ASSESSMENT OF THE ACTION OF DEPOSIT MYCOFLORA ON *PHASEOLUS VULGARIS* L . BEANS FROM SUCEAVA GENEBANK'S COLLECTION

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Abstract: *This study consisted in a phytopathological evaluation of epiphyte and endophyte mycological which appeared on Phaseolus vulgaris seeds, placed on two types of substrates (CGA medium and blotting paper) and emphasing corellations between the evolution of isolated micromycets on Phaseolus vulgaris samples and different periods of storage. The 30 populations of bean resulted from the active collection of Suceava Genebank, were conserved for different time intervals (6 and 15 years), in controlled atmosphere storages (T=+4^{0}C; relative air humidity = 30 - 40%).*

Micromycets were evaluated by counting the infected seeds, 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;*
- *to settle the influence of the substrate type - CGA medium (potato - dextrose - agar) and blotting paper - on the development of fungal pathogens;*
- *to establish a complementar action of identified micromycets on Phaseolus vulgaris seeds in two storage periods, by determining the correlation coefficients between the action of fungal pathogens identified on the samples taken in study.*

Keywords: *micromycetse, landraces, CGA medium, correlation coefficient*

1. Introduction

The transmission of fungal pathogens by seeds has always been the most rapidly spreading of diseases from one region to another and therefore it's need to know the symptoms, the biology and mode of transmission of pathogens, for once reported to be ensured effectively preventing and fighting them [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].

Viability of inoculum seed - borne is in some cases directly related to interspace, harvesting - storage - analysis. Some fungal pathogens are present and can be found in high percentages in freshly harvested seeds, but their infection it's reducing significantly after one year of storage [3].

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 [4]. 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 [5].

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;
- to establish the complementary action of identified micromycets on *Phaseolus vulgaris* seeds in two storage periods, by determining the correlation coefficients between the action of fungal pathogens identified on the samples taken in study.

2. Experimental

We have performed the phytopathological characterization of local germplasm represented by 30 populations of *Phaseolus vulgaris,* conserved for 6 and 15 years at T = +4°C, which come from collecting expeditions realized by the Collecting Department from Suceava Genebank during a term of 10 years (1993-2003).

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 *Phaseolus vulgaris* seeds, we implemented the following research methods :

- macroscopic analyses of the seeds;
- Ulster method [6] on CGA medium (potato - dextrose - agar).

Interpretation of results concerning identified micromycets evolutions on bean seeds taken in study was achieved by analyzing correlations and regressions accordingly with experimental factors [7].

3. Results and discussion

The seeds of *Phaseolus vulgaris,* 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 *Phaseolus vulgaris* seeds conserved at +4 ^{0}C temperature, for 6 and 15 years was different, as follows:

On the samples stored for 6 years at +4 ^{0}C temperature, we identified 10 fungal pathogens (*Penicillium sp., Aspergillus sp.*, *Rhizopus sp., Epicoccum sp., Cladosporium herbarum, Alternaria alternata, Trichothecium roseum, Trichoderma viride, Stachybotrys atra, Stemphylium botryosum*) which showed a different attack degree on each sample of the 21 analyzed, registering an infection rate of 70,5 % (444 infected seeds of 630 analyzed) (table 1).

On 9 samples stored at +4^{0}C temperature, for a period of 15 years we identified 9 fungal pathogens (*Penicillium sp., Aspergillus sp.*, *Rhizopus sp., Epicoccum sp., Cladosporium herbarum, Alternaria alternata, Trichothecium roseum, Trichoderma viride, Stemphylium botryosum*). The 270 seeds submitted to macroscopic and microscopic analysis presented an infection rate of 31,4 %, being infected 85 seeds. In these storage conditions, the genus *Stachybotrys* not expressed at all.

Table 1
Proportion of micromycets isolated on *Phaseolus vulgaris* beans placed on CGA medium

Experimental conditions	Seeds stored at T + 4^0C, for 6 years	Seeds stored at T + 4^0C, for 15 years
Isolated micromycets	**Attack frequency (%)**	
Penicillium sp.	11.1	11.8
Aspergillus sp.	0.8	1.5
Rhizopus sp.	20.3	6.3
Epicoccum sp.	5.5	0.7
Cladosporium herbarium	2.7	1.1
Alternaria alternata	17.1	2.6
Trichothecium roseum	5.8	1.5
Trichoderma viride	2.5	3.7
Stachybotrys atra	0.5	0
Stemphylium botryosum	3.9	2.2
TOTAL	**70.2**	**31.4**

b) blotting paper

For emphasing the role of substrate used for analysis of micromycets occuring on *Phaseolus vulgaris* seeds after different periods of storage, we used also blotting paper substrate.

Analyzing the 30 seed samples of *Phaseolus vulgaris* stored at +4^0C temperature for 6 and 15 years we have identified the following infection percentages caused by fungal pathogens:

On the samples stored for 6 years at +4^0C temperature we have identified 6 fungal pathogens (*Penicillium sp,. Rhizopus sp., Cladosporium herbarum, Alternaria alternata, Trichothecium roseum, Trichoderma viride*) which had a different attack degree on each sample of the 21 analyzed registering an infection rate of 57.7 % (table 2).

On 9 samples conserved at +4^0C temperature for a period of 15 years we have identified 7 fungal pathogens (*Penicillium sp., Aspergillus sp., Rhizopus sp., Cladosporium herbarum, Alternaria alternata, Trichothecium roseum, Trichoderma viride)*. The 135 seeds submitted to macroscopic and microscopic analysis presented an infection rate of 30.3 %.

Table 2
Proportion of micromycets isolated on *Phaseolus vulgaris* beans placed on blotting paper

Experimental conditions	Seeds stored at T + 4^0C, for 6 years	Seeds stored at T + 4^0C, for 15 years
Isolated micromycets	**Attack frequency (%)**	
Penicillium sp.	7.6	8.8
Aspergillus sp.	0	2.9
Rhizopus sp.	18.1	6.6
Cladosporium herbarium	3.2	3.7
Alternaria alternata	14.3	5.2
Trichothecium roseum	8.2	2.2
Trichoderma viride	6.3	0.7
TOTAL	**57.7**	**30.1**

Revealing correlations between isolated micromycets evolution on Phaseolus vulgaris samples taken in study and different storage periods (6 and 15 years)

For establishment complementary action of micromycets identified on *Phaseolus vulgaris* seeds in the two storage periods, we have determined correlation coefficients between fungal pathogens action identified on samples tahen in study (table 3).

In the analyzed samples of *Phaseolus vulgaris*, the results from the table related a few statistical correlations in both storage periods. After 6 years of storage of *Phaseolus vulgaris* seeds at +4°C temperature, it's noticed that there is one significant positive correlation between micromycets action: *Stemphylium botryosum x Alternaria alternata.*

Tabel 3
Correlation coefficients between micromycets action identified on *Phaseolus vulgaris* samples stored at +4°C, for 6 years

Related characters	*Penicillium sp.*	*Aspergillus sp.*	*Rhizopus sp.*	*Epicoccum sp.*	*Cladosporium herbarum*	*Alternaria alternata*	*Trichothecium roseum*	*Trichoderma viride*	*Stachybotrys atra*	*Stemphylium botryosum*
Penicillium sp.	1									
Aspergillus sp.	0.21	1								
Rhizopus sp.	0.10	0.18	1							
Epicoccum sp.	-0.03	-0.18	-0.21	1						
Cladosporium herbarum	-0.09	-0.00	-0.21	-0.19	1					
Alternaria alternata	0.01	0.11	-0.43	-0.04	-0.01	1				
Trichothecium roseum	-0.26	-0.24	-0.08	0.27	-0.09	-0.47	1			
Trichoderma viride	-0.40	-0.24	-0.42	-0.10	0.23	-0.05	0.27	1		
Stachybotrys atra	0.12	-0.12	-0.01	-0.06	-0.04	0.26	-0.13	-0.32	1	
Stemphylium botryosum	-0.02	0.57	-0.36	-0.06	-0.12	**0.56***	-0.06	0.03	-0.16	1

After 15 years of storage of *Phaseolus vulgaris* seeds at +4°C temperature, there is only one very significant positive correlation between fungal pathogens action *Rhizopus sp. x Aspergillus sp.* (table 4).

Therefore, after 15 years of conservation is observed a constant presence of two fungal pathogens *Rhizopus sp.* and *Aspergillus sp.*

For setting of the two micromycets action (*Aspergillus sp. x Rhizopus sp.)* present on seeds after 15 years of storage we traced the suitable regression straight (figure 1).

This regression straight line out complementary action of the two saprophytic micromycets, meaning that while maintaining seeds at $+4^0$C temperature alongside genus *Aspergillus* appears also *Rhizopus sp.*

Table 4
Correlation coefficients between micromycets action identified on *Phaseolus vulgaris* samples stored at +4°C temperature. for 15 years

Related characters	*Penicillium sp.*	*Aspergillus sp.*	*Rhizopus sp.*	*Epicoccum sp.*	*Cladosporium herbarum*	*Alternaria alternata*	*Trichothecium roseum*	*Trichoderma viride*
Penicillium sp.	1							
Aspergillus sp.	0.48	1						
Rhizopus sp.	0.43	**0.923 *****	1					
Epicoccum sp.	-0.16	-0.12	0.01	1				
Cladosporium herbarum	-0.63	-0.25	-0.38	-0.25	1			
Alternaria alternata	0.30	-0.30	-0.46	-0.30	0.17	1		
Trichothecium roseum	0.06	-0.16	-0.25	-0.16	-0.33	-0.14	1	
Trichoderma viride	-0.27	-0.06	-0.33	-0.06	0.69	0.47	-0.09	1

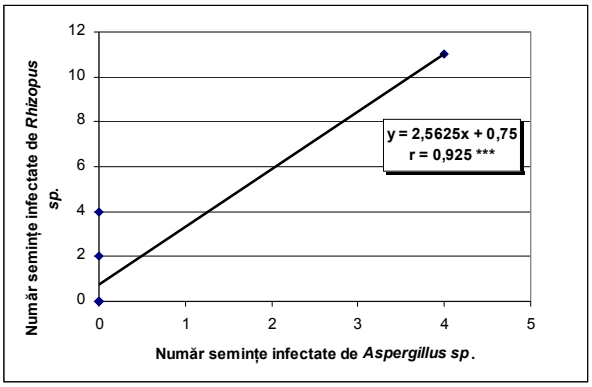

Fig. 1 - The regression straight for correlation between number of infected seeds of *Aspergillus sp.* and number of infected seeds of *Rhizopus sp.* on *Phaseolus vulgaris* samples stored in controlled environmental conditions (+ 4^0C) for 15 years

4. Conclusions

Deposit mycoflora developed on 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 seeds samples of *Phaseolus vulgaris* stored in 2 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 6 years at + 4^0C temperature and the other types of micromycets (*Penicillium sp., Aspergillus sp.,. Rhizopus sp., Epicoccum sp., Cladosporium herbarum, Alternaria alternata, Trichothecium roseum, Trichoderma viride, Stemphylium botryosum)* were detected in all storage conditions, but on a different number of seeds.

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

3. *Phaseolus vulgaris* seeds stored for 6 years at +4°C temperature presented additional infections with the following micromycets: *Stemphylium botryosum x Alternaria alternata,* existing significant correlations between the action of these two fungal pathogens.

4. After 15 years of storage of *Phaseolus vulgaris* seeds at +4°C temperature, there is a strong attack of *Rhizopus sp. x Aspergillus sp.,* being a very significant correlation between the action of these two fungal pathogens.

5. References

[1]. BAKER K.F., 1966. Dynamics of seed transmission of plant pathogens. *Ann. Rev. Phytopathol.*. 4. 311
[2]. HULEA A., NEGRU AL., SEVERIN V. 1973. *Main Seed Crop Diseases.* Ceres Publishing. Bucharest. 35 – 70
[3]. VAN DER SPEK J., 1965. *Botrytis cinerea als parasiet van vals*. Wageningen. 146
[4]. NAGY E., TRIF V., 1998. Research regarding prevention and control of seed borne diseases at cereals in terms of Transylvania. *Plant Protection issues.*Bucharest. 26: 48 - 53
[5]. BERATLIEF C., OPREA M. 1994. Ecosystem characteristics of agricultural products storages and health implications. *Plant Protection issues.* Bucharest. 22: 13. 44
[6]. MALONE J.P., MUSKETT A.E., 1941. The Ulster method for the examination of flax seed for the presence of seed borne parasites. *Annals Appl. Biol.*. 8 - 13. 28
[7]. CEAPOIU N., 1968. Statistical methods applied in agricultural and biological experiments. Agro-Forestry Publishing. Bucharesti