



## Application of the Ecologo-genetic Model in Broad Bean (*Vicia faba* L.) Breeding

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**Abstract.** The research was carried out at the Institute of Forage Crops (Pleven, Bulgaria) during the period 2016–2018. As methods of evaluation of 17 accessions of broad bean, the ecologo–genetic model for organization of the quantitative trait and the method of orthogonal regressions were applied. The estimation of source material of broad bean showed high productivity, expressed through seed weight per plant, in accessions BGE 029055 (40.57g), BGP (40.46g) and FbH 14 (34.85g) classified with ranks 4, 9 and 8, respectively. The traits of number of pods and seeds per plant were well combined in BGE 029055 (15; 3), BGP (12; 3) and Fb 2486 (12; 3), which also had the highest ranks (3–5). The estimation of source material through genetic–physiological systems allowed with a high probability to be selected appropriate parental forms. BGE 029055 had strong genes of the physiological systems (attraction and adaptability) in terms of productivity. BGE 046721, Fb 2486 and BGE 029055 were of interest and may be included in the combinative breeding for the development of adaptive and high–yielding cultivars.

**Keyword:** broad bean, genotype, phenotype, productivity.

### Introduction

Broad bean (*Vicia faba* L.) is an important legume crop which is mainly used as a protein source in human diets, as feed for animals as well as a source of available nitrogen in the biosphere [RUBIALES, 2010]. Expansion of broad bean areas is desirable, but it is hampered due to unstable yields [LINK *et al.*, 2010].

The genetics of quantitative traits have developed for many years mainly on postulates for polygenic inheritance, multifactority, strong dependence on genotype–environment effects, and the laws of variation statistics. The essential changes in genetics are related to the achievements of molecular biology and the ability to determine the loci of quantitative traits [HAVKIN, 1997; SYUKOV and CHESNOKOV, 2013, CAUNII, *et al.*, 2015].

Studies of different scientists have shown that different QTL (quantitative trait loci) are exhibited for one and the same characteristics under different environmental conditions. These data are in good consistency with the concept of redistribution of genetic formulas based

on the ideas of the complex ecologo–genetic organization of the quantitative signs in which the regulatory gene system plays a leading role [DRAGAVTSEV, 1983; CHESNOKOV, 2013]. The concept of the ecological–genetic model is not contrary to the idea of using marker genes for the purpose of quantitative signs breeding and using the molecular–genetic approach in different ecological and geographic areas [CHESNOKOV *et al.*, 2008; SYUKOV *et al.*, 2012]. So far, genetics researches have been primarily linked to the trait analysis, which is based on the popular postulate about unambiguous accordance "gene–trait" and the vision of genotype as a totality of potential signs. The biometric approach in the genetic analysis of quantitative signs, as a rule, does not satisfy all the requirements of theory and selection practice, as it uses models and methods that are often based on very inaccurate assumptions [IVANOV, 2011, SAMFIRA, *et al.*, 2015]. The phenomenological approach, on the contrary, uses precise methods of mathematical statistics to analyze quantitative signs but does not



take into account (so explicitly) the concrete mechanisms of inheritance. These approaches are not mutually exclusive, and each of them has advantages and disadvantages [DRAGAVTSEV, 2017].

The aim of the study was to make an attempt for a transition from breeding, based on the concept of "gene-trait", to breeding of genetic-physiological systems in broad bean.

### Material and methods

The research was carried out at the Institute of Forage Crops (Pleven, Bulgaria) during the period 2016–2018. The collection of broad bean (*Vicia faba* L.) included 17 accessions with different origin as follows: Spain (BGE 002106, BGE 029055, BGE 032012, BGE 041470, BGE 043776, BGE 046721), Portugal (Fb 1896, Fb 1903, Fb 1929, Fb 2481, Fb 2486, Fb 3270) and Bulgaria (FbH 13, FbH 14, FbH 15, FbH 16, BGP). The experiment was conducted in a field for organic production, without use of fertilizers and pesticides. A randomized block method was used, with a size of the plots of 4 m<sup>2</sup>. Sowing was manual, with a seed rate of 30 seeds per m<sup>2</sup>. The biometric characteristics included the following traits: number of pods and seeds per plant, number of seeds per pod, seed weight per plant (seed productivity), weight of a grain.

The modular organization of the quantitative trait was presented according to the model of Dragavtcev [DRAGAVTCEV, 1995]. According to this model, the genetic formula of a trait consisted of multiple mutually ordered components of a unified system. As a consequence of the integrity of elements of the genetic system within the organism, the phenotype can be represented as the 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 traits (one resultant trait and two component traits). The module reflects all stages of the realization of the genetic formulas depending on the level of ecological factors during ontogenesis.

In the modular organization, the resultant trait can be considered as a component in another next module. For example, component trait 1 × component trait 2 = resultant trait. For statistical processing of experimental data, including regression, dispersion and rank analysis, were used MS Excel (2003) and Statgraphics Plus for Windows Version 2.1 .

### Results and discussion

The study period covered three consecutive years, differing in climatic terms. The first two years were more favorable for the broad bean development due to a balanced combination of air temperature (15.7 °C on average for the vegetation period) and an optimum amount of rainfalls (278 mm on average). Relatively unfavorable was the third experimental year (2018) due to a higher average daily temperature (19.2 °C) during the active vegetation period and an uneven rainfall distribution, characterized by drought periods in April (19.6 mm) and May (47.7 mm), and a considerable amount in June (155.2 mm).

#### The modular organization of the quantitative trait and rank analysis

In the modern system of sustainable and environmentally friendly agriculture, an important requirement for the new cultivars is to be high yielding but at the same time ecologically stable. The redefining genetic formula of the trait as a result of genotype × environment interaction raises the question of revealing new models of the complex quantitative traits. The ecologo-genetic analysis is based on the fact that different genotypes have different genetic formulas that determine the expression of each trait. The phenomenon is known as a redefinition of the genetic formula of the trait under changing environmental conditions [DRAGAVTCEV and AVERYANOVA, 1983, BARBAT, et al., 2013]. In the present, the limiting factor of the environment was the year. Analysis of the elements of productivity (Table 1) showed a considerable variation by years in the values of the studied traits. With regard to module I (seed weight per plant), genotype differences were established both on the component traits



and on the resulting trait. The number of seeds per plant during 2016 and 2017 in almost all accessions (except for Fb

3270) was higher than in 2018. In Fb 3270, the plants managed to form 40 seeds per plant in 2018.

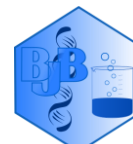
**Table 1.**

Influence of the environment conditions on modules "seed productivity/plant" and "number of seeds/plant" in broad bean accessions (by value)

Accessions	Years								
	2016	2017	2018	2016	2017	2018	2016	2017	2018
Module I: seed productivity/plant									
	Component trait 1			Component trait 2			Resultant trait		
	Number of seeds/plant			Weight of a grain (g)			Seed weight/plant (g)		
Fb 1896	25.13	31.13	22.54	1.403	1.194	0.515	35.25	37.17	11.6
Fb 1903	19.80	33.63	15.33	1.118	1.048	0.916	22.13	35.25	14.04
Fb 1929	27.38	24.43	14.50	1.119	0.985	0.483	30.64	24.08	7.00
Fb 2481	27.29	24.80	14.00	1.076	1.011	0.476	29.37	25.07	6.67
Fb 2486	29.58	37.70	29.00	1.032	1.241	0.466	30.54	46.80	13.50
Fb 3270	17.25	34.20	40.00	0.432	0.451	0.200	7.46	15.42	8.00
BGE 002106	38.80	41.90	16.00	0.859	0.745	0.688	33.33	31.20	11.00
BGE 029055	49.69	47.90	24.13	0.711	0.819	0.572	35.33	39.23	13.79
BGE 032012	41.90	31.42	19.50	0.947	0.989	0.518	39.68	31.08	10.10
BGE 041470	40.10	29.99	21.25	1.229	1.009	0.794	49.27	30.26	16.88
BGE 043776	20.70	27.60	23.25	0.937	0.873	0.559	19.40	24.10	13.00
BGE 046721	33.13	31.81	31.00	0.980	1.067	0.441	32.48	33.94	13.67
FbH 13	38.33	21.00	20.66	1.132	0.786	0.396	43.38	16.50	8.19
FbH 14	64.00	26.38	14.17	1.079	1.090	0.494	69.03	28.75	7.00
FbH 15	50.00	27.25	20.66	1.087	0.878	0.396	54.36	23.92	8.19
FbH 16	37.67	33.00	10.00	1.299	1.045	0.500	48.95	34.50	5.00
BGP	47.50	47.50	26.38	1.135	0.453	0.366	53.93	21.50	9.65
Module II: number of seeds/plant									
	Number of pods/plant			Number of seeds/pod			Number of seeds/plant		
Fb 1896	13.67	11.88	8.21	1.84	2.62	2.75	25.13	31.13	22.54
Fb 1903	9.50	12.88	6.33	2.08	2.61	2.42	19.80	33.63	15.33
Fb 1929	11.08	7.52	5.50	2.47	3.25	2.64	27.38	24.43	14.50
Fb 2481	12.64	8.54	7.33	2.16	2.90	1.91	27.29	24.80	14.00
Fb 2486	14.25	12.60	9.50	2.08	2.99	3.05	29.58	37.70	29.00
Fb 3270	7.50	11.13	11.00	2.30	3.07	3.64	17.25	34.20	40.00
BGE 002106	11.80	11.10	7.00	3.29	3.77	2.29	38.80	41.90	16.00
BGE 029055	17.31	15.38	11.17	2.87	3.12	2.16	49.69	47.90	24.13
BGE 032012	11.90	9.38	7.05	3.52	3.35	2.77	41.90	31.42	19.50
BGE 041470	11.05	7.88	6.04	3.63	3.81	3.52	40.10	29.99	21.25
BGE 043776	12.82	13.90	10.75	1.62	1.99	2.16	20.70	27.60	23.25
BGE 046721	10.35	7.75	8.67	3.20	4.10	3.58	33.13	31.81	31.00
FbH 13	12.00	9.31	9.61	3.19	2.26	2.15	38.33	21.00	20.66
FbH 14	27.00	9.75	7.33	2.37	2.71	1.93	64.00	26.38	14.17
FbH 15	13.00	7.88	9.61	3.85	3.46	2.15	50.00	27.25	20.66
FbH 16	12.00	12.40	7.25	3.14	2.66	1.38	37.67	33.00	10.00
BGP	16.00	9.50	11.16	2.97	5.00	2.36	47.50	47.50	26.38

In the formation of seed weight per plant, the weight of a single grain was used as the second component. Accessions Fb 1896 (1.403 g), BGE 041470 (1.229 g) and FbH 16 (1.299 g)

distinguished with the highest grain weight in 2016, and Fb 2486 (1.241 g) – in 2017. Also, Fb 1896, Fb 1903 and BGE 041470 exhibited the lowest variability (rank 3–4) of the trait in different limits of



the environment. In Fb 3270, BGP, FbH 13 and FbH 15, placed under unfavorable conditions, the weight of a grain sharply decreased (rank 14–17). For most of the other accessions, this instability was less pronounced. Regarding the resultant trait, variations between the accessions were

also observed. With the highest values, on average for the experimental period, were characterized BGE 029055, BGP and FbH 14 (40.57g, 40.46g and 34.85g respectively). The applied rank analysis classified these accessions with ranks 4, 9 and 8, respectively.

**Table 2.**

Influence of the environment conditions on modules “seed productivity/plant” and “number of seeds/plant” in broad bean accessions (by rank)

Accessions	Years											
	2016	2017	2018	average	2016	2017	2018	average	2016	2017	2018	average
Module I: seed productivity/plant												
	Component trait 1				Component trait 2				Resultant trait			
	Number of seeds/plant				Weight of a grain (g)				Seed weight/plant (g)			
Fb 1896	14	10	7	10	1	2	7	3	9	3	7	6
Fb 1903	16	6	13	12	7	5	1	4	15	4	2	7
Fb 1929	12	16	14	14	6	10	10	9	12	13	14	13
Fb 2481	13	15	16	15	10	7	11	9	14	11	16	14
Fb 2486	11	4	3	6	11	1	12	8	13	1	5	6
Fb 3270	17	5	1	8	17	17	17	17	17	17	13	16
BGE 002106	7	3	12	7	15	15	3	11	10	7	8	8
BGE 029055	3	1	5	3	16	13	4	11	8	2	3	4
BGE 032012	5	9	11	8	13	9	6	9	7	8	9	8
BGE 041470	6	11	8	8	3	8	2	4	4	9	1	5
BGE 043776	15	12	6	11	14	12	5	10	16	12	6	11
BGE 046721	10	8	2	7	12	4	13	10	11	6	4	7
FbH 13	8	17	9	11	5	14	14	11	6	16	11	11
FbH 14	1	14	15	10	9	3	9	7	1	10	14	8
FbH 15	2	13	9	8	8	11	14	11	2	14	11	9
FbH 16	9	7	17	11	2	6	8	5	5	5	17	9
BGP	4	2	4	3	4	16	16	12	3	15	10	9
Module II: number of seeds/plant												
	Number of pods/plant				Number of seeds/pod				Number of seeds/plant			
Fb 1896	5	6	9	7	16	14	6	12	7	10	14	10
Fb 1903	16	3	15	11	14	15	8	12	13	6	16	12
Fb 1929	13	17	17	16	10	7	7	8	14	16	12	14
Fb 2481	8	13	10	10	13	11	16	13	16	15	13	15
Fb 2486	4	4	7	5	14	10	4	9	3	4	11	6
Fb 3270	17	7	3	9	12	9	1	7	1	5	17	8
BGE 002106	12	8	14	11	4	4	10	6	12	3	7	7
BGE 029055	2	1	1	1	9	8	11	9	5	1	3	3
BGE 032012	11	11	13	12	3	6	5	5	11	9	5	8
BGE 041470	14	14	16	15	2	3	3	3	8	11	6	8
BGE 043776	7	2	4	4	17	17	11	15	6	12	15	11
BGE 046721	15	16	8	13	5	2	2	3	2	8	10	7
FbH 13	9	12	5	9	6	16	13	12	9	17	8	11
FbH 14	1	9	10	7	11	12	15	13	15	14	1	10
FbH 15	6	14	5	8	1	5	13	6	9	13	2	8
FbH 16	9	5	12	9	7	13	17	12	17	7	9	11
BGP	3	10	2	5	8	1	9	6	4	2	4	3

The specific biological possibilities of broad bean also were observed in regard to the module II (seeds number per plant). There was a certain analogy with the previous module. This is because the number of seeds participated in module II not as a component trait but as a resultant trait. Lower values of this trait negatively affected the total weight of seeds per plant, although a compensatory reaction by the plant was also possible through the second component trait.

On average for the study period, the highest rank (3) and corresponding seed numbers per plant were obtained for BGE 029055 and BGP (Table 2). Fb 2486 occupied third position with rank 6. Fb 1929 and Fb 2481 were assessed with ranks 14 and 15 and occupied the last places due to the smallest number of seeds which they formed. In the formation of module II (number of seeds per plant), two component traits were used: number of pods per plant and number of seeds in



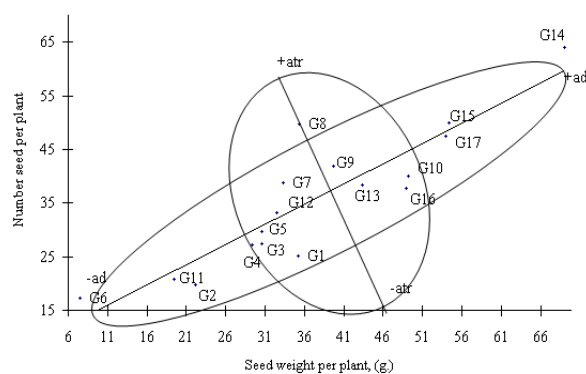
a pod. During 2016, accessions FbH 14 (27.00 g), BGP (16.00 g) and Fb 2486 (14.25 g) were distinguished with increased numbers of pods, and during the unfavorable year 2018 – these were BGE 029055 (11.17 g), BGP (11.16 g) and Fb 3270 (11.00 g). BGE 029055 (rank 1), followed by BGE 043776 (rank 4), Fb 2486 and BGP (rank 5), exhibited the lowest variability of the trait placed in different limits of the environment.

In terms of the second component trait (seeds number in a pod), particularly interesting were BGE 041470 and BGE 046721, as well as BGP, although the last one did not retain its productive possibilities when changing climatic conditions. In all accessions, it was found that by increasing the number of seeds in a pod, the stability decreases. FbH 15 and Fb 3270 were characterized by a little above the mean level of the trait (about 3 seeds in a pod) and a satisfactory level of variability.

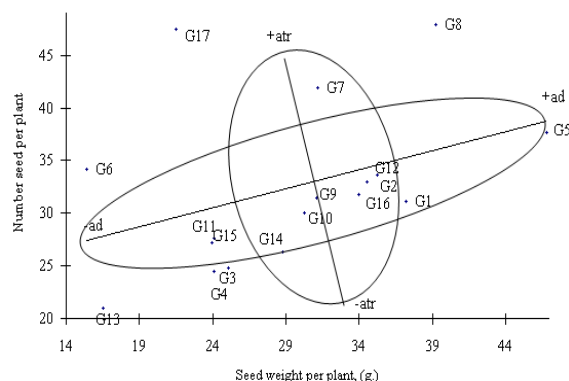
The average number of seeds in a pod for the period 2016–2018 varied in relatively narrow ranges (2–3). In most broad bean accessions studied, the increased number of pods per plant was determined by the number of seeds in a pod. A good combination between the number of pods per plant and seeds number in a pod was characterized for BGE 029055 (3) and BGP (3), which had the highest complex assessment in rank. Fb 2486 occupied the third with rank 6. The last places occupied accessions Fb 1929 (14), Fb 2481 (15) and BGE 046721 (12), which shows the limited possibilities of these genotypes to form more seeds per plant.

### Method of orthogonal regressions

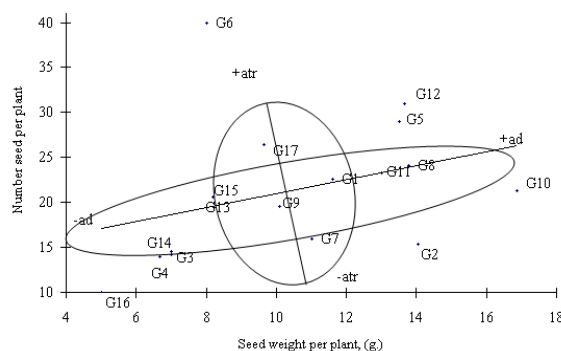
Unlike methods for genetic analysis of traits, the method of orthogonal regressions offered a study of 7 genetic-physiological systems.



2016



2017



2018

**G1** – Fb 1896, **G2** – Fb 1903, **G3** – Fb 1929, **G4** – Fb 2481, **G5** – Fb 2486, **G6** – Fb 3270, **G7** – BGE 002106, **G8** – BGE 029055, **G9** – BGE 032012, **G10** – BGE 041470, **G11** – BGE 043776, **G12** – BGE 046721, **G13** – FbH 13, **G14** – FbH 14, **G15** – FbH 15, **G16** – FbH 16, **G17** – BGP

**Figure 1.** Distribution of mean values of broad bean accessions

The use of this method in the breeding evaluation allows to optimize the process of selection of perspective material for the different directions of use as well as potential parental forms for the

needs of the combinative breeding. In the graph presented in **Figure 1**, so-called orthogonal regressions were used, from where the method takes its name. These regressions are different from those

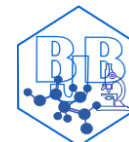


commonly used in the applied regression analysis, which are always two ( $A \times B$  and  $B \times A$ ). Orthogonal regression here is only one—this is the major axis of the ellipse of scattering or the geometric place of the points (straight line). The coordinate system (selection–background trait) allows identifying the genotype of the individual organism by phenotype. Moreover, the relative share of influence of genotype and environment is estimated on a scale of factual measurements of the trait. The method of orthogonal analysis suggests the identification of genotypes (varieties, hybrids) by "displacement" of the organism's genetic and physiological systems—attraction (attr), microdistribution of photosynthesis products, adaptability (ad), polygenic immunity, response to limiting factors of soil nutrition, tolerance to increased stand density, genetic variation of the duration of the ontogenetic stages. As selection criteria are used the direction and magnitude of the displacement of genotypes in the coordinate system (according to the trait studied) under change of the limiting factors of the environment.

In the present study, in case of worsening of the cenotic conditions (2018), accession BGE 041470, which was located in the quadrant, limited by the positive part of adaptability and the negative part of attraction, was the most adaptive one. This accession, regarding its low attractiveness, retained its position also under favorable conditions (2016 and 2017). In a better position were BGE 046721 and Fb 2486, which showed a good combination of genes for adaptiveness and attraction. BGE 046721 retained its good adaptability and in 2017. BGE 046721 (as demonstrated in the graph for 2017) had the highest adaptability, although (in the particular environment) its closeness to the regression line showed it did not exhibit strong genes for attraction. This accession did not respond positively to the improvement of the environment conditions (2016), moving to the negative part of the line of adaptability and occupying a position near above the regression line. This showed that genetic

control on the adaptability and attraction was redefined negatively at these limits of the environment. Accessions Fb 2481 and Fb 1929, which are located in the negative part of the adaptation line, were the least adaptable. They did not respond positively, even placed in a favorable growing environment. Of particular interest was BGE 029055, which was characterized by positive manifestations of the genes for adaptability and attraction in 2017. It retained its position in the same quadrant also in the other two years of study. In 2016, its adaptive ability was very low (approaching 0), but not negative, and in 2018 its attraction was weak but with positive value. The greater distance to the line of attraction (the line of orthogonal regression) in 2018 compared to 2016 showed that this accession had strong genes for adaptiveness. Accession FbH 14 was located in the quadrant, limited by the positive part of the lines for adaptability and attraction in 2016. In 2017 and 2018, it occupied a position in the opposite quadrant, which determined it as responsive to improving cultivation conditions and having weak genes for adaptability. The negative displacement in 2018 of FbH 16, which was among the highly productive accession regarding adaptability and its weak genes for attraction, defined it as a genotype with a predictable response to the environment. Under unfavorable conditions, it can cede to the rest in terms of productivity.

From the studied broad bean accessions, BGE 029055, BGE 046721 and Fb 2486 were of interest in combinative breeding. They were relatively well adapted to environmental changes and were characterized by satisfactory attraction [IANCULOV, *et al.*, 2004, PETRACHE, *et al.*, 2014]. Skuridin and Koval found that in hybrid populations the ecological (paratypical) variability of quantitative signs significantly exceeded the genotypic [SKURIDIN and KOVAL 2002, RASHED and BUTNARIU, 2014]. The authors considered that the selection of hybrids on phenotype, as well as the subsequent breeding assessment in creating cultivars, was a difficult task. In order to reduce the



paratyptic component in the total phenotypic dispersion, methods of standardization of the conditions by using background indices and corrections should be applied in the studies [BUTNARIU, et al., 2015]. Lutova pointed that over the past 50 years, the total volume of world agricultural production has grown about 2–3 times, and the area of agricultural land – only 12% (as a further increase is almost impossible) [LUTOVA, 2012]. According to the author in developed countries, the increase in grain legume yields was 95%, depending on the improvement of the breeding technologies and only 5% as a result of the crop technology improvement. As a result of longstanding researches, Dragavtsev concluded that the introduction of genetic engineering technologies has begun without much clarity and understanding of the ecological and genetic "device" of the traits determining the plant productivity and completely ignoring the ecological and genetic mechanisms of the interaction genotype  $\times$  environment, whose management determined the productivity of new cultivars [DRAGAVTSEV, 2012].

In some recent studies, Dragavtsev and collab. proposed to pay more attention to the theory of ecologo–genetic organization of the quantitative trait and good opportunities (thus created) for the introduction of new highly breeding technologies [DRAGAVTSEV et al., 2016, 2017, BUTNARIU, 2012]. The authors emphasized the need to solve the main problem in the initial stages of breeding, namely to create reliable methods for identifying the best genotypes in the early generations.

### Conclusions

The estimation of source material of broad bean showed high productivity, expressed through seed weight per plant, in accessions BGE 029055 (40.57g), BGP (40.46g) and FbH 14 (34.85g) classified with ranks 4, 9 and 8, respectively. The traits of number of pods and seeds per plant were well combined in BGE 029055 (15; 3), BGP (12; 3) and Fb 2486 (12; 3), which also had the highest ranks (3–5).

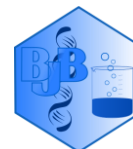
The estimation of source material through genetic–physiological systems

allowed with a high probability to be selected appropriate parental forms. BGE 029055 had strong genes of the physiological systems (attraction and adaptability) in terms of productivity.

BGE 046721, Fb 2486 and BGE 029055 were of interest and may be included in the combinative breeding for the development of adaptive and high–yielding cultivars.

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