

Evaluation of the genetic diversity in broad bean (*Vicia faba* L.) accessions

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Abstract

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In order to evaluate the level of genetic diversity in a collection of broad bean accessions for the needs of the combinative breeding, a field experiment was carried out during the period 2016-2018, at the Institute of forage crops (Pleven). The studied 17 accessions had different origin: Portugal, Spain, Bulgaria. It was used a randomized block method. Plants were grown under conditions of organic farming, without using fertilizers and pesticides. The results showed considerable differences between the accessions regarding the main quantitative traits. The impact of environmental conditions was well expressed and exceeded the impact of the factor of genotype. The level of genetic variance was lower for the traits of seed weight (6.84%), pods number (9.49%) and 1st pod height (9.77%). The coefficient of genetic variance was higher than the coefficient of phenotypic variance for pod length. In terms of the traits of pod length (84.32%, 1.33), 100 seeds mass (69.08%, 7.87) and 1st pod height (52.76%, 1.26) was found high heritability, coupled with medium to high genetic advance, which is a prerequisite for increasing the productive potential of the broad bean in these traits. Positive correlations were established between seed productivity and all quantitative traits, especially 100 seeds mass ($r = 0.574$), pod length ($r = 0.568$) and plant height ($r = 0.411$). From the breeding point of view, accessions BGE 041470 and Fb 2486, which were distinguished by high values of pods number, seeds number and 100 seeds mass, were of interest. Also, attention deserves to be paid to FbH 14, which formed a large number of pods and seeds per plant, as well as FbH 16, which was characterized by a good balance between the traits of seeds number, seed weight, and 100 seeds mass. These accessions are definitely of interest for hybridization programs and can be recommended as parent components in selection for increased productivity.

Keywords: broad bean; accessions; genetic diversity; breeding; heritability

Introduction

One of the oldest crops is broad bean (*Vicia faba* L.), belonging to family Fabaceae, order Fabales, class Magnoliopsida, division Magnoliophyta. The Middle East and the Mediterranean are considered to be its primary original center, after which it has spread to Central Asia, Afghanistan, India (Vishniakova, 2008, Potokina et al., 2008) and subse-

quently to the European countries. In countries like Great Britain and France, it is mainly used as feed for livestock, and in the Mediterranean countries, the Middle East and China – mainly in culinary industry (Duc et al., 2010).

Broad bean can be included in different growing systems, contributing to their sustainability through its ability to provide nitrogen via biological fixation; diversification of production, leading to pest reduction; its capacity to reduce en-

ergy consumption from fossil fuels; providing food and feed, which are rich in proteins (Jensen et al., 2010). In addition, the broad bean, along with other legumes, plays an important role in soil revegetation and therefore it is of importance to the modern systems of organic farming (Vishniakova, 2008; Balashova et al., 2011).

Broad bean is grown in more than 55 countries, as the harvested area is 2.46 million ha, and the total production is 4.84 million tons of dry grains. Over the past 50 years, the areas have been reduced by 34%. On the other hand, grain yields increase from 980 kg/ha (1961-1963) to 1964 kg/ha (2017) (FAOSTAT, 2017), mainly due to the replacement of the old cultivars with new ones (Fouad et al., 2013). Gene banks in the world conserve more than 36000 accessions (Fouad et al., 2013). As a whole, the total production is dominated by local landraces and cultivars, despite a number of improved cultivars which are developed by different national breeding programs. The main reason for the low yields and insufficient production is the sensitivity of landraces and cultivars to biotic and abiotic stress. The fluctuations in the harvested areas and the negative effects of different biotic and abiotic stress have led to a reduction in genetic diversity (Singh et al., 2013).

The purpose of the present study was to evaluate the genetic diversity in a collection of broad bean accessions for the needs of combinative breeding.

Materials and methods

The field trial was carried out during the period 2016-2018 at the Institute of Forage Crops (Pleven, Bulgaria) with 17 accessions of broad bean (*Vicia faba* L.): Fb 1896, Fb 1903, Fb 1929, Fb 2481, Fb 2486, Fb 3270 (originating in Portugal), BGE 002106, BGE 029055, BGE 032012, BGE 041470, BGE 043776, BGE 046721 (Spain), FbH 13, FbH 14, FbH 15, FbH 16, BGP (Bulgaria). Sowing was done manually, with a sowing rate of 40 seeds/m². It was used a randomized block method (Barov, 1982), with a plot size of 4 m². Plants were

grown under conditions of organic farming without using fertilizers and pesticides. At harvesting, biometric characteristic was done, which included the following traits: plant height, 1st pod height, pods number per plant, pod length, pod width, seeds number per plant, 100 seeds mass, seed weight per plant.

The following methods were used to process the experimental data: two-factor analysis of variance for determining the influence of factors genotype (accession) (G) and environment (E) for each trait; factor analysis by the method of principal components (Vandev, 2003); hierarchical cluster analysis by the method of Ward (1963) – for grouping accessions based on similarity as a measure of differences (the genetic distance); Euclidean distance between the studied accessions was calculated (as a measure for divergence) as the data were standardized preliminary. In addition, GGE biplot model was done, which uses singular value decomposition of first two principal components (Yan, 2002). Genetic advance in absolute unit (GA) and genetic gain (GG), assuming selection of the superior 5% of the genotypes, were estimated in accordance with the methods illustrated by Johnson et al. (1995).

All experimental data were processed statistically using the computer software GENES for 3a Windows XP (Cruz, 2009).

Results

The study of the patterns of the varying quantitative traits allows for a more precise selection of the hybridization source material, taking into account the influence of the modification variability.

Analysis of variance

The analysis of variance and obtained values of the mean squares in relation to the studied traits are presented in Table 1. The influence of the environmental conditions (years) was well expressed in all traits (except pod width) and exceeded the influence of the factor of genotype (accession). The lack of statistical significance for this trait determined similarity

Table 1. Analysis of variance regarding the studied traits in broad bean accessions

Source of variation	DF	Mean squares			
		plant height	1 st pod height	Pods number	seeds number
Years	2	4281.78*	234.76*	96.88*	975.35*
Accessions	16	59.48*	49.42***	10.86*	97.28*
Residuo	32	60.701	23.348	7.737	89.1863
Source of variation	DF	pod length	pod width	100 seeds mass	seed weight
Years	2	37.72*	1.43 ^{ns}	11826.59*	3189.20*
Accessions	16	5.95**	0.22 ^{ns}	766.53***	106.72*
Residuo	32	0.933	0.140	236.984	97.467

*significant at P < 0.1; ** at P < 0.05; *** at P < 0.01 ; ns = not significant

between the studied accessions. On the other hand, the data demonstrated that the mean squares of broad bean accessions were highly significant in terms of 1st pod height, pod length and 100 seeds mass. These results indicated the existence of considerable differences between the accessions and allowed an evaluation of genetic similarity.

Genetic parameters

The data in Tables 2 and 3 provide useful information on the general phenotypic variation of the traits (determined by the combined action of genetic and environmental factors), which is very important for the researcher under use of available genetic resources and to predict future breeding success. The values of the genetic variance of the traits (except pod length) were smaller than the corresponding phenotypic variances. The numerical expressions of the genotype variance ranged from 0.03 (pod width) to 176.52 (100 seeds mass). Compared with other traits, the genotype variances regarding 1st pod height (8.69), seeds number (2.70) and seed weight per plant (3.09) were relatively high. The highest phenotypic variance had the 100 seeds mass (236.98) and the lowest one (0.03) – pod width.

A high phenotypic variance was also observed for seed weight (97.47) and seeds number (89.19), while for 1st pod height and pod numbers, lower values were obtained. This indicates that the traits with a higher phenotypic variance are more strongly influenced by environmental conditions.

Genetic parameters are a function of environmental changes, and therefore broad bean accessions placed in another environment may receive an assessment different from that obtained in this study. The differences in values of the phenotypic variance versus the genetic variance were greater for seed weight, seeds number, 100 seeds mass and plant height, indicating that the expression of these traits was under polygenic control.

Coefficients of phenotypic and genotypic variance

In order to detect genotypic differences between particular varieties in main traits, breeders often use the coefficients of phenotypic (CV_p) and genotypic variance (CV_g). The values of CV_g were used as a part of the genetic variability evaluation. The calculated coefficients of CV_g for the studied traits are presented in Table 2, with ranging from 5.50% to 15.96% for seeds number and 100 seeds mass, respectively. The relative values of CV_g for seed weight (6.84%), seeds number (9.49%) and 1st pod height (9.77%) showed that the variation was low. Only in relation to pod length, CV_g was greater than CV_p. The ratio of CV_g to CV_p for it was greater than one, which implies an expected success in the breeding for this trait. In terms of 100 seeds mass, pod

length, 1st pod height, and pod width, there was a relatively small difference between CV_g and CV_p, indicating that the observed variation was mainly due to genetic factors rather than environmental factors.

Genetic advance

Genetic advance (GA), is a breeding parameter, whose value can be used to assess the expected progress in the breeding process (Table 2). From the tested characteristics, the 100 seeds mass of (7.87), pod length (1.33) and 1st pod height (1.26) had the best performance. In contrast, plant height (0.06), seed weight (0.09) and pod width (0.10) occupied the last positions.

The traits of pod width (606.23), pods number (141.21), and pod length (121.94) showed a higher genetic gain (GG) than 100 seeds mass (15.27) and plant height (13.55). When a combination of different breeding approaches is applied, the expected genetic gain would be higher than the individual use of one method.

Coefficient of heritability

The coefficient of heritability in a broad sense had high values 52.76–84.32%) with respect to the traits of 1st pod height, 100 seeds mass and pod length, suggesting that in

Table 2. Genetic component of variation for quantitative traits in broad bean accessions

Parameters	Plant height	1 st pod height	Pods number	Seeds number
Min	42.00	18.00	6.00	10.00
Max	101.00	46.00	27.00	64.00
CV _g (%)	–	9.77	9.49	5.50
CV _p (%)	–	16.02	25.65	32.35
V _g	0.41	8.69	1.04	2.70
V _p	60.70	23.35	7.74	89.19
GA	0.06	1.26	0.25	0.16
GG	13.55	39.55	141.21	55.52
H ² (%)	0.05	52.76	28.80	8.33
Parameters	Pod length	Pod width	100 seeds mass	Seed weight
Min	5.00	1.00	20.00	5.00
Max	13.00	2.00	141.00	69.00
CV _g (%)	15.31	10.17	15.96	6.84
CV _p (%)	11.43	22.11	18.56	38.00
V _g	1.67	0.03	176.52	3.09
V _p	0.93	0.14	236.98	97.47
GA	1.33	0.10	7.87	0.09
GG	121.94	606.23	15.27	71.39
H ² (%)	84.32	38.71	69.08	8.68

V_g – genotypic variance; V_p – phenotypic variance; GA – genet advance; GG – genetic gain; H² (%) – broad-sense heritability

Table 3. The Eigen values and vectors of the correlation matrix for traits in broad bean accessions

Variables/ Parameters	PC1	PC2	PC3
Plant height	-0.357	-0.329	-0.415
1 st pod height	-0.241	-0.619	-0.452
Pods number	0.317	0.427	-0.372
Seeds number	0.344	0.476	-0.211
Pod length	0.367	-0.056	0.373
Pod width	0.338	-0.190	0.470
100 seeds mass	0.402	-0.173	0.283
Seed weight	0.461	0.175	0.016
Parameter			
Variability (%)	0.532	0.191	0.128
Cumulative	0.532	0.723	0.851
EigenValues	4.253	1.529	1.024

PC1, PC2, PC3 – principal component 1, 2, and 3, respectively

most cases the differences between the accessions were due to the effect of the genotypic factors, not environmental ones (Table 2). In contrast, the lowest heritability was observed in plant height (0.05%), seeds number (8.33%) and seed weight per plant (8.68%).

The obtained results showed that high coefficients of heritability combined with medium to high genetic advance were found for pod length, 100 seeds mass and 1st pod height. This is a prerequisite for increasing the productive potential in these traits and a good opportunity to develop new forms of broad bean with such characteristics. Regarding pods number, the high value of genetic gain was coupled with a medium coefficient of heritability, indicating that this trait inherited additively and breeding directed to its improvement may also be effective.

Principal component analysis

The results of the main components analysis are presented in Table 3. Three major components were defined, which explained 85.10% of the total variation in the experiment. The first component (PC1) justified 53.20% of the total variation, the second component (PC2) – 19.10% and the third (PC3) – 12.80%. These percentages illustrated the existence of complex interrelationships between the studied traits. The strongest influence had the first component, which was determined by the performances of the traits of seed weight, 100 seeds mass, pod length, and plant height. The positive values of PC1 for most of the traits (except for plant height and 1st pod height) allow for parallel breeding for their improvement, especially those with higher values. The second component was in correlation dependences with the pods number, seeds number, and seed weight. The pod length, pod width and 100 seeds mass, were related to the third component.

When the factors to which the variation can be distributed are more than two (PC1, PC2, PC3, etc.), then the influence on a given trait is non-linear. In this case, under a positive change in the environmental conditions, it could not be predicted whether the accessions (or part of them) would react adequately or would change the values of their traits in the opposite direction. All this creates difficulties in assessing the individual genotype against the background of the whole sample of accessions.

The distribution of broad bean accessions in the coordinate system of the two components with the greatest influence (PC1 and PC2) presents the grouping of the accessions by similarity based on the selected quantitative traits (Figure 1 A). Accessions Fb 2486, BGE 002106, BGE 029055, FbH 14 and BGP were characterized by positive values of the first two major components. Their location (in one and the same quadrant) was mainly due to the traits of 1st pod height, seeds number and pod length. BGE 029055 occupied a place at the top of the quadrant and was quite distant from the other accessions in this group. Its distant location, within the quadrant gave reason to believe that phenotypically BGE 029055 was not very close to the other accessions regarding some of the traits.

Accessions Fb1896, Fb1903, BGE 032012, BGE 041470, BGE 046721, FbH 15 and FbH 16 referred to the quadrant, limited by the positive values of PC1 and the negative values of PC2. In the same quadrant were located the vectors of pod width, 100 seeds mass and seed weight. This shows the dependence of the accessions (under their grouping) by the numerical values of these traits. It is noteworthy that FbH 15 is almost at the boundary between these quadrants (PC2 \approx 0). Therefore, it can be assumed that, regarding some traits, FbH 15 exhibits characteristics of the accessions in the upper quadrant.

The Fb 3270 was located in the upper left quadrant of the coordinate system, with negative values on PC1 and positive ones on PC2. Its projection in the bipolar plane indicates that it is quite different from the other accessions in a large number of traits.

Fb 1929, Fb 2481, FbH 13 and BGE 043776 were located in the lower left quadrant (with negative values on PC1 and PC2). The first three accessions were very close to each other, whereas BGE 043776 was located close to the ordinary axis (PC2). This suggests that it will be near (in terms of some traits) with Fb 3270. On the other hand, Fb 1929 from the same quadrant will be similar to FbH 16 and Fb 1903, located in the adjacent quadrant.

The graphical representation of the indicators illustrates the correlation relationships between the traits designed in the frame of the sample of 17 accessions studied (Figure 1

B). The vectors of the traits with the greatest length determine the level of diversity. From the projections of the traits in the coordinate system, it is found that the seeds number, seed weight, 100 seeds mass, pod length and pod width have determining meaning.

The position of the vectors of pod numbers and seeds number was such that they formed sharp angles between them, indicating a strong positive dependence. Similar rela-

tionships with the same sign were also found between pod width, 100 seeds mass and seed weight. Negative was the correlation of 1st pod height with seeds number per plant.

Correlation analysis

The analysis of the relations between the structural elements of productivity is essential to establish a breeding criterion. The assessment of dependences between the traits is important in choosing appropriate source selection material to achieve the intended purpose. The results of the graphical analysis were confirmed by the correlations obtained (Table 4). The seed weight was in positive (statistically significant) dependence with all traits, the strongest being with 100 seeds mass ($r = 0.574$), pod length ($r = 0.568$) and plant height ($r = 0.411$).

High correlation coefficients were obtained between pod length and seeds number ($r = 0.791$) and pod numbers ($r = 0.707$). The trait of seeds number highly interacted with pods number ($r = 0.751$), and 100 seeds mass – with pod length ($r = 0.696$).

Cluster analysis

A hierarchical cluster analysis was applied to identify the similarity of the studied accessions, and the results were presented through a dendrogram (Figure 2). The bean accessions were grouped into two main clusters based on the quantitative characteristics examined. The first cluster (at the top of

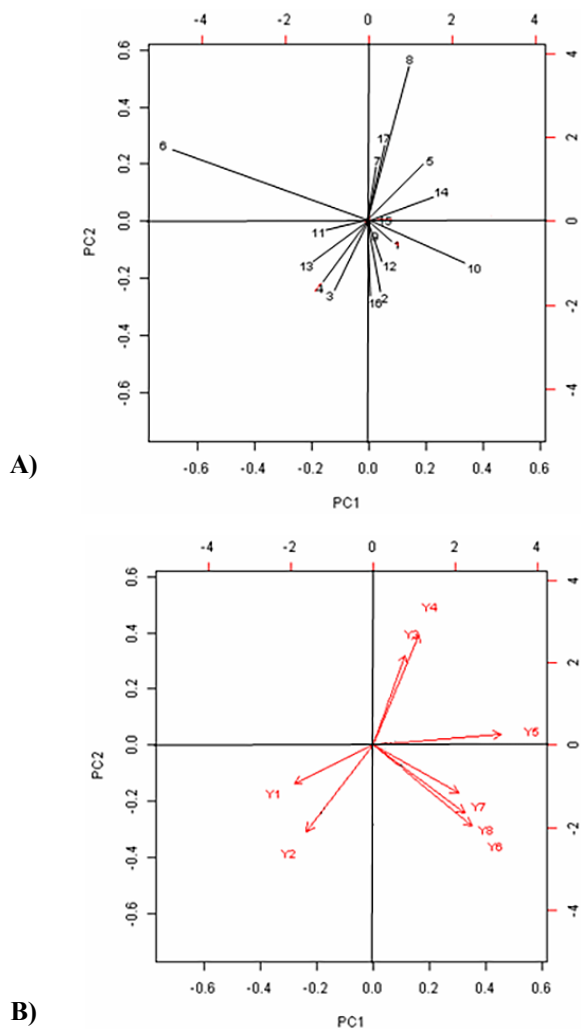


Fig. 1. Distribution of broad bean accessions and projection of the traits on the factorial plane

Variants: 1. Fb1896, 2. Fb1903, 3. Fb 1929, 4. Fb 2481, 5. Fb 2486, 6. Fb 3270, 7. BGE 002106, 8. BGE 029055, 9. BGE 032012, 10. BGE 041470, 11. BGE 043776, 12. BGE 046721, 13. FbH 13, 14. FbH 14, 15. FbH 15, 16. FbH 16, 17. BGP.

Y1 – plant height, Y2 – 1st pod height, Y3 – pods number, Y4 – seeds number, Y5 – pod length, Y6 – pod width, Y7 – 100 seeds mass, Y8 – seed weight

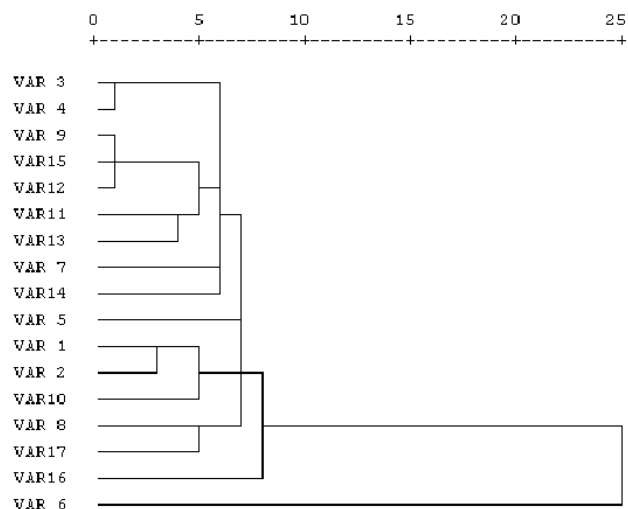


Fig. 2. Dendrogram of broad bean accessions

Variants: 1. Fb1896, 2. Fb1903, 3. Fb 1929, 4. Fb 2481, 5. Fb 2486, 6. Fb 3270, 7. BGE 002106, 8. BGE 029055, 9. BGE 032012, 10. BGE 041470, 11. BGE 043776, 12. BGE 046721, 13. FbH 13, 14. FbH 14, 15. FbH 15, 16. FbH 16, 17. BGP

Table 4. Correlation coefficients (r) among the quantitative traits in broad bean accessions

Traits	Plant height	1 st pod height	Pods number	Seeds number	Pod length	Pod width	100 seeds mass
1 st pod height	0.683**						
Pods number	0.391**	0.001					
Seeds number	0.380**	-0.069	0.751**				
Pod length	0.570**	0.186*	0.707**	0.793**			
Pod width	0.556**	0.299**	0.374**	0.310**	0.791**		
100 seeds mass	0.440**	0.191*	0.181*	0.527**	0.696**	0.621**	
Seed weight	0.411**	0.210**	0.271**	0.219**	0.568**	0.407**	0.574**

*/ ** significant at the 0.05 / 0.01 level

the dendrogram) was more numerous, and almost all accessions were involved. In the first sub-cluster, Fb 1929 and Fb 2481 were similar, and they formed a subgroup with the least distance units. Fb 1929 and Fb 2481 were distinguished by relatively low plants, formed fewer pods and seeds per plant, and occupied one of the last positions regarding seed mass (Table 5). Below them was located a second subgroup which included BGE 032012, FbH 15, BGE 046721, BGE 043776 and FbH 13. The last two accessions were genetically more distant compared to the first two. The third subgroup consisted of accessions BGE 002106 and FbH 14, which were distinguished by the pod length, seeds number and seeds weight. Their values were around and above the average for the study.

In the second sub-cluster, Fb1896 and Fb1903 were the closest related. They had very near values in terms of plant height, 1st pod height, pods number and 100 seed mass. Accessions Fb 2486 and the subgroup, including BGE 029055 and BGP, were genetically the most distant.

The third sub-cluster included FbH 16, which is characterized by high plants, high-located 1st pod, a fewer number of seeds, but with a greater seed weight than the average for the accessions studied.

The second cluster consisted of one accession (Fb 3270). Its particular position in the dendrogram was due to the fact that it had the lowest values in terms of seed weight per plant, 100 seeds mass, pod length and width, while at the same time it formed a very long stem.

It should be noted that accessions BGE 041470 and Fb 2486 were distinguished by high values of seeds number, seed weight and mass per 100 seeds. Also, attention should be paid to FbH 14, which formed a large number of pods and seeds per plant, as well as FbH 16, which was characterized by a good balance between the traits of seeds number, seed weight, and 100 seeds mass. These accessions were definitely of interest to hybridization programs and can be recommended as parent components in breeding for increased productivity.

Table 5. Distinctive features of the investigated accessions

Accessions	Plant height	1 st pod height	Pods number	Seeds number	Seed weight	100 seeds mass	Pod length	Pod width
Fb 1896	72	28	11	26	28.01	105.48	7.82	1.60
Fb 1903	71	30	10	23	23.81	102.34	8.38	1.74
Fb 1929	69	34	8	22	20.57	86.68	8.04	1.58
Fb 2481	71	35	10	22	20.37	85.46	7.36	1.62
Fb 2486	65	24	12	32	30.28	91.31	8.34	1.53
Fb 3270	78	33	10	30	10.29	36.28	6.12	1.09
BGE 002106	64	26	10	32	25.18	76.34	8.74	1.37
BGE 029055	68	22	15	41	29.45	71.72	7.16	1.51
BGE 032012	72	31	9	31	26.96	81.60	9.40	1.56
BGE 041470	69	25	8	30	32.14	101.38	11.49	1.73
BGE 043776	78	29	12	24	18.83	78.84	6.95	1.70
BGE 046721	72	34	9	32	26.70	82.95	11.54	1.55
FbH 13	79	35	10	27	22.69	80.70	7.95	1.54
FbH 14	71	33	15	35	34.93	88.70	8.45	1.78
FbH 15	71	31	10	33	28.82	81.33	8.38	1.64
FbH 16	79	35	11	27	29.48	95.17	9.06	1.73
BGP	73	28	12	40	28.36	68.60	8.55	1.58

Discussion

Khazaei et al. (2013) considered that the genetic diversity and breeding of new varieties appropriate to modern conditions were key elements in addressing the challenges of climate change. According to the authors, agronomic science and practice mainly used the available genetic resources to develop new genotypes adapted to the specific environmental conditions. More and more topical is the question of testing germplasm with a broader genetic basis and pronounced tolerance to abiotic stress.

A number of researchers (Jeberson et al., 2016; Sharma et al., 2017) reported that the mass of 1000 seeds, plant height and seeds number per plant had a high inheritance coefficient combined with high genetic advance. They concluded that in these traits predominated additive gene effects, and the environment had a lesser impact on them, so the selection for improvement of these traits would be quite effective.

Fikreselassie (2012) received results according to which the yield index had a medium (in value) heritability coefficient in a broad sense, combined with moderate genetic advance and slight impact of the environment. The author also established a high heritability coefficient associated with low genetic advance regarding different yield components. He considered that this was due to the low values of genotypic and phenotypic variance. In a study of pea genotypes in terms of some main quantitative characteristics, Ahmad et al. (2014) reported a high heritability coefficient and genetic advance, as well as a stronger impact of the environment compared to the genotype. The authors considered that in the investigated genotypes, the selection regarding these characteristics will be less effective. In addition, on the basis of values of the heritability coefficient and genetic advance in pea cultivars, Saxesena et al. (2014) found both an additive and non-additive gene effect on the manifestation of main quantitative traits.

Hefny (2013) concluded that the trait of pods number per plant in white lupine genotypes was under non-additive gene control because of very low values of the heritability coefficient and genetic advance. The environmental impact on this trait was great, and it would be difficult to make an effective selection to increase seed productivity. Other researchers (Bhuvaneswari et al., 2017) found that the yield index showed a large indirect positive effect on seed yield by the number of seeds per plant. Similar were the dependencies of seed yield with seeds number per plant and 1000 seeds mass.

Conclusions

The studied 17 accessions of broad bean showed considerable differences with respect to the main quantitative traits.

The impact of environmental conditions was well expressed and exceeded the impact of the factor of genotype.

The level of genetic variance was lower for the traits of seed weight (6.84%), pods number (9.49%) and 1st pod height (9.77%). The coefficient of genetic variance was higher than the coefficient of phenotypic variance for pod length.

In terms of the traits of pod length (84.32%, 1.33), 100 seeds mass (69.08%, 7.87) and 1st pod height (52.76%, 1.26) was found high heritability, coupled with medium to high genetic advance which is a prerequisite for increasing the productive potential of the broad bean in these traits.

Positive correlations were established between seed productivity and all quantitative traits, especially 100 seeds mass ($r = 0.574$), pod length ($r = 0.568$) and plant height ($r = 0.411$), which implies an opportunity to improve productivity through the indicated traits.

From the breeding point of view, accessions BGE 041470 and Fb 2486, which were distinguished by high values of pods number, seeds number and 100 seeds mass, were of interest. Also, attention deserves to be paid to FbH 14, which formed a large number of pods and seeds per plant, as well as FbH 16, which was characterized by a good balance between the traits of seeds number, seed weight, and 100 seeds mass. These accessions are definitely of interest for hybridization programs and can be recommended as parent components in selection for increased productivity.

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