INVESTIGATIONS ON THE RESISTANCE OF SOME BULGARIAN COMMON BEAN GENOTYPES TOWARDS BEAN WEEVIL (*ACANTHOSCELIDES OBTECTUS* SAY)

ПРОУЧВАНЕ УСТОЙЧИВОСТТА НА НЯКОИ БЪЛГАРСКИ ГЕНОТИПИ ФАСУЛ СПРЯМО ФАСУЛЕВИЯ ЗЪРНОЯД (*ACANTHOSCELIDIS OBTECTUS* SAY)

Elena APOSTOLOVA[1], Nedyalka PALAGACHEVA[2], Diyana SVETLEVA[1]* and Antonia MATEEVA[2]

[1] Depdrtment of Genetics and Plant Breeding, Agricultural University, 4000 Plovdiv, Bulgaria

[2] Depdrtment of Entomology, Agricultural University, 4000 Plovdiv, Bulgaria

*Coresponding author: E-mail: svetleva@yahoo.com

ABSTRACT

The most effective, environmentally sound and safety way to fight pests with biological means is the use of resistant varieties to them. In the present study were indicated the reactions of 30 Bulgarian common bean genotypes to the most economically important enemy – bean weevil (*Acanthoscelidis obtectus* Say). For this purpose, the following indicators were traced – seed damages and young adult insects, which largely characterized the response of different common bean genotypes to that biological pest enemy. The results of this investigation present a sensitive response to the sustainability of different genotypes to the bean weevil. The Bulgarian common bean varieties Plovdiv 11M, Abritus, Crystal and Bulgari can be used in breeding programs as donors of resistance to the bean weevil.

Keywords: *Acanthosceides obtectus* Say., bean weevil, common bean genotypes, *Phaseolus vulgaris* L.

РЕЗЮМЕ

Най-ефективният, екологосъобразен и безопасен начин за борба с биологичните неприятели е използването на устойчиви към тях сортове. В тази връзка, с настоящото проучване е установена реакцията на устойчивост на 30 сорта фасул спрямо икономически най-важния им неприятел – фасулевия зърнояд (*Acanthoscelidis obtectus* Say.). За целта са проследени следните показатели – повреда на семената и новоимагинирали възрастни, характеризиращи в голяма степен реакцията на различните сортове фасул спрямо изследвания биологичен обект. В резултат на изследването е установена чувствителна реакция по отношение устойчивостта на отделните сортове фасул спрямо фасулевия зърнояд. Сортовете Пловдив 11М, Абритус, Кристал и Българи могат да се използват в селекционни програми, като донори на устойчивост спрямо фасулевия зърнояд.

Ключови думи: *Acanthosceides obtectus* Say., фасулев зърнояд, генотипи фасул, *Phaseolus vulgaris* L.

РАЗШИРЕНО РЕЗЮМЕ

Фасулът е важна селскостопанска култура, която е обект на нападение от много неприятели, както при отглеждането му на полето, така и при съхранение. Особено важно място сред тях има фасулевият зърнояд *Acanthoscelides obtectus* Say. Възрастните насекоми отлагат яйцата си върху повърхността на зърната, а излюпените ларви се вгризват във вътрешността им, там какавидират, след което имагинират.

Един от най-ефикасните екологосъобразни и безопасни начини за борба със зърноядите по фасула е отглеждането на устойчиви към тези неприятели сортове. В тази връзка целта на настоящето изследване е да се проучи степента на устойчивост на 30 линии и сортове фасул спрямо този неприятел.

Проучването е проведено при контролируеми лабораторни условия с 30 генотипа фасул. Експериментът е заложен в четири повторения, с по 10 семена от всеки генотип и колонизирани по една двойка – самец и самка.

Отчетени са показателите - смъртност на насекомите (на $14^{\text{-тия}}$ и $21^{\text{-вия}}$ ден от залагането на експеримента), повреда на семената ($21^{\text{-ви}}$ ден) и новоимагинирали възрастни ($62^{\text{-ри}}$ ден). При проведеното проучване не се установява зависимост между броя на живите насекоми, отчетени на $14^{\text{-тия}}$ и $21^{\text{-вия}}$ ден от залагане на експеримента.

Смъртността при първото отчитане варира от 47 до 83%, докато при второто отчитане (на $21^{\text{-вия}}$ ден) тя е 100% при 12 генотипа (40% от общия брой), а при останалите генотипи тя варира от 83 до 97%. Установява се, че за повреждането на семената и за излюпването на нови насекоми основно значение (40,83%) имат живите бръмбари, отчетени на $14^{\text{-тия}}$ ден от залагането на експеримента.

Като основна причина за по-слабото предпочитане от фасулевия зърнояд на семената от генотипите ИЗК-ДК-4, А 195, Пловдив 15 М и Добруджански 7, вероятно са техните по-специфични морфологични и физико-биохимични бариери.

Тестираните 30 генотипа фасул реагират специфично спрямо фасулевия зърнояд. Сортовете Пловдив 11М, Абритус, Кристал 137 и Българи са със сравнително добра устойчивост на фасулев зърнояд. Те могат да се използват в селекционни програми като източници на тази устойчивост ог-чиста хранителна продукция.

INTRODUCTION

Beans are an important agricultural crop, which is subjected to attacks from many biological enemies, both in the field of cultivation and storage. One of the most important is the responsibility of bean weevil (*Acanthoscelides obtectus* Say). Adult insect deposited their eggs on the surface of grains. The hatched larvae are fretting inside the seeds and they are turn into a pupa and adult insect.

For the conservation of beans stored products is mainly used a set of insecticides. Unilateral application of these pesticides against bean weevil rise to resistance in the populations [1, 6, 13, 18].

Therefore, in relation to environmental protection and consumption of pure pesticide food a global trend for the limited application has been adopted.

Modern plant protection programs like special hermetic packaging for storage, use insecticide action on plant products, powders, oils, etc. has been developed [1, 7, 11, 12, 14, 16].

One of the most efficient and environmentally safety methods for protection from bean weevil in beans is growing of resistant varieties [3].

In this context, the aim of this study was to examine the level of resistance in 30 lines and varieties of common beans to the bean weevil *Acanthoscelides obtectus* Say.

MATERIAL AND METHODS

The study was conducted in controlled laboratory conditions with 30 common bean genotypes (Table 1). The experiment was set up in four iterations with 10 seeds of each genotype and infected by a couple - male and female of *Acanthoscelides obtectus* Say.

Reported indicators were the mortality of insects (on the 14th and 21st day of set up the experiment), seed damage (21st day) and the number of young adult insects (62nd day).

Table 1. Tested Bulgarian common bean genotypes.
Таблица 1. Проучвани български генотипи фасул

№	GENOTYPES	№	GENOTYPES	№	GENOTYPES
1.	Ludogorie	**11.**	A 195	**21.**	1028
2.	Garmen	**12.**	Dunav 1	**22.**	Dobrudjanski ran
3.	Padesh 1	**13.**	Hitovo	**23.**	Plovdiv 11M
4.	Samoranovo	**14.**	Trudovetz	**24.**	Plovdiv 15M
5.	IZK-DК-4	**15.**	IIRR 75 85	**25.**	Trakijski
6.	80-7-11-12	**16.**	Cristal 137	**26.**	Abritus
7.	Pokrovnik	**17.**	84-34-1	**27.**	Dobrudjanski 7
8.	Plovdiv 10	**18.**	Bisser	**28.**	1026
9.	Medkovetz 98-1	**19.**	Oreol GP	**29.**	Prissad
10.	Dobrudjanski 2	**20.**	IIRR 1426	**30.**	Bulgari

The obtained results were analyzed by Single Factor (ANOVA) Analysis of Variance [21]. By Student's “t” criterion was established warranted to determine the differences between genotypes and the standard variety Plovdiv 10 [19].

Morphological data were analyzed by numerical taxonomy technique using NTSYS-pc version 2.01b (1986-1997, Applied Biostatistic Inc.). The principal component analysis was used to derive the variance from data associated with the first three principal components. Two-dimensional graphics for the studied traits were presented with vectors [5].

The DIST coefficient was used to cluster individuals applying the SAHN procedure, which uses the unweighted pair group method with arithmetic averages (UPGMA) [15]. Phenograms for similarity were produced using the TREE DISPLAY sub-program.

RESULTS AND DISCUSSION

The data presented in Table 2 demonstrated that there is warranted (at level P = 0.001) between the comparison of insect mortality and survival rate recorded on the 14th and 21st day of set up the experiment.

Table 2. The results of Single Factor Analysis.
Таблица 2. Резултати от еднофакторния дисперсионен анализ.

Source of variation	*SQ*	*FG*	*S^2*	*F exp.*	*F crit*
	Reporting on the 14th day				
Between Groups	143,7316	1	143,7316	167,6875	11,97259
Within Groups	51,42839	60	0,85714		
Total	195,16	61			
	Reporting on the 21st day				
Between Groups	1253,701	1	1253,701	4101,673	11,97259
Within Groups	18,33935	60	0,305656		
Total	1272,04	61			

No correlation between the numbers of live insects recorded on the 14th and 21st day of set up the experiment was found in this study. Relatively large number (10) genotypes were seriously damaged - 50% to 70 %. The high mortality has been recorded in the number of the new transformed adult insects (Table 3).

In the first reporting (on 14th day) the highest number of live insects were for the seeds of genotypes - Bulgari and IIRR 75 85, and on 21st day for Padesh 1, Pokrovnik, IIRR 75 85 and Dunav 1.

Mortality rate on the 14th day varied from 47% to 83 %, while in the second reading (on the 21st day) it is 100% in 12 variants (40% of the total number of variants), while in other genotypes ranged from 83% to 97 %.

Warranted to determine the differences, established by the Student’s "t" criterion, to the standard variety Plovdiv 10 is different.

Table 3. Recorded mortality and survival rate of beetles (Average of the 4 replications).

Таблица 3. Смъртност и преживяемост на възрастните (средно от 4 повторения).

N°	GENOTYPES	14th day		21st day	
		Alive, No.	Dead, No.	Alive, No.	Dead, No.
1.	Ludogorie	$3,7^{n.s.}$	$6,3^{n.s.}$	$0^{n.s.}$	$10,0^{n.s.}$
2.	Garmen	$4,3^{n.s.}$	$5,7^{n.s.}$	$0^{n.s.}$	$10,0^{n.s.}$
3.	Padesh 1	$3,7^{n.s.}$	$6,3^{n.s.}$	$1,5^{---}$	$8,5^{+++}$
4.	Prelom	$3,3^{++}$	$6,7^{--}$	$0^{n.s.}$	$10^{n.s.}$
5.	Samoranovo	$3,0^{+++}$	$7,0^{+++}$	$0,3^{-}$	$9,7^{+}$
6.	IZK-DK-4	$4,3^{n.s.}$	$5,7^{n.s.}$	$0,3^{-}$	$9,7^{+}$
7.	80-7-11-12	$3,3^{++}$	$6,7^{--}$	$0,7^{---}$	$9,3^{+++}$
8.	Pokrovnik	$4,3^{n.s.}$	$5,7^{n.s.}$	$1,7^{---}$	$8,3^{+++}$
9.	**Plovdiv 10 (St)**	**4,0**	**6,0**	**0**	**10,0**
10.	Medkovetz 98-1	$2,0^{+++}$	$8,0^{---}$	$0,7^{---}$	$9,3^{+++}$
11.	Dobrudjanski 2	$1,7^{+++}$	$8,3^{---}$	$1,0^{---}$	$9,0^{+++}$
12.	A 195	$4,0^{n.s.}$	$6,0^{n.s.}$	$0^{n.s.}$	$10,0^{n.s.}$
13.	Dunav 1	$4,3^{n.s.}$	$5,7^{n.s.}$	$1,3^{---}$	$8,7^{+++}$
14.	Hitovo	$3,0^{+++}$	$7,0^{---}$	$0^{n.s.}$	$10,0^{n.s.}$
15.	Trudovetz	$3,3^{++}$	$6,7^{--}$	$0,7^{---}$	$9,3^{+++}$
16.	IIRR 75 85	$5,0^{---}$	$5,0^{+++}$	$1,7^{---}$	$8,3^{+++}$
17.	Cristal 137	$4,7^{--}$	$5,3^{++}$	$0^{n.s.}$	$10,0^{n.s.}$
18.	84-34-1	$2,3^{+++}$	$7,7^{---}$	$1,0^{---}$	$9,0^{+++}$
19.	Bisser	$3,3^{++}$	$6,7^{--}$	$0,7^{---}$	$9,3^{+++}$
20.	Oreol GP	$2,7^{+++}$	$7,3^{---}$	$0^{n.s.}$	$10,0^{n.s.}$
21.	IIRR 14 26	$3,3^{++}$	$6,7^{--}$	$0,7^{---}$	$9,3^{+++}$
22.	1028	$3,0^{+++}$	$7,0^{---}$	$1,0^{---}$	$9,0^{+++}$
23.	Dobrudjanski ran	$4,3^{n.s.}$	$5,7^{n.s.}$	$1,0^{---}$	$9,0^{+++}$
24.	Plovdiv 11M	$4,3^{n.s.}$	$5,7^{n.s.}$	$0^{n.s.}$	$10,0^{n.s.}$
25.	Plovdiv 15M	$4,0^{n.s.}$	$6,0^{n.s.}$	$0^{n.s.}$	$10,0^{n.s.}$
26.	Trakijski	$3,7^{n.s.}$	$6,3^{n.s.}$	$0,3^{-}$	$9,7^{+}$
27.	Abritus	$2,3^{+++}$	$7,7^{---}$	$0^{n.s.}$	$10,0^{n.s.}$
28.	Dobrudjanski 7	$2,7^{+++}$	$7,3^{---}$	$0,7^{---}$	$9,3^{+++}$
29.	1026	$1,7^{+++}$	$8,3^{---}$	$0^{n.s.}$	$10,0^{n.s.}$
30.	Prissad	$3,0^{+++}$	$7,0^{---}$	$0^{n.s.}$	$10,0^{n.s.}$
31.	Bulgari	$5,3^{---}$	$4,7^{+++}$	$0,3^{-}$	$9,7^{+}$
GD $_{5,0\%\ (+;\ -)}$		**0,48**		**0,28**	
GD $_{1,0\%\ (++;\ --)}$		**0,64**		**0,37**	
GD $_{0,1\%\ (+++;\ ---)}$		**0,83**		**0,48**	

NOTE: n.s. – Less than GD $_{5,0\%}$

There are different seed damages among different bean genotypes (Fig. 1).

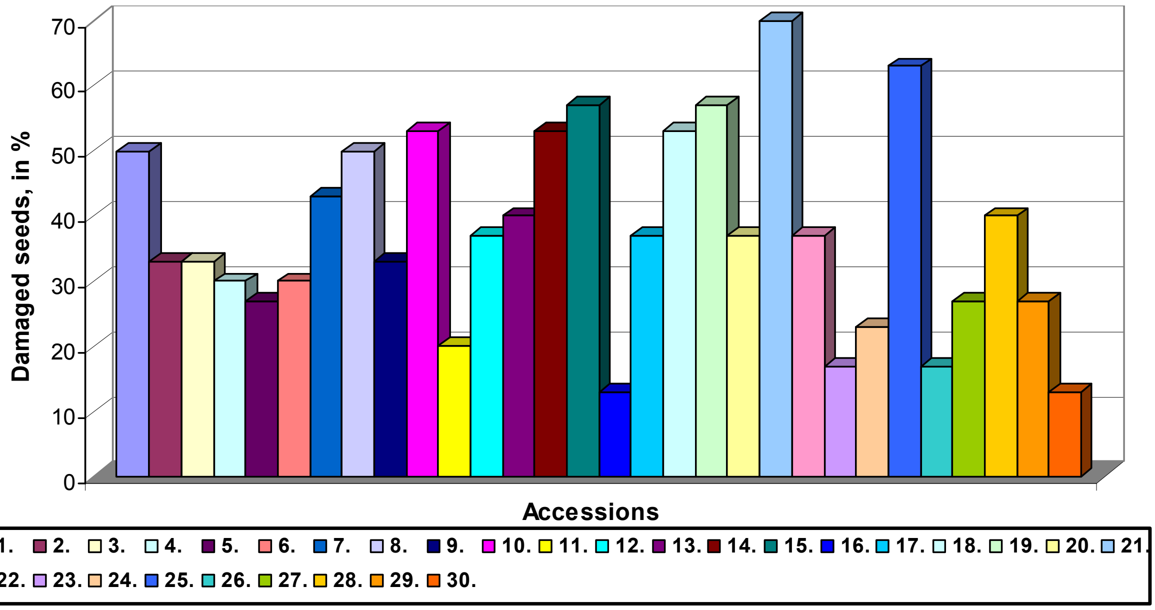

Figure 1. Seed damages recorded on the 21st day of set up the experiment.
Фигура 1. Повреди на семената, отчетени на 21-я ден от залагане на експеримента

Varieties Crystal 137, Plovdiv 11M, Bulgari and Abritus show the best resistance to damage by bean weevil. The extent of seed damages does not exceed 8% - 13%. Slightly preferred host insect species are the genotypes - IZK-DK-4, A 195, Plovdiv 15M and Dobrudjanski 7. The seed damages are range from 17% to 26 %. These varieties are object of interest since they differ in many morphological traits.

The third group include the genotypes (Garmen, Padesh 1, Samoranovo, 80-7-11-12, Medkovets 98-1, Dunav 1, Hitovo, 84-34-1, IIRR 1426, Dobrudjanski ran and 1026), less preferred by *Acanthosceides obtectus* Say. The seed damages are ranging from 30% to 43 %.

It is interesting to noted that Schoonhoven et al. [17] found low levels of resistance in cultivated genotypes of the common bean, *Phaseolus vulgaris* L., to the bean weevil, *Acanthoscelides obtectus* (Say), but high levels of resistance in noncultivated wild forms of beans. Resistance was expressed as reduced oviposition, a prolonged larval developmental period, and reduced progeny weight.

It is noteworthy that almost all of our genotypes with colored seeds are attacked by *Acanthosceides obtectus* Say. less than those, with white seeds (table 4, fig. 1).

Seed's size has also an important feature. It is shown on figure 1, that the seeds of big sized genotypes are less damaged than those with smaller seeds (table 4). Schoonhoven et al. [17] found that weevil resistance in studied of them genotypes was related to small seed size, but they concluded that other factors were probably more important.

Table 4. Color and size of the seeds in tested Bulgarian common bean genotypes.
Таблица 4. Оцветяване и едрина на семената на проучваните български генотипи фасул.

№	GENOTYPES	COLOR	SIZE	№	GENOTYPES	COLOR	SIZE
1.	Ludogorie	White	Small	**16.**	Cristal 137	White	Small
2.	Garmen	White	Big	**17.**	84-34-1	Colored	Small
3.	Padesh 1	White	Small	**18.**	Bisser	Colored	Small
4.	Samoranovo	Colored	Small	**19.**	Oreol GP	Colored	Small
5.	IZK-DК-4	White	Small	**20.**	IIRR 1426	Colored	Small
6.	80-7-11-12	Colored	Small	**21.**	1028	White	Small
7.	Pokrovnik	White	Small	**22.**	Dobrudjanski ran	White	Big
8.	Plovdiv 10	White	Small	**23.**	Plovdiv 11M	White	Small
9.	Medkovetz 98-1	White	Small	**24.**	Plovdiv 15M	White	Small
10.	Dobrudjanski 2	White	Small	**25.**	Trakijski	Colored	Small
11.	A 195	Colored	Big	**26.**	Abritus	Colored	Small
12.	Dunav 1	White	Small	**27.**	Dobrudjanski 7	White	Small
13.	Hitovo	Colored	Small	**28.**	1026	White	Small
14.	Trudovetz	White	Small	**29.**	Prissad	White	Big
15.	IIRR 75 85	Colored	Small	**30.**	Bulgari	White	Big

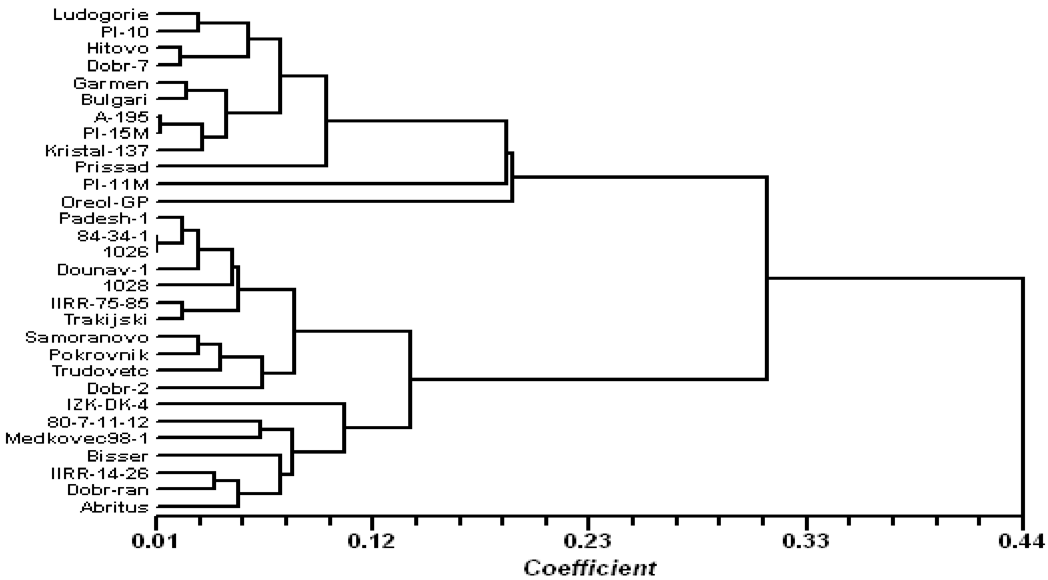

Figure 2. Phenogram of 30 common bean accessions based on the UPGMA method.

The cluster analysis in Figure 2 presents two main cluster groups formed. The first one includes 12 and the second - 18 genotypes. First cluster group, with some exceptions also consist of genotypes with relatively less damaged seeds (13 to 26%).

Figure 3 reveals the essential role of the live beetles and the correlation with the seed damages and the hatching of new insects (40.83%), recorded on the 14th day of the experiment. These three attributes - live beetles on the 14th day (Life 1) seed damages (Damseeds) and young adult insects (New Adults) are pointed out on the right of the graphs (Fig. 3. 1-2 and 1-3). Less influential are the live insects recorded on the 21st day (15.83%), and at least - the young adults (2.78%).

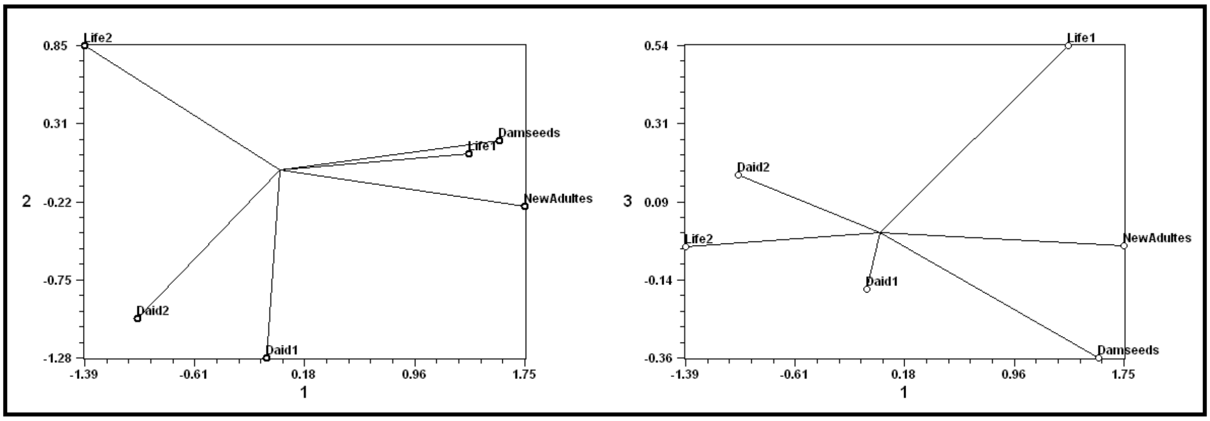

Figure 3. Principal component analysis of the 30 accessions and the projection of the traits in axes 1-2 and 1-3.

According to [10] host food that is less damaged by an insect species in relatively constant environmental conditions, can be considered as sustainable. It is known that the level of resistance varies between hosts in two extremes - immunity and high sensitivity [2].

We can assume that the reason for the sustainability of the genotypes IZK-DK-4, A 195, Plovdiv 15M and Dobrudjanski 7 to the weevil been is based on their specific morphological, physical and biochemical barriers.

Physical barriers, such trichomes, surface waxes, solid and tissue sclerotinisation are genetically regulated biochemical processes.

According to Nietupski et al. [9] the natural resistance of the bean varieties to bean weevil is a complex process that is governed by several physical and chemical factors. The main role lies on the lectin protein group who break down the nutrition of bean weevil by inactivation of digestive enzymes.

Known hypotheses explaining host specialization in insects used as an argument of the existence of genetic transformations in larva development under the influence of host varieties [8].

Since Camargo Lezama et al. [3] testing 6 bean lines and varieties on preference, mortality and fertility of the *Acanthosceides obtectus* Say. and defined the line CPG 0131 as the most resistant to that enemy.

Chipollini and Stiles [4] concluded that indirect size-selective seed predation can have significant effects on maternal plant fitness both by altering the number of progeny produced, and by altering the fitness of surviving progeny.

CONCLUSIONS

Tested 30 bean genotypes react specific against weevil bean.

Varieties Plovdiv 11M, Abritus, Crystal 137 and Bulgari had a relatively good resistance to bean weevil. They can be used in future breeding programs as sources of resistance for clean food production.

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