ha^{-1}) (3). The control Mir, although statistically significant, yields only the Wt6803, occupies a middle position among the sample group of 4 (shows greater variability) and accumulates a fresh root mass of 9.2 kg ha^{-1}. X07P54, X06PWY and CA1P are low-productive and high-variable, and they occupy the last positions in rank (5-7).

In the same samples, the lowest and unstable green mass yields were reported, respectively 219.6 kg ha^{-1}, 283.0 kg ha^{-1}and 239.7 kg ha^{-1}. The collection of samples with the highest values for this feature is characterized by Wt6803 (388.0 kg ha^{-1}) and Mir (375.8 kg ha^{-1}). The high rank (1-2), especially on the Wt6803, indicates the level of their stability. The Wt6803 is particularly impressive due to its high productive capabilities in terms of accumulation of fresh root and green mass. This variety is definitely of interest to the needs of combinatorial selection.

From the results so obtained, it can be assumed that the manifestation of the same genetic control of the quantitative sign under different environmental conditions indicates that these conditions do not result in a change in the function of the genes controlling the sign. The expression of the genes changes when the nature of the variation changes.

Orthogonal regression method. Identification of genotype by phenotype.

The ecological variability of the trait is the basis of the change in polygene activity in the stable set of genes of the polygenic system of the trait. It is believed that fluctuations in the environment give rise to ecological variability, which changes only the activity of the loci constant in the set of genes in the polygenic system. Therefore, it is not necessary to control the specific conditions of the environment. It is only necessary to measure the ecological variability, to deduce its assessment from the phenotypic, in order to obtain an estimation of the genetic variability.

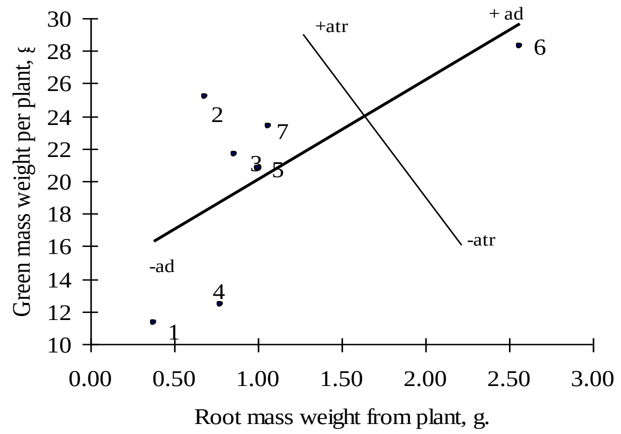
Table 3. Impact of environmental conditions on the modules fresh aboveground and fresh root mass productivity in pea varieties (at book value and rank)

Cultivar	**Limit (years)**			**Yield (kg ha^{-1})**	**Ranks**			
	2014	**2015**	**2016**	**2014-2016**	**2014**	**2015**	**2016**	**Average**
	Module Fresh root mass accumulation							
X07P54	74	46	7.4	6.4^{a}	6	7	5	7
X06PWY	123	83	6.8	9.1^{ab}	3	6	6	5
NDPO80138-B-2	133	103	9.1	10.9^{ab}	2	4	4	2
CA1P	115	94	5.2	8.7^{ab}	4	5	7	6
L020140	90	120	12.1	10.5^{ab}	5	2	3	3
Wt6803	142	116	15.9	12.9^{b}	1	3	2	1
Mir	56	128	19.5	9.2^{ab}	7	1	1	4
	Module Green mass yield							
X07P54	135.7	379.4	143.7	219.6^{a}	7	5	5	6
X06PWY	301.7	436.8	110.4	283.0^{a}	2	7	7	5
NDPO80138-B-2	259.5	447.0	159.9	288.8^{a}	4	4	4	4
CA1P	149.2	427.1	142.9	239.7^{a}	6	6	6	6
L020140	249.3	400.3	231.6	293.7^{a}	5	3	3	4
Wt6803	338.9	505.3	319.7	388.0^{b}	1	1	1	1
Mir	279.8	535.7	311.9	375.8^{b}	3	2	2	2

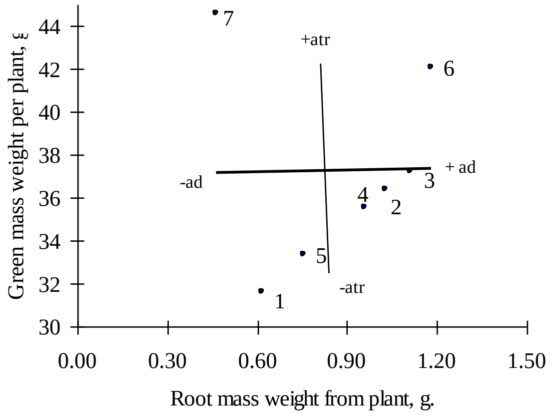
Numbers followed by the same letters are not significantly different at the 5% level of significance.

In this study, performance-related features were analyzed. In this respect, the genetic adaptability-attractiveness system was evaluated by analyzing the two-dimensional orthogonal coordinate system for the weights of green biomass and fresh root mass of the plants. The productivity of the varieties is represented graphically (Figure 2), and according to their biological capabilities, genotypes can fall into the corresponding quadrants of the coordinate system as a result of different combinations of positive and negatively changing genetic systems.

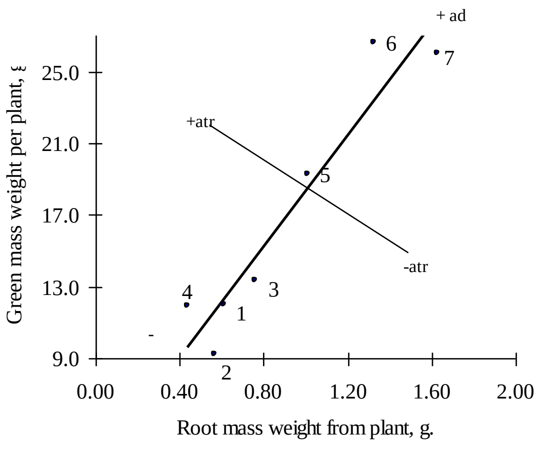
The first quadrant includes samples that have positive characteristics, both in attractiveness and adaptability. A good combination of attractiveness and adaptation genes exhibits Wt6803 for 2015 and 2016, and L020140 for the third year of the study. Under favorable environmental conditions in 2014, only the Wt6803 variety demonstrates its adaptive capabilities to the maximum extent, the expression of which is the high value of the traits tested. The remaining samples are located close to the origin of the coordinate system with a negative adaptation. It can be assumed that they will not positively react to improving the growing conditions (favorable environment).



2014 - flowering stage



2015 - flowering stage



2016 - flowering stage

Figure 2. Distribution of mean values of varieties from peas

When limiting the conditions of cultivation (2015) the patterns change their position, and adaptively valuable are X07P54, X06PWY and NDPO80138-B-2 falling within the quadrant, limited by positive adaptability and negative attraction. The layout of L020140, Wt6803 and Mir above the orthogonal coordinate system for 2016 shows their very well-defined adaptability to specific environmental conditions. For 2016, samples are located very close to the regression line and show little attractiveness. The Mir variety as well as X07P54, X06PWY and NDPO80138-B-2, can be referred to genotypes characterized by weaker genetic systems of attraction.

The analysis of changes in genetic systems of attraction and adaptability makes it possible to identify with high probability the models that are carriers of valuable genetic plasma on the studied signs. According to genetic systems attractiveness and adaptability in terms of green biomass productivity and fresh root mass weight Wt6803, Mir and L020140 are of interest for selection.

Of all the possible tasks of the genetic models used, the greatest interest in geneticists is the problem of identifying genotypes of the observed phenotype. This problem is directly related to the task of general identification and the mathematical model itself. The significance of this is that, without sufficient reliability of the model, it is impossible to carry out the identification of the genotype.

DISCUSSION

According to Morozova (2014) under the same conditions of the experiment, the main reason for differences between genotypes is the unequal physiological and biochemical state of the seeds (germ, endosperm, and perisperm). This reality determines different starting conditions for the growth and development of plants throughout the growing season. In varietal populations as well as in linear mixtures of different genotypes, the variation of the quantitative trait further includes the genetic features. Shtark et al. (2006) found that double inoculation - fungi arbusculyar mycorhisis and nodule-bacterial symbiosis increased seed productivity and dry mass of plants (selected as basic parameters for the evaluation of symbiotic activity) and in most of the studied pea genotypes exceeded the effect of mineral fertilization.

Tyurin (2014) determines the adaptability of the plant to exogenous factors in agrophytocenosis as the most important criterion for the economics value of the variety which largely determines the level and stability of yields in different climatic years. His results of studies in vetch show that the creation of high-yielding varieties in the green mass direction can be achieved by forming a larger number of leaflets on the complex leaf prior to formation of the first fertile node. Therefore, the seed productivity may increase by the higher number of seeds in pod, the number of pods per plant and the 1000 seeds weight.

Tyurin and Zolotaryov (2013) results showing a differentiated response of samples of mossy vetches of different ecological and geographical origin to environmental conditions. The authors express the need for a planetary selection of varieties, taking into account the particular phytocoenoses and the specific climatic conditions in which they will be grown.

Yessimbekova and Mukin (2014) believe that knowledge of the major environmental limits does not automatically lead to a higher level of selectivity but can be used to determine the level of genetic variability and responsiveness of a particular population.

According to Dragavtcev (2010) the Mendel model does not use the set of key environmental variables. This model can describe the effect of only 1% of the genes that determine the phenotypic manifestation of the sign and 6% gene action on the very short way gene - product. The author believes that a large percentage of genes found in eukaryotes and their effect on quantitative signs are not described by Mendel's model. Moreover, genetics have not yet found, localized and identified specific genes for productivity, yield value, horizontal immunity, and homeostasis of yield (plasticity of the variety), dry resistance, winter and cold resistance.

It is these properties that can radically increase plant productivity and yield, but still little is known about their specific genes. Therefore, Mendel's classical genetics can help genetic enhancement of quantitative signs only in rare cases where a "large" gene in a particular environment influences productivity. Through molecular genetics models, only in recent years, significant attempts have been made to "link" genes defining quantitative signs with limiting environmental factors. Kocherina (2007) considered that the use of the method of orthogonal regressions reveals significant perspectives to the selection of cultural plants.

In their studies Naumkin (2007) and Vishnyakova (2008) prove the suitability of the selection aimed at increasing the symbiotic potential and the selection of promising genotypes for inclusion in the selection process. We confirm this thesis.

The ecological-genetic model allows predicting the change of the genetic parameters in the polygene model, due to changes in the environment in which the individual development of the particular organism takes place. Within this model, the possibility of their prognosis is of great theoretical interest and is of significant practical significance. However, the ecological-genetic theory of the quantitative trait is capable of adequately describing the complex ecological-genetic 'device' of the given trait due in part to polymorphism of polygene for resistance to drought and partly to polygene for cold resistance.

As a result of the study, plants from Wt6803 and Mir show good results on the modules productivity of fresh aboveground and root mass, and from NDPO80138-B-2 by nodule weight and fresh root mass weight per plant.

The varieties Wt6803, Mir and L020140 are of interest to the selection due to the successful balance of physiological systems - attraction and adaptability to the weight of the fresh aboveground and root mass. They can be included in hybridization programs to obtain high-yield genotypes for these signs.

Author Contributions: Conceived and designed the experiments: VK and VV. Performed the experiments: VK and VV. Analyzed the data: AK, VK and VV. Wrote the paper: AK, VK and VV. Revised the paper: AK, VK and VV. All authors read and approved the final manuscript.

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