EVALUATION OF THE CYTOGENETIC EFFECTS PRODUCED BY *BOTRYTIS ALLII* FUNGUS TO *ALLIUM CEPA*

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ABSTRACT

The purpose of this paper was to evaluate the cytological effects produced by Botrytis allii fungus (gray rottenness) on the meristematic cells of onion (Allium cepa). In case of the samples studied (onion bulbs affected by the disease, together with a healthy control), the following cytogenetic characteristics were analysed: the mitotic index, the frequency of mitosis phases in the roots apex, the frequency and types of chromosomal aberrations and nuclear abnormalities.

After analysing results were found large differences between samples; thus, the mitotic index decreased from 11.88% for the control to 2.24-4.01% for samples affected by Botrytis allii. Also, it has been found the following main types of chromosome aberrations and nuclear abnormalities: sticky and laggard type chromosomes, fragments of chromosomes, as well as cells with nuclear erosion. From this point of view, the frequency of chromosomal aberrations and nuclear abnormalities was significantly higher in the case of variants affected by Botrytis allii, compared to the control variant (1.41% for control variant and 10.43-14.74% for variants affected by Botrytis allii).

The results show that Botrytis allii (gray rottenness) has the ability to cause a large number of mitotic abnormalities to Allium cepa, affecting the growth and development of plants. In addition, the cytogenetic effects of infection with Botrytis allii can probably be similar to those produced by the action of a mutagenic agent.

INTRODUCTION

Onion (Allium cepa L.) belongs to the genus Allium of the family Alliaceae and is originated in southwestern Asia. Onions are an important food crop worldwide. It is grown mainly as addition for different food dishes. Onions not only provide flavour, they also provide important nutrients and health-promoting phytochemicals. Thus, onion, along with garlic and leek, is part of the functional food category that abounds in antioxidant vitamins and phytonutrients (allylsulfides), very healthy for the daily diet of consumers. From this point of view, a niche segment consisting of different functional foods and dietary supplements - very necessary for the consumer whose daily stress level is increased - has been developed (Săvescu P., 2017; Săvescu P., 2016). Vitamin C, or the ascorbic acid, is one of the most valuable substances; to onion, vitamin C is the most important minor component (9 mg/100 g fresh onion) (Belitz H.D. and Grosch W., 1992). Onions are a good source of dietary fiber and folic acid. They also contain calcium, iron, and have a high protein quality (ratio of mg amino acid/gram protein).

In countries with high-performance farming, the focus and specialization of

onion-producing farms has been made in the most favourable areas. In Romania, the most farms where onions are grown are located in counties in the southeastern part of the country, where are the best pedoclimatic conditions for this species. The onion culture is also suitable in the Oltenia region, though this area is often affected by drought and heat that strongly influence other plant (like maize, peanuts) development and yield (Bonea D., 2016; Bonea D. and Urechean V., 2017; Olaru L.A., 2009; Popescu C.V. and Bora C., 2009). However, there is emphasized the rational use of water as a main method to combat drought (Popescu C.V. and Bora C., 2009).

Fungal pathogens may be present on plants before harvesting, in the time of harvest or after harvesting, in the time of storage, depending on storage conditions. Botrytis allii is a plant pathogen, a fungus that causes gray rottenness to stored onions (Chilvers M.I. and Du Toit L.J., 2006). The species was first described scientifically by Mancel Thornton Munn in 1917. Botrytis allii it is a common disease in onion crops and produces major damage, 15-20%, both in field crops and in deposits.

Latent Botrytis infections in onion bulbs are typically detected by storing harvested bulbs for 8 to 16 weeks, and then cutting the bulbs vertically to assess for internal symptoms (Chilvers M.I. et al., 2004). A real-time fluorescent PCR assay based on SYBR green chemistry has been developed to quantify the amount of neck rot Botrytis spp. (B. allii, B. aclada, and B. byssoidea) present in onion seed (Chilvers M.I. and Du Toit L.J., 2006).

Higher plants provide valuable genetic assay systems for screening and monitoring environmental pollutants. For this purpose, the Allium cepa is one of the most frequently used higher plant species. The Allium test for genotoxicity was introduced by L. Evan (1938). The vegetal meristematic tissues that are used for testing the effects of some mutagens on chromosomes should be easy to obtain and less expensive. From this point of view, Allium cepa are well suited to cytogenetic studies because the meristematic roots appear lightly, have relatively large chromosomes in small numbers and can be easily observed by microscope.

MATERIALS AND METHODS

The experiment was maintained in laboratory conditions at 24±2°C. For cytogenetic analyses, five bulbs of a commercial variety of onion (Allium cepa, 2n = 16) were selected; one of which was healthy (Control) and four bulbs were affected by Botrytis allii (samples V1, V2, V3, V4). The onion bulbs were allowed to produce roots in tap water for 72 h.

After germination, the roots from each onion bulbs were immediately cut and fixed in solution of ethanol and glacial acetic acid (3:1) for 24 h; next day, the roots were transferred to 70% alcohol and stored in refrigerator until use. For microscopic preparation, the root tips were treated with 1N HCl for 5 min and HCl 50% for 16 min; after that, the roots were washed with distilled water. To carry out the chromosomes view using the optic microscope, these were colorized with the basic fuchsine decolorized solution (Schiff reactive), prepared in the laboratory. The effective colorized operation was the introduction to 3-4 cc colorant solution from onion roots placed in glass ampoules, at room temperature. After max 30 minutes, the meristematic tissues were colored in purple-red.

Microscopic slides were done by pressing. The measurements were made under an optic microscope (MBL-2000 Kruss type) by considering 15 areas per slide. The mitotic index and the number of abnormal cells were counted in each phase of mitosis. The mitotic index was determined by scoring more than 100 cells per slide and was calculated as the

percent ratio of dividing cells and total numbers of cells scored.

The different phases of mitosis were calculated as following: Prophase frequency % = number of cells in prophase x 100/total number of dividing cells; Metaphase frequency % = number of cells in metaphase x 100/ total number of dividing cells; Anaphase frequency % = number of cells in anaphase x 100/ total number of dividing cells; Telophase frequency % = number of cells in telophase x 100/ total number of dividing cells.

The rates of mitotic abnormalities were also calculated: number of cells with chromosomal aberrations/ nuclear abnormalities x 100/ total number of dividing cells.

The analysis of variance was used to assess the significant differences between control and each treatment. The data were expressed as mean ± standard error of the means for all parameters. To calculate and to graphically represent the statistical parameters, the Microsoft Office Excel 2010 software of Windows XP operating system was used.

RESEARCH RESULTS

The effects produced by Botrytis allii fungus (gray rottenness) on the meristematic cells of onion (Allium cepa) are reported in Table 1 and Figure 1. Analysis of the results showed a decrease in % MI of between different samples and control. Thus, the mitotic index decreased from 11.88% (Control) to 2.24 % (V1); 3.08% (V2); 4.01% (V3) and 2.83% (V4).

Mitotic index is considered a parameter that allows one to estimate the frequency of cellular division; inhibition of mitotic activities is often used for tracing cytotoxic substances. Generally cytotoxicity means a decrease in mitotic index and as an increase of the chromosomal aberrations and nuclear abnormalities. Regarding the different phases of mitosis, they recorded the following values: prophase 54.03% (Control) and 40.08-45.90% (V1-V4); metaphases 19.69% (Control) and 28.82-35.95% (V1-V4); anaphase 11.25% (Control) and 13.36-14.95% (V1-V4); telophase 15.03% (Control) and 9.24-14.55% (V1-V4).

The changes in the organization and morphology of the chromosomes in the root tips affected by Botrytis allii were observed (Table 2). They identified some types of chromosomal aberrations and nuclear abnormalities, as follows: sticky and laggard type chromosomes, fragments of chromosomes, as well as cells with nuclear erosion (Figure 3). From this point of view, the frequency of chromosomal aberrations and nuclear abnormalities was significantly higher in the case of variants affected by Botrytis allii, compared to the control variant: 1.41% (Control); 10.43% (V1); 14.74% (V2); 11.91% (V3) and 12.22% (V4).

Regarding the frequency of different types of mitotic abnormalities (Figure 2), sticky chromosomes recorded values between 0.71% (Control) and 3.85-5.91% (V1-V4); aberrant fragments chromosomes recorded values between 0.30% (Control) and 0.92-3.62% (V1-V4); laggard chromosomes recorded values between 0.40% (Control) and 1.95-2.92% (V1-V4).

In mitosis, laggards chromosomes may be lead to genetic disequilibrium between daughter cells. On the other hand, from the point of view of nuclear anomalies, the control variant did not recorded such cases, whereas at the samples affected by Botrytis allii, was detected cells with nuclear erosion with a frequency of 2.74% (V1); 3.23% (V2); 2.81% (V3) and 3.12% (V4).

The appearance of chromosomal anomalies in plants is generally regarded as a potential danger, even if, sometimes, the mutagens create variability (Bonciu E., 2012; Bonea D. et al., 2018; Bonea D. and Bonciu E., 2017).

Table 1

The mitotic index and frequency of mitosis phases to *Allium cepa* affected by *Botrytis allii* fungus

	Mitotic index (MI%)*	Frequency of mitosis phases (%)*			
		P	M	A	T
Control	11.88±0.65	54.03±2.58	19.69±2.05	11.25±0.41	15.03±1.90
V1	2.24±0.21	40.08±4.51	32.01±4.02	13.36±1.82	14.55±2.03
V2	3.08±0.42	43.01±2.57	28.82±0.58	14.95±2.82	13.22±4.08
V3	4.01±0.51	40.26±3.05	35.95±3.01	14.55±2.03	9.24±1.68
V4	2.83±0.34	45.90±2.60	29.55±0.61	14.31±2.46	10.24±1.79

*mean ± standard error of the means
P=Prophase; M=Metaphase; A=Anaphase; T=Telophase

Table 2

Frequency of mitotic abnormalities induced by *Botrytis allii* fungus to *Allium cepa*

	Total CA and NA (%)	Type (%)			
		S	F	L	NE
Ct	1.41	0.71	0.30	0.40	0
V1	10.43	3.85	0.92	2.92	2.74
V2	14.74	5.44	3.62	2.45	3.23
V3	11.91	5.91	1.24	1.95	2.81
V4	12.22	4.85	2.23	2.02	3.12

CA=Chromosomal aberrations; NA=Nuclear abnormalities; S=Stickiness; F=Fragments; L=Laggards; NE=Nuclear erosion

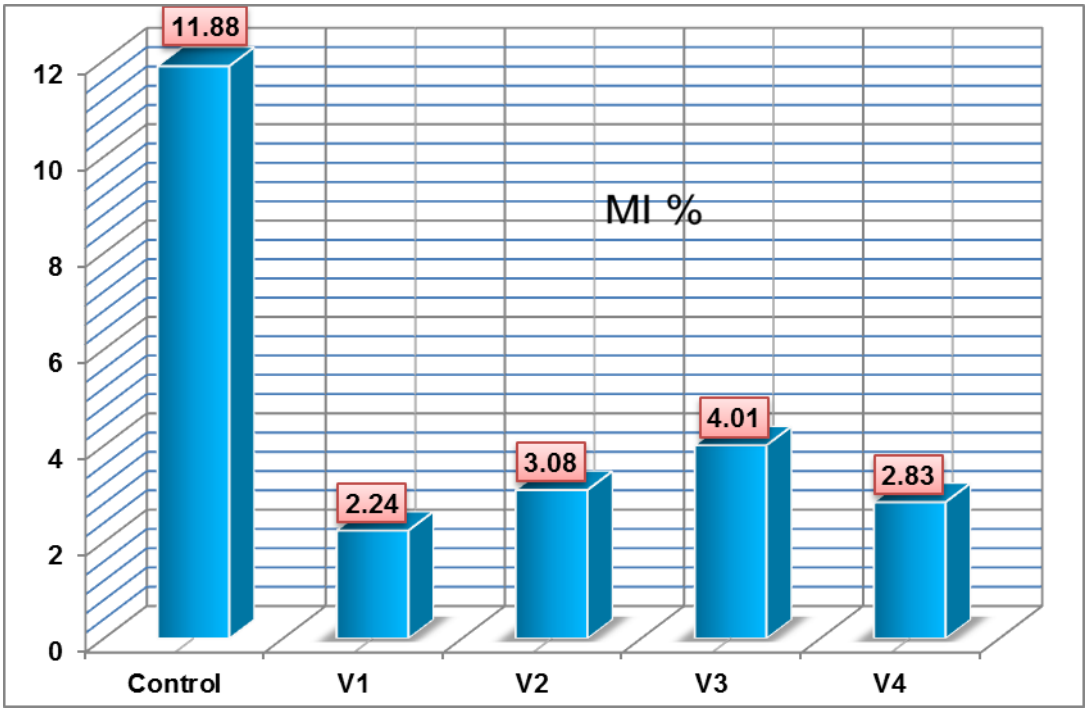

Figure 1. Graphical representation of the mitotic index (MI%) to *Allium cepa* affected by *Botrytis allii* fungus

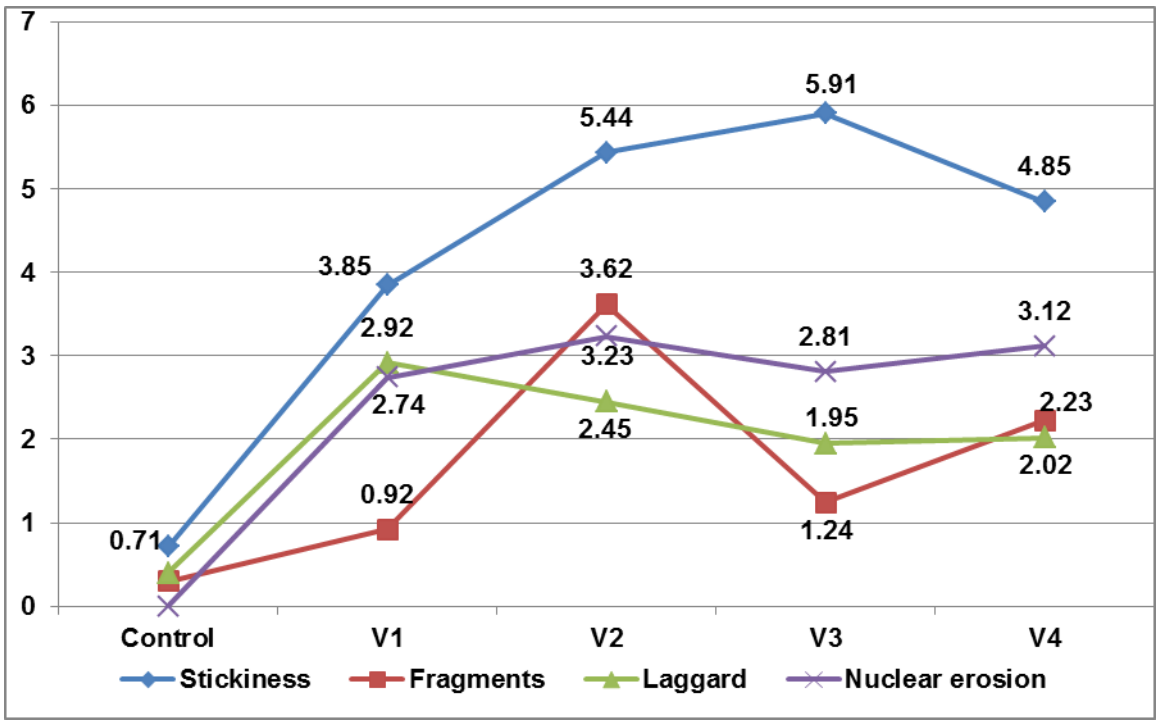

Figure 2. Graphical representation of the chromosomal aberration (%) and nuclear abnormalities (%) to *Allium cepa* affected by *Botrytis allii* fungus

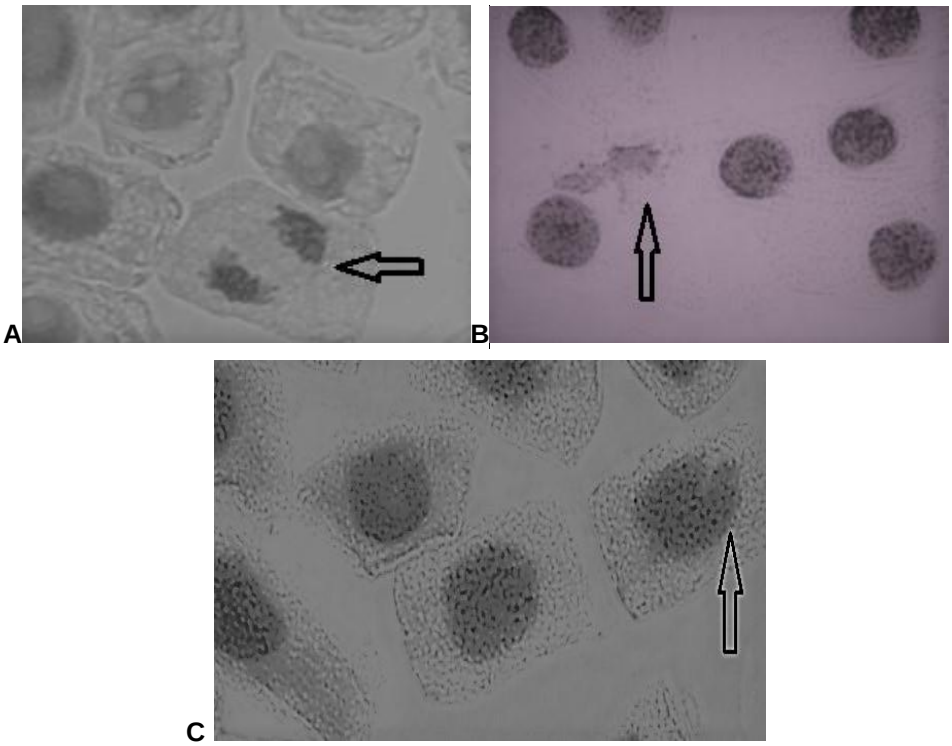

Figure 3. Some mitotic abnormalities caused by action of *Botrytis allii* fungus to *Allium cepa*: stickiness in ana-telophase (A); laggard in metaphase (B); cell with nuclear erosion (C)

CONCLUSIONS

In our experience, *Botrytis allii* fungus has a significant influence on the mutagenic activity to *Allium cepa* and can produce negative effects on mitotic divisions in *Allium cepa* cells. The mitotic inhibition will lead to growth inhibition of active protective response when plants are exposed to this pathogen. Also, the appearance of various chromosomal aberrations illustrates the cytotoxic potential of this disease.

The fungal pathogen caused a relatively high number of chromosomal aberrations and nuclear abnormalities in all analysed samples compared to the control sample. In conclusion, we can say that *Botrytis allii* fungus can behave as a weak mutagen

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