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STUDY REGARDING THE CELLULAR ACTIVITY IN GARLIC (*A. sativum*) BULBS AFFECTING BY *Sclerotium cepivorum*

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Abstract

The purpose of this study was to evaluate the cellular activity in meristematic roots of garlic bulbs (Allium sativum) affected by Sclerotium cepivorum, from the point of view of the mitotic index, the frequency of mitosis phases and the frequency and types of chromosomal and nuclear abnormalities. Results obtained indicated large differences between samples; thus, the mitotic index decreased from 14.64% for the control to 4.36-10.25% for samples affected by S. cepivorum. Also, it has been found the following main types of chromosome aberrations and nuclear abnormalities: laggards, stickiness, rings, micronucleus and nuclear dissolutions. From this point of view, the frequency of chromosomal aberrations and nuclear abnormalities was significantly higher in the case of variants affected by S. cepivorum, compared to the control variant (1.04% for control variant and 8.65-11.24% for variants affected by S. cepivorum). The results suggest that S. cepivorum has the ability to disturbance the cellular activity in A. sativum meristematic roots, which affecting the growth and development of plants.

Key words: *A. sativum, Sclerotium cepivorum, mitotic index, chromosomal anomalies.*

INTRODUCTION

The production and the economic efficiency of the agricultural crops are in strong correlation with a good management of the respective vegetable genotype (Butnariu and Caunii, 2013; Dragomir and Partal, 2016; Roșculete and Roșculete, 2016; Roșculete and Roșculete, 2018, Pandia et al., 2019).

Garlic (*Allium sativum*) belongs to the genus *Allium* of the family *Alliaceae*. The generic name *Allium* is the Latin word for garlic, and the type species for the genus is *Allium sativum*, which means "cultivated garlic" (Block, 2009).

Some sources refer to Greek (*aleo* = to avoid) by reason of the smell of garlic (Block, 2009). Various *Allium* species have been cultivated from the earliest times, and about a dozen species are economically important as crops, or garden vegetables, and an increasing number of species are important as ornamental plants.

Garlic is grown around the world. It is related to onion, leeks, and chives. It is thought that garlic is native to Siberia, but spread to other parts of the world over 5000 years ago. Garlic is most commonly used for conditions related to the heart and blood system (Borrelli et al., 2007; Ahmadi et al., 2013). These conditions include high blood pressure, high levels of cholesterol or other fats (lipids) in the blood (hyperlipidemia), and hardening of the arteries (atherosclerosis).

In foods and beverages, fresh garlic, garlic powder, and garlic oil are used to add flavor. Garlic produces a chemical called allicin. This is what seems to make garlic work for certain conditions. Allicin also makes garlic smell. Some products are made "odorless" by aging the garlic, but this process can also make the garlic less effective. It's a good idea to look for supplements that are coated (enteric coating) so they will dissolve in the intestine and not in the stomach (Garlic, Uses & Effectiveness, https://www.webmd.com/vitamins/ai/ingredientmono-300/garlic).

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.

Sclerotium cepivorum is the causal agent of the disease commonly known as *Allium* root rot. It is a problem found all over the world on *Allium* spp. that can be very devastating since it can result in large crop losses. Once a field has *S. cepivorum* it is difficult and costly to continue growing *Allium* spp. there, if possible at all. The fungus is positively influenced mainly by cool weather and survives in the soil as small, round structures known as sclerotia. These sclerotia can survive in the soil for decades (Sammour et al., 2012).

During cool weather there may be white, fluffy mycelia growth at the base of the stem plate when the leaves are yellowing. Mycelia growth is favoured by cool and moist soil, and in the right conditions the mycelia can cover the whole bulb. On these mats of mycelium black sclerots will form that will be about the size of a poppy seed (Sammour et al., 2012).

Higher plants provide valuable genetic assay systems for screening and monitoring environmental pollutants. For this purpose, the *A. cepa* and *A. sativum* are the most frequently used higher plant species (Bonciu et al., 2018). The vegetal meristematic tissues that are used for testing the effects of some mutagens on chromosomes should be easy to obtain and less expensive.

MATERIALS AND METHODS

For this study, bulbs of garlic (*A. sativum*, 2n = 16) affected by *S. cepivorum* were selected, together with a control without traces of diseases or pests. After germination in distilled water, for fixing meristematic cells, Carnoy's fixator was used (ethanol and glacial acetic acid, 3:1 ratio). The biological material was then hydrolysed and stained with basic fuchsine 1%.

The mitotic activity was calculated as the percent ratio of number of cells in mitotic division and total numbers of cells.

The percent of the chromosomal aberrations and nuclear anomalies was determined as the percent ratio of number of cells with mitotic abnormalities and total number of cells in mitotic division.

For cytogenetic study it was used the Kruss Optronic microscope.

Statistical analysis was done using MS Excel 2007. The analysis of variance (ANOVA) was used to assess the significant differences between the control variant and each treatment.

RESULTS AND DISCUSSIONS

Species of the *Allium* genus are used very often in genetic researches, being easy to trace from a cytogenetic point of view (Hao et al., 1994; Dolatyari et al., 2018; Rosculete et al., 2019). However, there are other species used as genetic models for research, such as: *V. faba* (Kosev and Georgieva, 2019); *C. libani* (Mercimek Takci et al., 2019); alfalfa (Nikolova and Georgieva, 2019) and mistletoe plants (Samfira et al., 2013). Regarding the present study, *S. cepivorum* fungus has ability to induce several effects to garlic (Table 1 and Figure 1). Analysis of the results showed a decrease of the MI between 14.64% (Control) and 10.25% (V4); 9.21% (V1); 8.23% (V5); 6.18 (V2) and 4.36% (V3).

Table 1. The mitotic activity to *A. sativum* bulbs affected by *S. cepivorum*

	Mitotic index (MI%±SE)	Frequency of mitosis phases (%)*			
		P	M	A	T
Ct	14.64±0.2	54.11±2.5	18.64±2.1	12.21±0.1	15.04±2.0
V1	9.21±0.2	40.21±4.5	30.21±2.1	13.16±1.2	16.42±2.0
V2	6.18±0.6*	43.31±2.5	26.42±0.4	15.05±2.1	15.22±3.1
V3	4.36±0.5*	40.25±3.0	31.15±2.4	14.51±2.1	14.09±1.5
V4	10.25±0.2	43.32±2.4	29.55±0.6	13.21±2.5	13.92±1.8
V5	8.23±0.2*	45.92±2.6	31.25±0.4	15.21±2.3	7.62±3.0

SE=standard error of the means; P=Prophase; M=Metaphase; A=Anaphase; T=Telophase *Significant at level 5% (p=0.05)

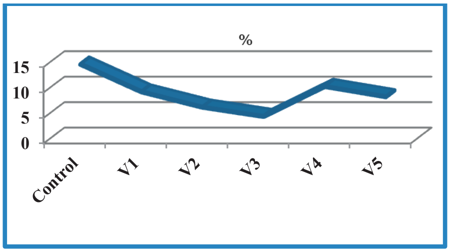

Figure 1. The depression of the mitotic index (MI%) in *A. sativum* cells exposed to *Sclerotium cepivorum*

The *S. cepivorum* can resists in the soil 4-5 years by sclerots that are carried by irrigation water or by the maintenance works, ensuring the spread of the pathogen. The sclerots can also be find on the bulbs intended for planting and can maintain their viability for 4-5 years. They can produce new root infections even one month after their formation. In crops there are two periods of maximum sensitivity of plants to this fungus. One in March-April and another after the formation of bulbs and until harvesting, the attack being favoured by the wounds that appear on the roots or bulbs. Plants in cold and moist soils are more sensitive, as the fungus can attack from 2°C to 35°C, when the mycelium is destroyed.

The mitotic index is important criteria used in the assessment of cells proliferation processes (Bonciu, 2012).
The analysis of the mitosis stages allowed finding some fluctuations in the experimental variants, compared to the control. Thus, they recorded the following values: prophase 54.11% (Ct) and 40.21-45.92% (V1-V5); metaphases 18.64% (Control) and 26.42-31.25% (V1-V5); anaphase 12.21% (Ct) and 13.16-15.21% (V1-V5); telophase 15.04% (Ct) and 7.62-16.42% (V1-V5).
Frequency of cellular abnormalities induced by *S. cepivorum* fungus to *A. sativum* is showed in Table 2.

Table 2. The genotoxicity effects induced by *S. cepivorum* to *A. sativum*

	Total CA and NA (%)	Specification (%)				
		L	S	R	MN	ND
Control	1.04	0.11	0.30	0.00	0,42	0.21
V1	8.94	3.15	1.02	0.89	1.74	2.14
V2	11.24	3.04	2.18	2.08	2.42	1.52
V3	9.45	3.11	1.20	1.09	2.96	1.09
V4	8.65	2.64	2.86	0.93	1.10	1.12
V5	10.12	2.81	3.23	0.99	2.02	1.07

CA = Chromosomal aberrations; NA=Nuclear abnormalities; L=Laggards; S=Stickiness; R=Rings; MN=Micronucleus; ND= Nuclear dissolutions

The cytological profile of the garlic meristematic roots analysed revealed a series of chromosomal and nuclear anomalies, represented by: laggards, stickiness, rings, micronucleus and nuclear dissolutions (Figure 3). The recorded values ranged between 8.65% (V4) and 11.24% (V2), while in the control variant, cellular abnormalities were identified only as a percentage of 1.04%. These results indicate the strong genotoxicity potential of *S. cepivorum* fungus in the garlic cells and suggest the possibility of impairing normal growth and development of the future plantlets.
Chromosomal anomalies are changes in the number or structure of the chromosomes, while nuclear anomalies represent changes in the structure of the nucleus. In some cases, the structural anomalies of the plant cells dose not manifest at the plant in which they were identified, but the manifestations can occur at the descendants who inherited the respective modification (Bonciu, 2018; Bonciu et al., 2018).
Chromosomal abnormalities can be defined as chromosomes restructuring, after which whole groups are moved from one chromosome to another. They do not usually affect the structure of the genes but especially the links between them. Any change in the structure of the chromosomes can mean a change in the amount or arrangement of genetic information. These changes may induce some disturbances in plants development.
As can be seen in Figure 2, the limits of quantifiable values of the cellular anomalies identified were: 2.64-3.15% (laggards), 1.02-3.23% (stickiness), 0.89-2.08% (rings), 1.10-2.96% (micronuclei) and 1.07-2.14% (cells with nuclear dissolutions).
Some changes in chromosomes structure occur when the genetic material is broken and rearranged into a certain one way. For example, in the case of ring chromosomes, the terminations of a chromosome usually bind when the two endings of the chromosome are deleted. The ends that remain are "Sticky" and it is unite in the form of a ring. The effects that this chromosomal aberration will have it on the plant depend usually by the amount of genetic material that was lost before the ring is formed.
Some previous studies in the literature explain the correlation of the diseases and pests attack depending on the environmental conditions or the culture management (Butnariu, 2012; Partal et al., 2013; Partal et al., 2014; Paraschivu et al. 2015; Paraschivu et al., 2017; Cotuna et al., 2018; Pandia et al., 2018). In order to limit the attack and the damages caused by *S. cepivorum* fungus, preventive and curative measures are required, such as: 5-6 years of crop rotation, deep ploughing preceded by burning of diseased plants, balanced fertilization, use of healthy bulbs for planting and their preventive treating, treatments with specific fungicides in the vegetation etc.
Many authors have described techniques for testing the viability of sclerotia and for measuring their germination in response to host plants (Coley-Smith, 1985; Banks and Edgington, 1989; Davis et al., 2007; Esler and Coley-Smith, 2007; Amin et al., 2014).

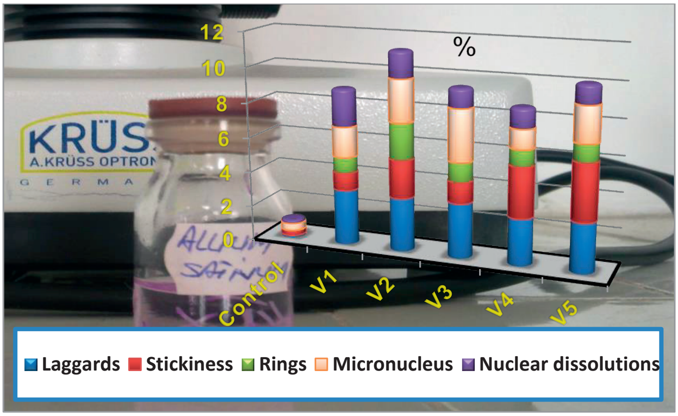

Figure 2. Frequency of the main types of cellular abnormalities identified in *A. sativum* meristematic roots under influence of *S. cepivorum*

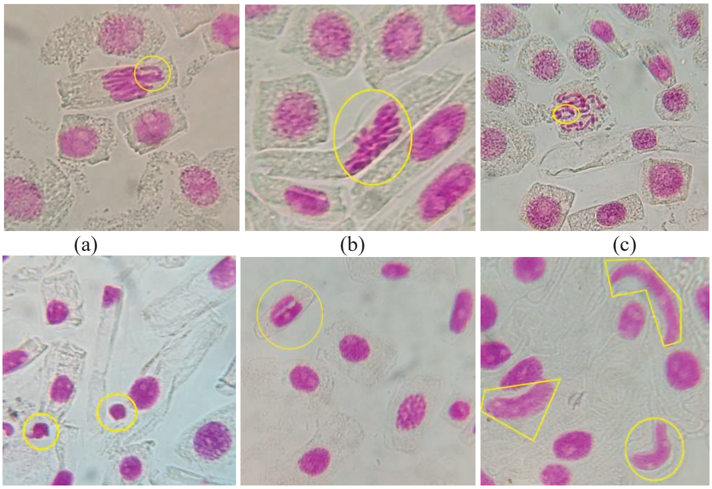

Figure 3. The aberrant appearance of *A. sativum* cells affected by *S. cepivorum*: laggards (a), stickiness (b), ring chromosome (c), micronuclei (d), nuclear dissolutions (e, f)

CONCLUSIONS

The evaluation of the influence of the *S. cepivorum* on the *A. sativum* cells suggests the depression of the mitotic index and the mitotic activity respectively. This can affect the growth and development of the garlic plants. The reduction of mitotic activity was correlated with the increase of the cellular abnormalities frequency.

Comparative cytological analysis in the garlic bulbs affected by the *S. cepivorum* and healthy bulbs, reveal specific features of inhibition of the mitotic activity process and phenotypic induction of the some types of chromosomal and nuclear anomalies that indicate the strong cytotoxicity and genotoxicity of this fungus in *A. sativum* cells.

In order to limit the attack and the damages caused by this fungus, preventive and curative measures are required, such as use of healthy bulbs for planting and treatments with specific fungicides in the vegetation.

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