CYTOGENETIC EFFECTS INDUCED BY DEPOSIT MYCOFLORA IN *ZEA MAYS* L. SEEDS FROM THE COLLECTION OF SUCEAVA GENEBANK

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Key words: *Zea mays* seeds, deposit mycoflora, cytogenetics

Abstract: The purpose of our work was to study the cytogenetic effects induced by deposit mycoflora in *Zea mays* L. (2n = 20) seeds from the collection of Suceava Genebank. Cytogenetic effects were studied by means of classical plant chromosomes methods of study. We have observed that the values of the mitotic indexes are decreasing in accordance with the increasing of the storage age and also with the number of mycoflora species that are infesting the grains. Infestation with specific mycoflora produced a relatively large number, statistically significant by comparison to the controls, of interphasic aberrations and also of chromosomal aberrations in mitosis ana-telophase in all studied probes. Comparing our results with similar published data, we can strongly state that the cytogenetic effects induced by deposit mycoflora in *Zea mays* L. seeds are similar with those produced by the action of a weak mutagenic agent

INTRODUCTION

The purpose of our work is to study the cytogenetic effects induced by deposit mycoflora in various species seeds from the collection of Suceava Genebank. This paper shows the cytogenetic effects induced by deposit mycoflora in *Zea mays* L. (2n = 20) seeds from the above mentioned collection. Cytogenetic effects were studied by means of: calculation of the mitotic index and frequency of mitosis phases, registration of the frequency and types of abnormal interphases and identification of chromosomal aberrations in mitotic ana-telophases, in accordance with classical plant cytogenetics methods.

MATERIALS AND METHODS

The biological material consisted of five probes of seeds selected from the collection of Suceava Genebank:

- The control: SVGB-1654, 17 years old, germination ratio 100%, not infested by mycoflora,
- SVGB – 13769, 8 years old, germination ratio 92%, infested by *Penicillium* sp. and *Rhizopus* sp.
- SVGB – 13797, 8 years old, germination ratio 82%, infested by *Penicillium* sp., *Aspergillus* sp. and *Alternaria alternata*
- SVGB – 3829, 17 years old, germination ratio 83%, infested by *Penicillium* sp., *Rhizopus* sp. and *Aspergillus* sp.
- SVGB – 1627, 17 years old, germination ratio 84%, infested by *Penicillium* sp., *Rhizopus* sp., *Cladosporium herbarum* and *Alternaria alternata*

The grains germinated in Petri dishes, on filter paper, wetted with distilled water for all variants till the roots reached 10-15 mm in length. We calculated the germination percent and the duration of germination for all experimental variants. When the roots reached the length of 10-15 mm they were fixed in Battaglia for 30 minutes. We have performed fast cytological slides according to Feulgen method (Cimpeanu at al., 2002).

We have studied:

1. The mitotic index and frequency of mitosis phases
2. The frequency and types of aberrant interphases
3. The frequency and types of chromosomal aberrations in mitotic ana-telophases (A-T).

We have analysed this 3 steps on 5 fresh preparations. For each slide we have studied: 10 microscopic fields (objective 40x) on which we have counted all the cells in interphase, prophase, metaphase, anaphase and telophase; 10 microscopic fields on which were counted all the cells in normal and abnormal interphases and the type of interphasic aberrations and 50 ana-telophases were analysed, counting the normal, aberrant ana-telophases and the type of chromosomal aberrations. All data were statistically processed (Fowler and Cohen, 1990). Microphotographs were performed with the digital camera of Nikon research microscope with the 100X objective.

RESULTS AND DISCUSSIONS

The infestation with fungi generally determined a significant decrease of the mitotic index (Table 1). Only for the SVGB-13769 probe the frequency of dividing cells is 6,45%, a very close value to the mitotic index registered for the control (4,81%) (Fig. 1). The lowest value of the mitotic index (2%) was observed for the SVGB-1627 probe.

The values of the mitotic index are proportionally decreasing with the increase of the number of infesting species of fungi and with the storage age of grains.

For the majority of probes, and also for the controls, we have recorded a predominance of prophases, followed by metaphases and telophases. Anaphases were recorded only for controls and SVGB-13769 and SVGB-13797, but in a very small percentage (Table 1 and Figure 2). An exception was noted for the probe SVGB-1627 where the frequency of prophases was low and metaphases were predominant. The frequency of prophases is decreasing proportionally with the increase of infestation and of the storage age of the grains (Fig. 2). The frequency of telophases is decreased for all probes in comparison to the controls. An exception was noted for the SVGB-13769 probes in which we have registered a high frequency of telophases in comparison to the controls.

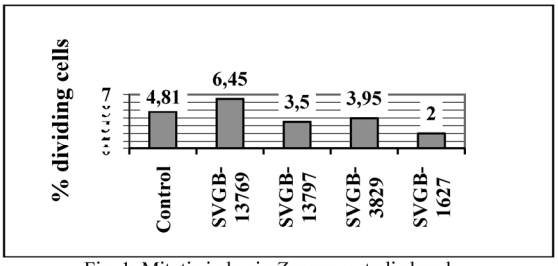

Fig. 1: Mitotic index in *Zea mays* studied probes

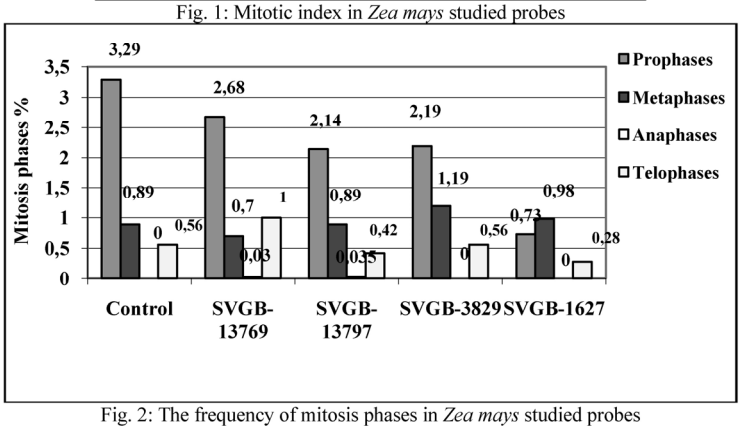

Fig. 2: The frequency of mitosis phases in *Zea mays* studied probes

Infestation with fungi induced the increase of interphasic aberrations in all the studied probes (Table 2 and Fig. 3).

The highest frequency of aberrant interphases (1,76%) was registered for SVGB-1627 probe. For the other probes the registered values are similar and quite low, but, in comparison to the very small value of aberrant interphases counted in the control probes (0,03%), we can state that the infestation with mycoflora induced a statistically significant increase of anaphase aberrations, without regard to the type of fungi or to the age of probes.

The most frequent interphasic aberration is represented by the binucleated cells, which frequency is increasing proportionally with the age of probes and with the number of infesting mycoflora species (Fig. 4).
In SVGB-13769 probe the infesting mycoflora induced some interphases with one micronucleus and, in a very low percentage, interphases with two micronuclei. Analysing all the mentioned data in comparison with data from literature (Maniu et al. 2002, 2005) we can strongly state that the cytogenetic effects induced by deposit mycoflora in *Zea mays* beans are similar with those produced by the action of a weak mutagenic agent.

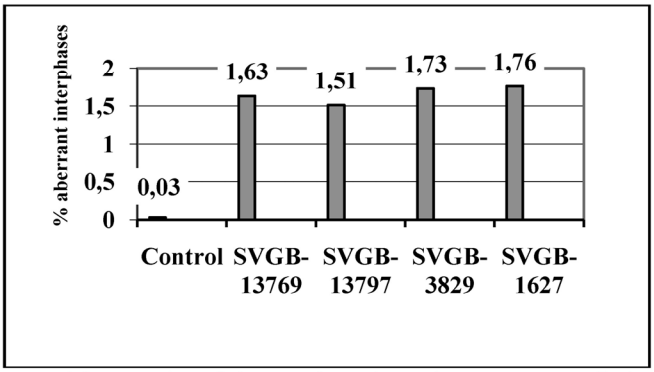

Fig. 3: Total frequencies of aberrant interphases in *Zea mays* studied probes

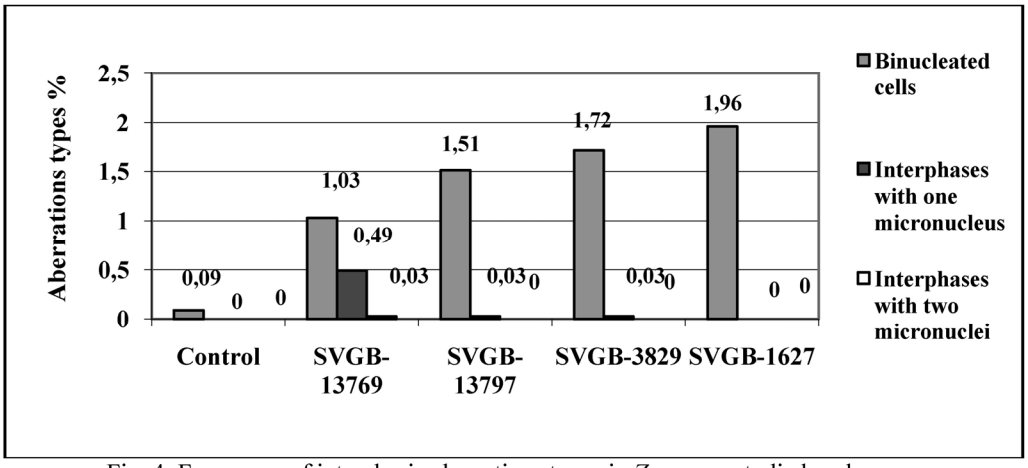

Fig. 4: Frequency of interphasic aberrations types in *Zea mays* studied probes

Analysing the data from table 3 one can observe that both for the control, SVGB-3829 and SVGB-1627 probes were registered chromosomal aberrations in mitosis ana-telophase. In the rest of two probes the number of observed ana-telophases was too small to perform statistic analysis.
The highest percentage of aberrant ana-telophases (2,50%) was observed for the SVGB-1627 probe (Fig. 5). The chromosomal aberrations types show a great variety, being distributed randomly (Fig.6).

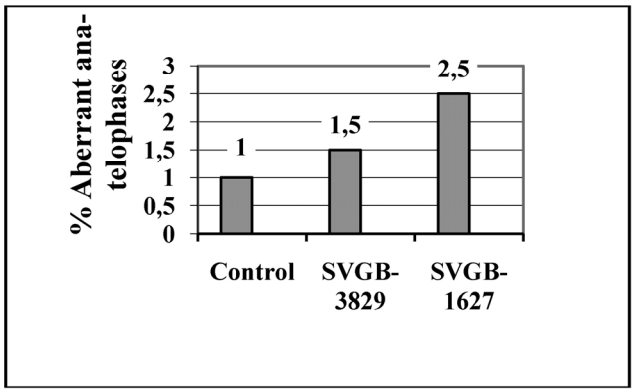

Fig. 5: Total frequency of aberrant ana-telophases in *Zea mays* studied probes

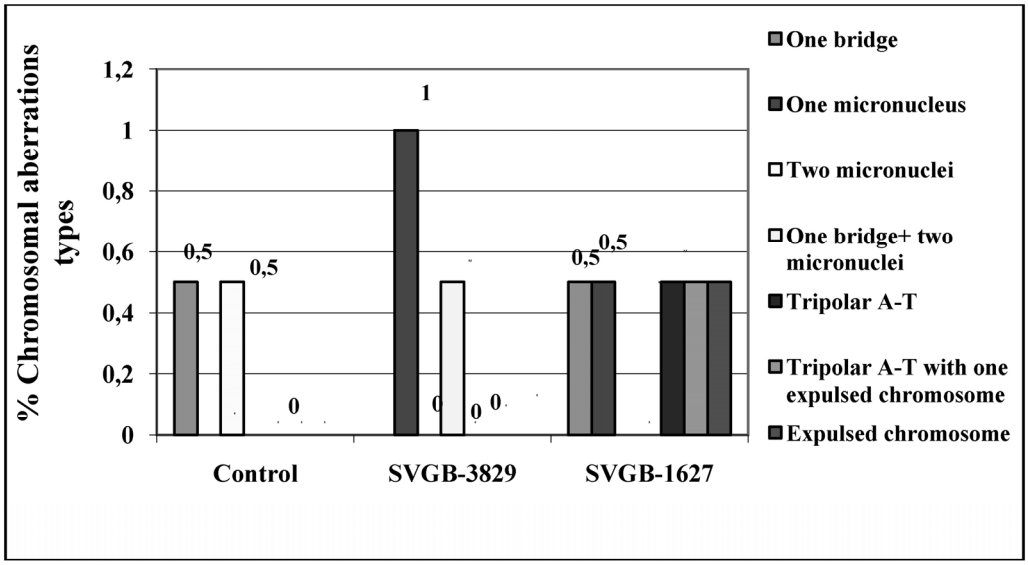

Fig. 6: Frequency of chromosomal A-T aberrations in *Zea mays* studied probes

We have observed and counted many simple chromosomal aberrations such as: one bridge, one micronucleus, two micronuclei, tripolar ana-telophases, expulsed chromosomes and some complex chromosomal aberrations such as: one bridge with two micronuclei, tripolar A-T with expulsed chromosomes, etc. The highest frequency was registered for tripolar ana-telophases and expulsed chromosomes.

The increase of the number of aberrations in ana-telophase is statistically significant reported to the controls; comparing our results with similar published data (Maniu et al. 2002, 2005) we can strongly state that the cytogenetic effects induced by deposit mycoflora in *Zea mays* seeds are similar with those produced by the action of a weak mutagenic agent.

CONCLUSIONS

The values of the mitotic indexes are decreasing in accordance with the increasing of the storage age and also with the number of mycoflora species that are infesting the seeds.
Infestation with specific mycoflora produced a relatively large number, statistically significant by comparison to the controls, of interphasic aberrations and also of chromosomal aberrations in mitosis ana-telophase in all studied probes.
Comparing our results with similar published data, we can strongly state that the cytogenetic effects induced by deposit mycoflora in *Zea mays* L. seeds are similar with those produced by the action of a weak mutagenic agent.

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Table 1: Frequency of mitoses and of mitosis phases in *Zea mays* studied probes

Probes	Total studied cells	Total interphases		Total mitoses		Total prophases		Total metaphases		Total anaphases		Total telophases	
	Nr.	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%
Control	2122	2020	95,19	102	4,81	70	3,29	19	0,89	1	0,05	12	0,56
SVGB-13769	3127	2989	93,55	138	6,45	84	2,68	22	0,70	1	0,03	31	1,00
SVGB-13797	2800	2702	96,50	98	3,50	60	2,14	25	0,89	1	0,035	12	0,42
SVGB-3829	3019	2900	96,05	119	3,95	66	2,19	36	1,19	-	-	17	0,56
SVGB-1627	2856	2799	98,00	57	2,00	21	0,73	28	0,98	-	-	8	0,28

Table 2: Frequency of interphasic aberrations in *Zea mays* studied probes

Probes	Total studied cells	Total normal interphases		Total aberrant cells			*Aberration types*					
							Binucleated cells		Interphases with one micronucleus		Interphases with two micronuclei	
	Nr.	Nr.	%	Nr.	%	$\pm s\bar{x}$	Nr.	%	Nr.	%	Nr.	%
Control	2020	2017	99,91	2	0,03	0,0009	2	0,09	-	-	-	-
SVGB-13769	2989	2940	98,37	49	1,63	0,030	31	1,03	17	0,49	1	0,03
SVGB-12769	2702	2660	98,66	42	1,51	0,003	41	1,51	1	0,03	-	-
SVGB-3829	2900	2849	98,47	51	1,73	0,004	50	1,72	1	0,03	-	-
SVGB-1627	2799	3081	98,24	55	1,76	0,004	55	1,96	-	-	-	-

Table 3: Frequency of chromosomal aberrations in mitosis ana-telophase (A-T) in *Zea mays* studied probes

Probes	Studied A-T	Total normal A-T		Total abnormal A-T		Types of chromosomal aberrations in mitosis A-T													
						One bridge		One micro-nucleus		Two micro-nuclei		One bridge + 2 micro-nuclei		Tri-polar A-T		Tripolar A-T with expulsed chromosome		Expulsed chromosome	
	Nr	Nr	%	Nr	%	Nr	%	Nr	%	Nr	%	Nr	%	Nr	%	Nr	%	Nr	%
Control	200	198	99,00	2	1,00	1	0,50	-	-	1	0,50	-	-	-	-	-	-	-	-
SVGB-3829	200	197	98,50	3	1,50	-	-	2	1,00	-	-	1	0,50	-	-	-	-	-	-
SVGB-1627	200	195	97,50	5	2,50	1	0,50	1	0,50	-	-	-	-	1	0,50	1	0,50	1	0,50