ASSESSMENT OF THE ACTION OF DEPOSIT MYCOFLORA ON *VICIA FABA* L BEANS FROM SUCEAVA GENEBANK'S COLLECTION

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Abstract: *This study consisted in a phytopathological evaluation of epiphyte and endophyte mycological flora which appeared on Vicia faba seeds placed on two types of substrates (CGA medium and blotting paper). The 30 populations of faba bean resulted from the active collection of Suceava Genebank and conserved for different time intervals (8, 18 and 23 years), in controlled atmosphere storages (T=+4^{0}C; relative air humidity = 30 - 40%).*
Micromycets were evaluated by counting the infected seeds and the attack frequency was expressed as a percentage, by visual estimation of seeds surface.
The target objectives of the study were to establish the influence of the conservation period on the activity of micromycets placed on stored seeds and to settle the influence of the substrate type - CGA medium (potato - dextrose - agar) and blotting paper - on the development of fungal pathogens.

Keywords: *micromycets, landraces, CGA medium*

1. Introduction

Size variation of pathogen colonies kept in constant environmental conditions reflects the differences in quantity, viability and location of inoculums on seed. The development of colony around each seed on growth medium, and the intensity of symptoms on germs in case of blotting paper tests are closely dependent on the amount of inoculum on seed - number of spores or mycelium abundance [1].

Generally, the correlation between inoculum (spores load/seed) and colony size (the amount of mycelium) developed on CGA medium (potato - dextrose - agar) is very significant [2].

Micromycets existing on stored legume seeds can cause during storage a wide range of changes, with negative consequences from a technological, nutritional, hygienic and commercial point of view [3]. Beratlief and collaborators in a study concerning the deposit ecosystem characteristics, revealed the mycological flora evolution and sequence on legume seeds stored with high moisture content [4].

The purposes of this study are:

- to establish the influence of the conservation period on the activity of micromycets placed on stored seeds
- to settle the influence of the substrate type - CGA medium (potato - dextrose - agar) and blotting paper - on the development of fungal pathogens.

2. Experimental

We have performed the phytopathological characterization of local germplasm represented by 30 populations of *Vicia faba,* conserved for 8, 18 and 23 years at T = +4°C, which come from collecting expeditions realized by the collecting department from Suceava Genebank during a term of 15 years (1986-2001).

Lab experiments were carried on Suceava Genebank by using the genetic

seminal material from the active collection of the institution, which was placed on the CGA medium and blotting paper.

To make possible the assessment of the micromycets present on *Vicia faba* seeds, we implemented the following research methods:

- macroscopic analyses of the seeds;
- Ulster method [5] on CGA medium (potato - dextrose - agar).

3. Results and discussion

The seeds of *Vicia faba,* placed on CGA medium and blotting paper, presented after the incubation period the following characteristics concerning the presence of fungal microorganisms:

a) CGA medium (potato - dextrose - agar)

On CGA medium, the presence of deposit mycoflora on the 30 samples of *Vicia faba* seeds conserved at $+4^0$C temperature, for 8, 18 and 23 years was different, as follows:

On the samples stored for 8 years at $+4^0$C temperature, we identified 11 fungal pathogens (*Penicillium sp., Aspergillus sp.*, *Rhizopus sp., Epicoccum sp., Cladosporium herbarum, Alternaria alternata, Trichothecium roseum, Trichoderma viride, Botrytis fabae, Stachybotrys atra, Stemphylium botryosum)* which showed a different attack degree on each sample of the 7 analyzed, registering an infection rate of 78% (table 1).

On 17 samples stored at $+4^0$C temperature. for a period of 18 years we identified 11 fungal pathogens (*Penicillium sp., Aspergillus sp.*, *Rhizopus sp., Epicoccum sp., Cladosporium herbarum, Alternaria alternata, Mucor sp., Trichothecium roseum, Trichoderma viride, Botrytis fabae, Stemphylium botryosum*). The 510 seeds submitted to macroscopic and microscopic analysis presented an infection rate of 66 %.

Table 1

Proportion of micromycets isolated on *Vicia faba* beans placed on CGA medium

Experimental conditions	Seeds stored at T+ 4^0C, for 8 years	Seeds stored at T+ 4^0C, for 18 years	Seeds stored at T+4^0C, for 23 years
Isolated micromycets	**Attack frequency (%)**		
Penicillium sp.	13.3	16	11.6
Aspergillus sp.	6.2	10.6	6.1
Rhizopus sp.	25.7	18.4	7.2
Epicoccum sp.	8.1	0.4	2.7
Cladosporium herbarum	3.8	1.9	3.3
Alternaria alternata	11.4	11.4	5.5
Mucor sp.	0	0.4	0
Trichothecium roseum	0.5	2.7	2.7
Trichoderma viride	0.9	0.8	1.1
Botrytis fabae	2.8	2.9	0
Stachybotrys atra	0.9	0	0
Stemphylium botryosum	4.3	0.2	0
TOTAL	**77.9**	**65.7**	**40.2**

Other 6 seed samples. conserved for a period of 23 years, have been infected by a smaller number of fungal microorganisms (*Penicillium sp., Aspergillus sp.*, *Rhizopus sp., Epicoccum sp., Cladosporium herbarum, Alternaria alternata, Trichothecium roseum, Trichoderma viride)* and the infection percentage on the 180 seeds analyzed was much lower (40.5 %).

In table 2 are presented the micromycets placed in three experimental

conditions (8. 18 and 23 years) at +4^{0}C temperature:

Table 2

Action mode of micromycets identified on *Vicia faba* beans in 3 experimental conditions on CGA medium

Experimental conditions	Seeds stored at T +4^{0}C, for 8 years	Seeds stored at T +4^{0}C, for 18 years	Seeds stored at T +4^{0}C, for 23 years
Isolated micromycets	**Total number of infected seeds**		
Penicillium sp.	**28**	**82**	**21**
Aspergillus sp.	13	54	11
Rhizopus sp.	**54**	**94**	**13**
Epicoccum sp.	17	2	5
Cladosporium herbarum	8	10	6
Alternaria alternata	**24**	**58**	**10**
Mucor sp.	0	2	0
Trichothecium roseum	1	14	5
Trichoderma viride	2	4	2
Botrytis fabae	6	15	0
Stachybotrys atra	2	0	0

b) blotting paper

Analyzing the 30 seeds samples of *Vicia faba* stored at +4^{0}C temperature for 8, 18 and 23 years, we have identified the following infection percentages caused by fungal pathogens:

On the samples stored for 8 years at + 4^{0}C temperature we have identified 6 fungal pathogens (*Penicillium sp., Aspergillus sp., Alternaria alternata, Rhizopus sp., Cladosporium herbarum, Botrytis fabae*). which had a different attack degree on each sample of the 7 analyzed, registering an infection rate of 68.6% (table 3).

Table 3

Proportion of micromycets isolated on *Vicia faba* beans placed on blotting paper

Experimental conditions	Seeds stored at T +4^{0}C, for 8 years	Seeds stored at T + 4^{0}C, for 18 years	Seeds stored at T +4^{0}C, for 23 years
Isolated micromycets	**Attack frequency (%)**		
Penicillium sp.	19	10.9	10
Aspergillus sp.	2.8	2.7	6.6
Rhizopus sp.	13.3	5.0	5.5
Cladosporium herbarum	7.6	1.6	0
Alternaria alternata	20.9	3.9	2.2
Trichothecium roseum	0	4.7	0
Botrytis fabae	4.7	1.9	0
TOTAL	**68.3**	**30.7**	**24.3**

On 17 samples conserved at +4^{0}C temperature for a period of 18 years, we have identified 7 fungal pathogens **(***Penicillium sp., Aspergillus sp., Rhizopus sp., Cladosporium herbarum., Alternaria alternata., Trichothecium roseum, Botrytis fabae***)**. The 255 seeds submitted to macroscopic and microscopic analysis presented an infection rate of 30.9 %.

The 6 seed samples with a storage period of 23 years have been infected by a smaller number of micromycets (*Penicillium sp., Aspergillus sp., Rhizopus sp.* and *Alternaria alternata***)**. the infection percentage on the 90 seeds analyzed being more low (24.4 %).

In table 4 are presented the micromycets placed in 3 experimental conditions (8, 18 and 23 years) at +4^{0}C temperature. Analyzing the number of

infected seeds, we can observe that all micromycets genus of legume seeds were isolated on a more smaller number of seeds when samples were incubated on blotting paper, in comparison to the number of seeds placed on CGA medium.

Table 4

Action mode of micromycets identified on *Vicia faba* in 3 experimental conditions on blotting paper

Experimental conditions	Seeds stored at T+4^{0}C, for 8 years	Seeds stored at T+4^{0}C, for 18 years	Seeds stored at T +4^{0}C, for 23 years
Isolated micromycets	**Total number of infected seeds**		
Penicillium sp.	**20**	**28**	**9**
Aspergillus sp.	3	7	6
Rhizopus sp.	14	13	5
Cladosporium herbarum	8	4	0
Alternaria alternata	**22**	**10**	**2**
Trichothecium roseum	0	12	0
Botrytis fabae	5	5	0

4. Conclusions

Deposit mycoflora developed on faba bean seeds taken in this study was analyzed according to genotype period of seed conservation and type of substrate used.

From our study resulted the following conclusions:

1. The seed samples of *Vicia faba* stored in 3 experimental conditions placed on CGA medium, were infected in different proportions by fungal pathogens. The species *Stachybotrys atra* was identified only on the samples conserved for 8 years at +4^{0}C temperature and the species *Botrytis fabae, Stemphylium botryosum* were identified only on the seeds with a storage period of 8 and 18 years. Other types of micromycets were detected in all storage conditions, but on a different number of seeds. The species *Mucor sp*. was isolated only on the seed samples with a storage period of 18 years.

2. By placing the same seed samples of *Vicia faba* in 3 experimental conditions on blotting paper, we observed that samples were infected in a smaller proportion compared to CGA medium. The fungal pathogens *Mucor sp., Epicoccum sp., Trichoderma viride, Stachybotrys atra, Stemphylium botryosum* identified on CGA medium, were not isolated on blotting paper.

5. References

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