DIAGNOSIS AND EVALUATION OF *VICIA FABA* L. GERMPLASM RESISTANCE TO BIOTIC STRESSES

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Rezumat

Bobul (*Vicia faba* L.) este una dintre cele mai vechi culturi din lume. Cu toate că în Rusia se cultivă din secolul al VI-lea, în prezent există doar 5 soiuri pentru boabe. Primul pas în elaborarea unui program de cercetare pe această specie, în Institutul de Selecţie şi Seminologie ale Culturilor Leguminoase din Federaţia Rusă a fost realizat diagnosticul bolilor ce atacă bobul. În zonele centrale ale Rusiei, *Vicia faba* L. se infectează cu *Uromyces fabae* (Grev.) de Bary ex Fuckel, *Ascochyta fabae* SPEG. şi *Broad bean mottle virus*. Între gradul de îmbolnăvire al plantelor şi gradul de germinare al seminţelor a fost stabilită corelaţie negativă semnificativă (r=-0,49; tr=3,50 > T05=2,01). Studiile preliminare ale efectului imunomodulator indus de glicozidele steroidice denotă că acesta depinde de soi. Glicozidul steroidal *Ecostim* (c=0,-005%) stimulează germinaţia, creşterea rădăciniţelor embrionare şi tulpinilor la soiul *Russkie chiornie.*

Cuvinte cheie: Vicia faba L., patogeni, glicozide steroidale
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Introduction

Broad bean (*Vicia faba* L.) is one of the most ancient crops in the world. It is represented in collections by cultivated forms only, because the wild ancestor of its species hasn't yet been discovered [5]. The world production of broad beans was 4,9 Mt from 2,8 Mha in 2005, which is smaller, then the world production of soybean (220 Mt) and pea (12 Mt). The main broad beans producers are China (2Mt), Europe (1Mt – principally United Kingdom, France, Spain, Portugal, Greece), Ethiopia (0,4 Mt), Egypt (0,4 Mt) and Australia (0,2 Mt) [4]. The feed market for dry seed has mostly been developed in Europe (0,7 Mt consumed by pigs and poultry), but the using of broad beans as a source of protein for foods is usual for Mediterranean countries and China [5]. *Vicia faba* L. have been planted in Russia from VI century [7, 12]. Investigation of *Vicia* subgenus phylogeny based on analysis of RAPDs and RFLP of PRC-amplified chloroplast genes show, that broad bean could been introduced in Russia from 2 centers of origin: from Asia (India, Afghanistan) and from Europe (The Mediterranean region) [14]. Broad beans are cultivated in the northern and the central areas of Russian Federation now, and we have only 5 varieties of broad bean at present [13]. The importance of broad bean is increasing now, because: 1) it's seeds are rich in protein and starch, 2) its play the vast role in the improvement of the soil, 3) its are need for private vegetable-gardens in Russia. But *Vicia faba* L. are infected with biotic stressors as all *Fabaceae*. And that moment is weakening of its positions on the food *Fabaceae* market [9]. So, we began the special program for the broad beans breeding on resistance to biotic stresses in All-Russian Research Institute for Vegetable Breeding and Seed Production.

Materials and methods

Germplasm of *Vicia faba* L. in our Institute was the material of our study. It has been included in 3 traditional varieties (Russkie chiornie, Byelorusskie and Velena), 20 samples from the collection of All-Russian Institute for Crop Production (VIR – from the N.I. Vavilov-collection) and 20 breeding samples. It has been planted in the field in experimental plots of the laboratory of *Fabaceae* breeding in 2009 and in 2011 years. Immunomodulating effect of steroid glycosides has been studied with the moldstim and the ecostim (in concentrations 0,001%; 0,005%; 0,01% and 0,05%) in 2013.

Methods of investigation included in:

Diagnosis of diseases by symptoms with help of Russian [11], Moldavian [15] and European Handbook of Plant Diseases [3].

Identification species of disease's agent with Karl Zeiss –microscope, using Canon video-system. The treatment of video-figures on Personal Computer with the Scope Photo-program, version 3.0.

Phytopathological evaluation the degree of disease's spread.

Correlation and dispersion analysis by Dospekhov B.A. [10].

Study of immunomodulating effect of the moldstim and the ecostim.

Results and Discussion

The first step of our program was the diagnosis of diseases infected broad bean in the Middle part of Russia and preliminary evaluation of plants resistance to diseases. We evaluated the germplasm broad bean of our Institute: 3 varieties, 20 samples from the collection of VIR and 20 breeding samples. Broad beans were infected with *Uromyces fabae* (Grev.) de Bary ex Fuckel, *Ascochyta fabae* Speg. and broad bean mottle virus.

Uromyces fabae **(**Grev.) de Bary ex Fuckel (syn. *Uromyces vicia-fabae* (Pers.) Schröter). The fungus commonly attacks broad bean, but has a wider host range on various species of *Vicia*, *Lathyrus* and *Lens* [1]. Plants show typical rust pustules on the upper and under sides of leaves and on stems (Fig.1). Aescospores may overwinter in the Mediterranean region, but can't to survive in severe winters of our region. Mycelium, uredinio- and teliospores in leaves and stems could to remain viable for 1-2 years. The degree of disease spreading was 76% in 2009 (the humid summer) and 6, 5% in 2011 (the dry summer). Effective chemical control has been achieved in field trails against natural infection with both contact and systemic fungicides [6].

Ascochyta fabae Speg. *A. faba* resembles *A. pisi* , but it is largely specialized to *Vicia faba* L. It causes large lesions (up to 10 cm in diameter) on leaves and more small by diameter spots on stems and beans. Lesions have reddish-brown margins and grayish centers with black dots of pycnidia in the center (Fig.2). Colorless pycnospores have cylindrical form and 1-2 partitions (Fig.3). Infected seeds are covered with circular dark brown spots (Fig.4). Pathogen can remain viable into seed for up to 3 years and for 4-5 months on infected trash [2]. The degree of disease spreading was 100% in 2009 (the humid summer) and 1, 5% in 2011 (the dry summer).

Figure 1. Typical uredo-pustules of *Uromyces fabae* (Grev.) de Bary ex Fuckel on leaves and stems of *Vicia faba* L Russkie chiornie – variety (5.08.2009y.)

Figure 2. Symptoms of *Ascochyta vicia-fabae* Speg. on leaves and on pods of broad beans. Russkie chiornie – variety | (13.07. 2011y.).

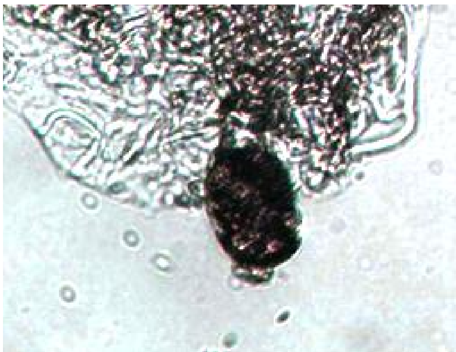

Figure 3. Pycnidia and pycnospores of *Ascochyta vicia-fabae* Speg. – preparation the leaf of *Vicia faba* L. Laboratory of gamete's selection (14.07.2011y.).

Broad bean mottle virus caused vein clearing and chlorotic mosaic of leaves (Fig.5). The upper part of leaf was pointed. The degree of disease spreading was 23% in 2009 and 46, 3% in 2011. Unfortunately, we couldn't to support of our visual diagnosis with help of ELISA. So, we think that our studies will in progress.

As we could see, *A. faba* could to infect the seeds (Fig.4), remain viable into seeds and transmitted by seeds [2]. So, we decided to test the bond between the degree of plants affection with *A. faba* and the degree of seeds germination of these plants with correlation analysis. Correlation analysis show, that there is significant negative correlation between the degree of plants affection and the degree of seed germination: the more the degree of plants affection, the smaller of its seeds germination degree. R = - 0,49; t_r = 3,50> t_{05} = 2,01 (Tab.1).

Figure 4. Symptoms of *Ascochyta vicia-fabae* Speg. on seeds of *Vicia faba* L. Breeding material. Laboratory of gamete's selection (6-14.04.2012y.).

Figure 5. Symptoms of virus infection on leaves of *Vicia faba* L. Broad bean mottle virus? Breeding material. Laboratory of gamete's selection (14.07.2011).

Table 1. Investigation the dependence between the degree of plants affection with *A. faba* (X) and the degree of seeds germination of these plants (Y). VNIISSOK (2009, 2011ys.).

№ of pair	Means of traits, %		X^2	Y^2	XY
	The degree of plants affection, X	The degree of seeds germination, Y			
1	*2*	*3*	*4*	*5*	*6*
1.	50	40	2500	1600	2000
2.	50	34	2500	1156	1700
3.	41	49	1681	2401	2009
4.	69	87	4761	7569	6003
5.	50	77	2500	5929	3850
6.	47	79	2209	6241	3713
7.	100	12	10000	144	1200
8.	75	57	5625	3249	4275
9.	61	66	3721	4356	4026
10.	73	31	5329	961	2263
11.	100	22	10000	484	2200
12.	100	29	10000	841	2900
13.	56	45	3136	2025	2520`
14.	100	33	10000	1089	3300
15.	67	21	4489	441	1407
16.	100	9	10000	81	900

Table 1. (Continuation)

1	*2*	*3*	*4*	*5*	*6*
17.	50	30	2500	900	1500
18.	33	15	1089	225	495
19.	86	35	7396	1225	3010
20.	69	37	4761	1369	2553
21.	50	20	2500	400	1000
22.	83	17	6889	289	1411
23.	50	57	2500	3249	2850
24.	64	52	4096	2704	3328
25.	36	52	1296	2704	1872
26.	46	37	2116	1369	1702
27.	40	14	1600	196	560
28.	29	67	841	4489	1943
29.	50	34	2500	1156	1700
30.	50	8	2500	64	400
31.	70	29	4900	841	2030
32.	35	66	1225	4356	2310
33.	30	56	900	3136	1680
34.	46	42	2116	1764	1932
35.	83	33	6889	1089	2739
36.	100	0	10000	0	0
37.	75	33	5625	1089	2475
38.	33	43	1089	1849	1419
39.	20	57	400	3249	1140
40.	100	0	10000	0	0
Σ	2467	1525	174179	76279	84315

$$r = \frac{\Sigma XY - (\Sigma X \Sigma Y) : n}{\sqrt{(\Sigma X^2 - (\Sigma X)^2 : n)(\Sigma Y^2 - (\Sigma Y)^2 : n)}} = \frac{84315 - 94054}{\sqrt{(174179 - 152152)(76279 - 58141)}} = -0,49$$

$S_r = 0,14$; $t_r = 3,50 > t_{05} = 2,01$ – correlation is significant.

It is known, that secondary metabolites of plants - steroid glycosides – possess of immunomodulating effect [8]. We decided to use some of its to stimulate of broad bean resistance to biotic stresses. We evaluated biological effects of two steroid glycosides in different concentration at the first step of our study. Seeds of two broad bean varieties have been exposed in water solutions of moldstim and ecostim in different concentrations during 24 hours. Water without glycosides served as standard. Then its have been placed on filter paper in Petri diches, which have been placed in incubator (t= + 24^{0}C) before the seeds germination. Length of embryo roots and stems was measured after week of incubation. Data have been tested with dispersion analysis. Results of dispersion analysis show, that effect of steroid glycosides is depended on variety. Moldstim and ecostim inhibited of embryo roots and stems growth of Byelorusskie variety, and stimulated of embryo roots and stems growth of Russkie chiornie variety (tab.2,3). The significant stimulating effect was observed for ecostim – 0,005% for embryo roots growth (tab.2), and for moldstim-0,005% and ecostim – 0,005% for embryo stem growth (tab. 2,3). So, we can to select the ecostim in concentration 0,005% for the test of it's immunomodulating effect in the field.

Table 2. Effect of steroid glycosides on the length of the broad bean embryo root. Laboratory of gamete's selection. VNIISSOK (2013y.)

Steroid glycosides – concentration (B)	Length of embryo root, X, mm, by varieties (A)				LSD_{05A} = 4,04
	Byelorusskie	deviation from St	Russkie chiornie	deviation from St	
Water - standard	22,6	St	19,4	St	- 3,2
Moldstim – 0,001%	16,1	- 6,2	16,8	- 2,6	+ 0,7
Moldstim – 0,005%	12,9	-9,7	20,3	+0,9	+7,4
Moldstim – 0,01%	10,0	-12,6	19,8	+0,4	+9,8
Moldstim – 0,05%	13,7	-8,9	14,9	-4,5	+1,2
Ecostim – 0,001%	9,9	-12,7	25,0	+5,6	+15,1
Ecostim – 0,005%	14,1	-8,5	29,3	+9,9	+15,2
Ecostim – 0,01%	10,6	-12,0	24,6	+5,2	+14,0
Ecostim – 0,05%	14,9	-7,7	23,5	+4,1	+8,6
X, mm, by glycosides (B)	13,9	-	21,5	-	+7,6
LSD_{05B} = 6,08					

Table 3. Effect of steroid glycosides on the length of the broad bean embryo stem. Laboratory of gamete's selection. VNIISSOK (2013 y.)

Steroid glycosides – concentration (B)	Length of embryo stem, X, mm, by varieties (A)				LSD_{05A} = 3,7
	Byelorusskie	deviation from St	Russkie chiornie	deviation from St	
Water - standard	27,8	St	19,8	St	- 8,0
Moldstim – 0,001%	11,9	-15,9	16,1	- 3,7	+4,2
Moldstim – 0,005%	13,4	-14,4	29,4	+9,6	+16,0
Moldstim – 0,01%	7,9	-19,9	21,6	+1,8	+13,7
Moldstim – 0,05%	14,3	-13,5	7,5	-12,3	-6,8
Ecostim – 0,001%	10,2	-17,6	23,1	+3,3	+12,9
Ecostim – 0,005%	11,4	-16,4	25,3	+5,5	+13,9
Ecostim – 0,01%	13,8	-14,0	21,8	+2,0	+8,0
Ecostim – 0,05%	20,7	-7,1	14,8	-5,0	-5,9
X, mm, by glycosides (B)	14,6	-	19,9	-	+5,3
LSD_{05B} = 7,9					

Conclusion

The broad bean germplasm of VNIISSOK included in 3 varieties, 20 collecting samples and 20 breeding samples infected with *Uromyces fabae* (Grev.) de Bary ex Fuckel, *Ascochyta fabae* Speg. and virus disease (Broad Bean Mottle Virus –

proposed). The degree of diseases spreading was varied from 1,5-6,6% (in the dry year, 2011) to 76-100% (in humid year, 2009). Significant negative correlation between the degree of plants affection and the degree of seed germination has been established (r=-0,49; t_r=3,50> t_{05}=2,01). In preliminary studies of immunomodulating effect of steroid glycosides we show that it depends on variety. Steroid glycoside Ecostim (c=0,005%) stimulated the embryo roots and stems growth of Russkie chiornie variety.

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