CYTOGENETIC EFFECTS INDUCED BY BARK EXTRACT FROM *HIPPOPHAE RHAMNOIDES* ON *SECALE CEREALE L.*

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Keywords: polyphenols, *Secale cereale L.,* germination, chromosomal abberations

Abstract: plants development it is influenced by various exogenous and endogenous factors. In this context, a group of substances of particular interest are the phytoregulators of growth. Polyphenols are a group of natural products of great diversity. The significance of the accumulation of large amounts of polyphenols in plants is unknown. Their presence has significant effect on humans due to their widespread use in medical, food and beverages, etc. Duet o the informations presented in the literature we have decided to study the influence of these kind of extracts on the mitotic division. Investigated material comes from the seeds of rye, untreated (control) and treated with polyphenols (from *Hippophae rhamnoides* bark) in the following concentrations: 1%, 0.5% and 0.1%. To trace the effects of extracts on the cell division, we considered necessary to determine the germination capacity, growth rate and chromosomal abberation.

INTRODUCTION

In agricultural practice, phytoregulators are used in small quantities, and have an important effect on the processes of plant growth and development, especially in young organisms, if applied during some specific phenophase and climate (germination, flowering, fertilization, ripening, drought, humidity, senescence, etc.) in order to contributing to the improving the quantity and quality of agricultural products.

Previous studies carried out by standard procedures made either under greenhouse and in fields, have shown that natural products with aromatic structure have favorably influence on plants growth. It is also noted that plant growth regulating action is differentiated according to the nature of the product (lignins and polyphenols), methods of separation, the concentration of chemical modification of the product and not least by the nature of the biological material used.

It is known that a number of plant secondary metabolites may be involved in the development of plants during their life or after death in the natural recycling of carbon in humus formation conditions. These metabolites are involved in various defense responses against pathogenic or nonpathogenic microorganisms.

MATERIAL AND METHODS

Biological material comes from rye harvest from 2007, untreated (control) and treated with polyphenols (extracts obtained from the *Hippophae rhamnoides* bark), in the following concentrations: 1%, 0.5%, 0.1%.

Before germination, seeds were immersed for 24 hours in solutions having a concentration of 1% polyphenol, 0.5% and 0.1% for each source (*Hippophae rhamnoides* bark), made available by S.C.Plantavorel S.A. A number of 200 seeds /Petri dish were germinated in the thermostat at a temperature of 20^0C. In addition to these three experimental variantes, we have used a control, which was immersed in distilled water, 24 hours before germination.

After 5-6 days, most seeds have germinated. When roots reached 10-15 mm in length, the material was fixed for the cytogenetic analysis. In order to study the division cycle and chromosomal aberrations Carr staining technique was used Carr (carbolic-fuxin).

For the germination capacity, an identically prepared experimental group was studied in terms of time required to start germination and germination percentage, using the formula:

$$\% \frac{\text{total number of seeds - ungerminated seeds}}{\text{total number of seeds}} \cdot 100$$

Determinations of the germination percentage was achieved at 96 hours after the onset of this process. Another investigated parameter was the increase in length of plantlets. Study on lenght growth rate of plantlets (in mm) was performed 96 hours after germination (4 days). After 4 days, we measured the length of plantlets both blank and the three concentrations. Within three days we made the measurement. The data were interpreted statistically and graphically.

RESULTS AND DISCUSSIONS

Germination under polyphenols treatment in Secale cereale L.

Results obtained from investigation of germination capacity for seeds treated with

polyphenols, are presented in Table 1 and Fig. 1:

Table 1 Germination capacity under *Hippophae rhamnoides* bark extract

Nr.	Concentration (%)	Germinated seeds (%)
1	control	26,5
2	1	44,5
3	0,5	47,0
4	0,1	34,0

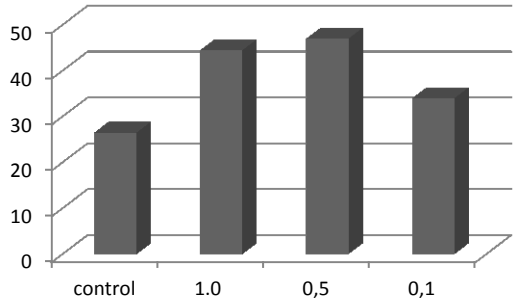

Fig. 1 Germination capacity, *Hippophae rhamnoides* bark extract treatment consequently

From these data, it appears that the untreated seeds (control) germinate in a rate of 26.5%. On polyphenols treated seed we recorded an increase of germination capacity at all concentrations used, as 34% in seeds treated with polyphenols at a concentration of 0.1, 47% in those treated with polyphenols at a concentration of 0.5% and 44.5% in those treated with the polyphenol concentration of 1%.

It appears therefore that at concentration of 0.5%, the polyphenols used in experiments have the most favorable effect, behaving as true phytostimulators.

Rate of lenght increase

As it is evident from the table and graphs above (Table 2 and Fig. 2, 3) plantlets growth was stimulated compared to the control. These certify, once again, the effect of polyphenols as biostimulator of growth of rye plantlets. The most effective concentration of polyphenols used to be 0.5%, the same concentration that gave good results as biostimulator for germination.

Table 2 Rate of growth in size from seed treated with seedlings polyphenols

	Average length of plantlets (mm)				Average length sum
	24 h	48 h	72 h	96 h	
Control	0,050	0,055	0,065	0,07	0,215
0,1%	0,065	0,085	0,100	0,09	0,430
0,5%	0,100	0,135	0,075	0,16	0,470
1 %	0,150	0,105	0,080	0,11	0,445

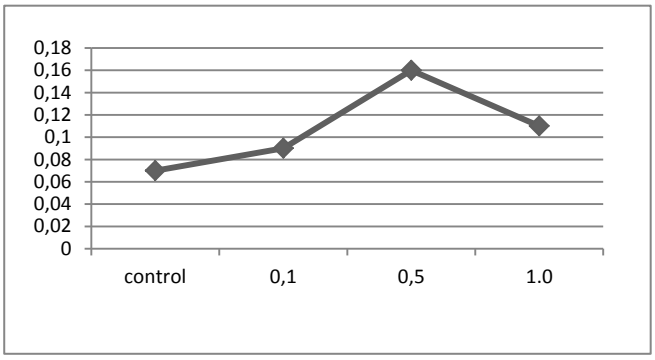

Fig. 2 The rate of length increase in *Secale cereale L*. plantlets germinated from seeds treated with polyphenols (*Hippophae rhamnoides* bark) and untreated, after 96 hours

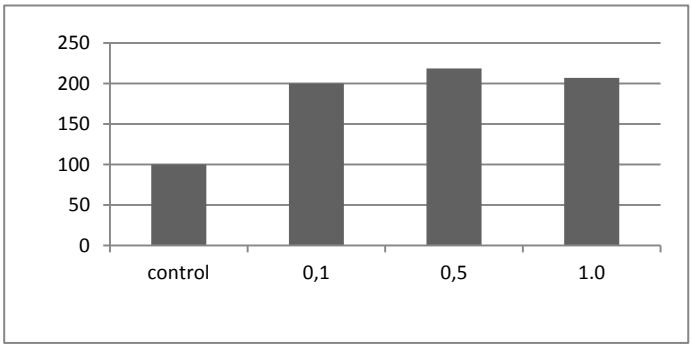

Fig. 3 The percentage of length increase rate of plantlets from seeds treated with compared with control

Chromosomal aberrations induced by treatment with polyphenols extracts in Secale cereale L.

Effect of extracts possessing bio-stimulator effects used in our experiments was shown on the capacity of these extracts to induce germination enhance on rye and to enhance the growth of plantlets from treated seeds. However, such compounds may induce chromosomal aberrations in mitosis, a matter investigated in our experiments.

A „perfect” biostimulator should only have beneficial effects on plant material, but do not produce chromosomal aberrations and, in a word, have no mutagenic capacity, at least not a significant capacity to induce mutations.

The first aspect investigated, coupled with the ability to induce mutations, was the effect of polyphenols on the different stages of mitotic division. The results from this study are summarized in Fig. 4, 5, 6, 7 and 8.

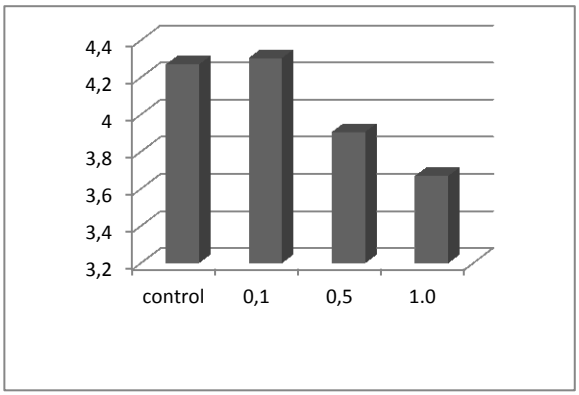

Fig. 4 The rate of cell division in root meristem of rye, treated with polyphenols (*Hippophae rhamnoides* bark)

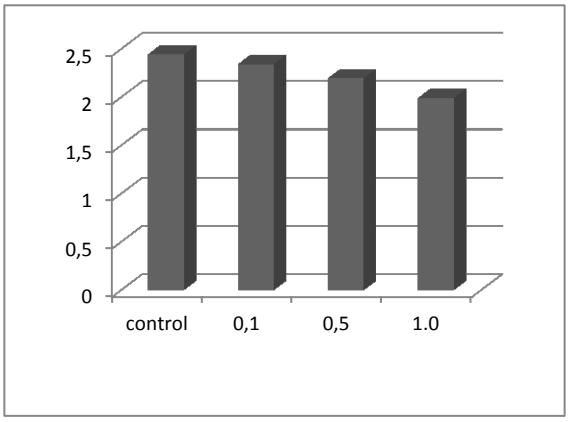

Fig. 5 The rate of prophases in root meristem of rye, treated with polyphenols (*Hippophae rhamnoides* bark)

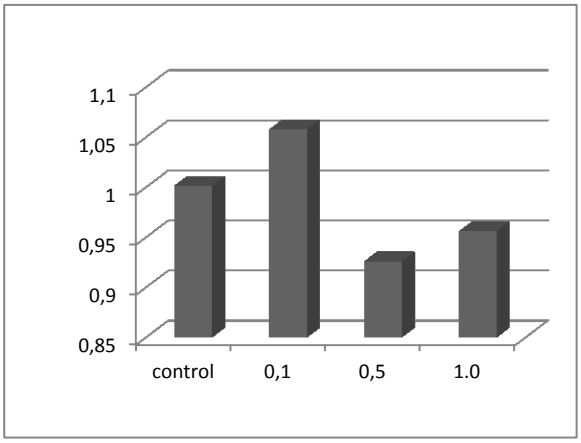

Fig. 6 The rate of metaphases in root meristem of rye, treated with polyphenols (*Hippophae rhamnoides* bark)

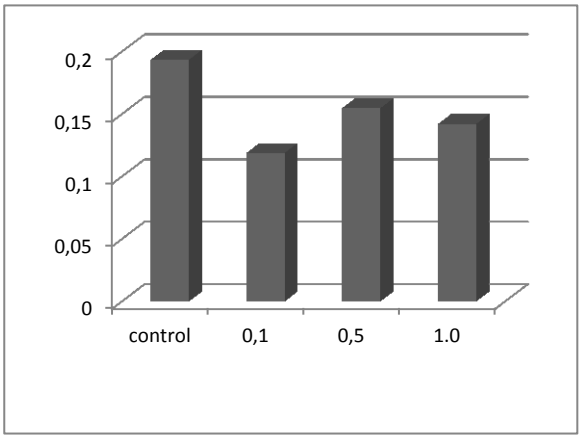

Fig. 7 The rate of anaphases in root meristem of rye, treated with polyphenols (*Hippophae rhamnoides* bark)

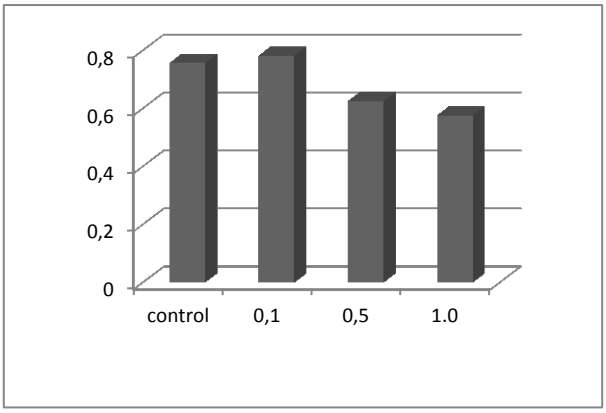

Fig. 8 The rate of telophases in root meristem of rye, treated with polyphenols (*Hippophae rhamnoides* bark)

As seen in Fig. 4, the number of dividing cells decreases, directly proportional manner with increasing concentration of polyphenolic extracts. It appears therefore that although exert a biostimulator, as evidenced in the first experiments, polyphenols inhibit cell division. Alone, a concentration of 0.1%of extracts inducing a very slight increase in cell division percentage compared with control. The following graphic is shown for each phase of the division situation (Fig. 5,6,7,8). For these evaluations, the pattern of variation is not as balanced as if the total number of cells in division, respectively prophase. We believe that in every stage of mitotic division, cells exhibit a specific response to the action of external agents such as polyphenolic growth regulators. But in all cases, concentrations of 0.1 and 0.5% proving their biostimulator ability, unlike the concentration of 1.0% that showed an inhibitory influence.

In the graphics above are presented data obtained by recording the number of anatelophase, normal and aberrant metaphases and the proportion of the main types of

aberrations encountered.

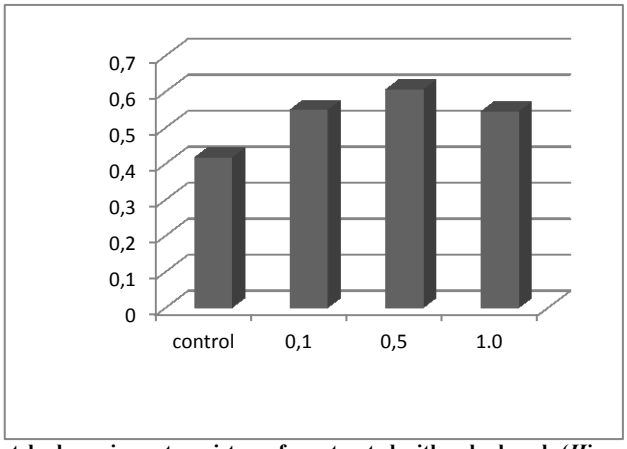

Fig. 9 Abberant anatelophases in root meristem of rye, treated with polyphenols (*Hippophae rhamnoides* bark)

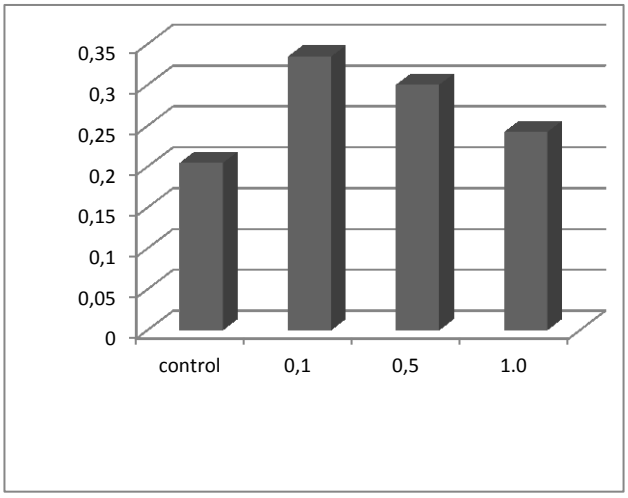

Fig. 10 Abberant anatelophases with fragments in root meristem of rye, treated with polyphenols (*Hippophae rhamnoides* bark)

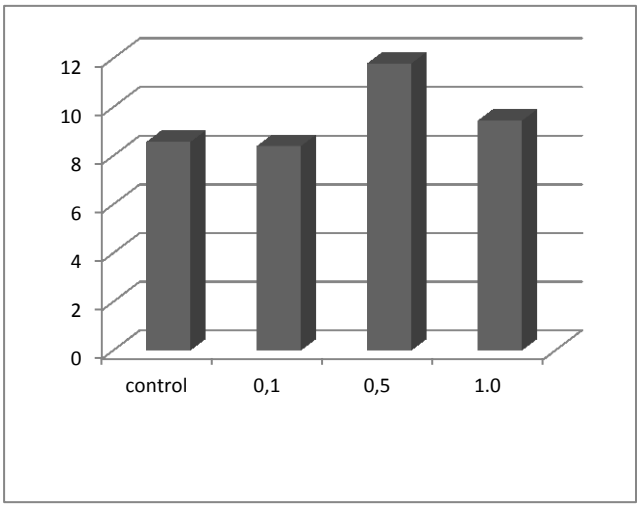

Fig. 11 Abberant anatelophases with bridges in root meristem of rye, treated with polyphenols (*Hippophae rhamnoides* bark)

As we can see from the chart shown in Fig. 9, the number of aberrant ana-telophases is directly proportional to the increase in polyphenolic extract concentration used. These results attest mutagenic capacity, dependent on the concentration of the extracts, despite their very efficiency of biostimulation activities, both in terms of seed germination, as well as capacity of growth in plantlets. In the aberrant metaphases encountered, most intense effect had treatment with polyphenols in a concentration of 0.5%. Chromosomal aberrations found were mainly represented by bridges, fragments, and micronuclei (interphase aberration). The most „effective” inducing mutations concentrations of extracts are 0.5 and 1.0%. We assume that extracts have specific effects depending on concentration, acting on different chromosomes or chromatid level.

The appearance of chromosomal aberrations recorded in micro-photographs is shown in the following:

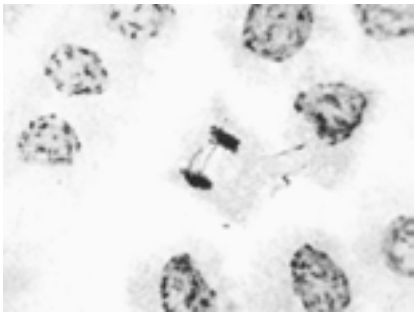

Fig. 12 Anatelophase with bridges (*Hippophae rhamnoides* bark extract 0,1%)

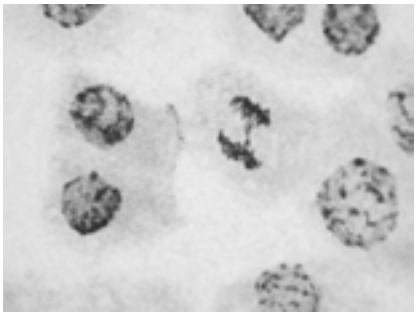

Fig. 13 Anatelophase with bridges (*Hippophae rhamnoides* bark extract 0,1%)

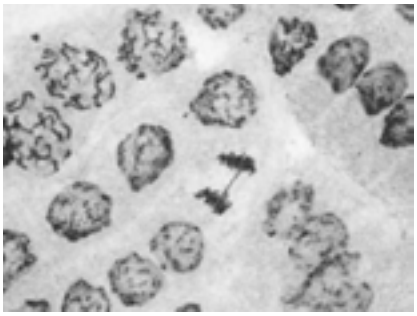

Fig. 14 Anatelophase with bridges (*Hippophae rhamnoides* bark extract 0,1%)

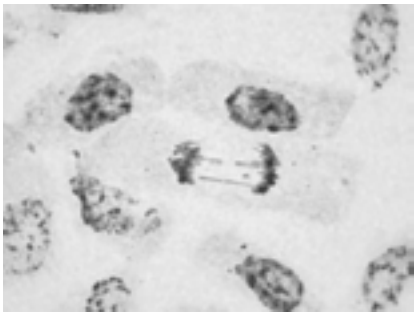

Fig. 15 Anatelophase with bridges (*Hippophae rhamnoides* bark extract 0,1%)

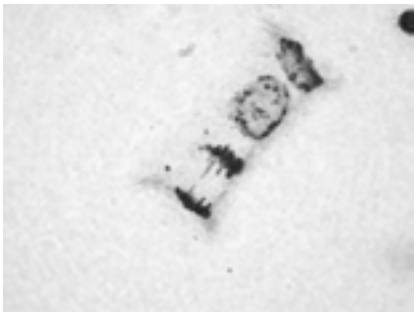

Fig. 16 Anatelophase with bridges (*Hippophae rhamnoides* bark extract 0,5%)

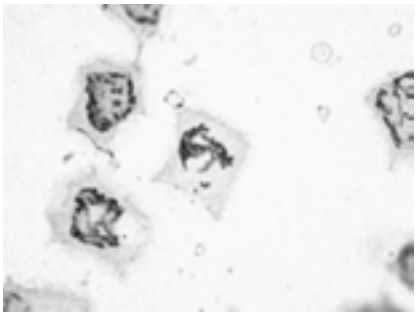

Fig. 17 Anatelophase with bridges and fragments (*Hippophae rhamnoides* bark extract 0,5%)

CONCLUSIONS

Investigations on the germination capacity showed an increase of this parameter, at the concentration of 0.5% polyphenols extract (*Hippophae rhamnoides* bark extract).

Measurements of the rate of growth in length have proved that the most effective concentration of polyphenols is also 0.5%.

Studies have revealed the emergence of mitotic division chromosomal aberrations in ana-telophase. Number of aberrant ana-telophase is directly proportional to the increase in polyphenolic extract concentration used. Chromosomal aberrations were represented mainly by bridges and fragments. The most effective polyphenolic extracts concentration were found to be 0.5% and 1%.

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