SOME CYTOGENETIC EFFECTS INDUCED IN BARLEY BY THE TREATMENTS WITH HYDROALCOHOLIC ROSEMARY EXTRACT

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Abstract: The paper presents some results regarding the cytogenetic effects induced in barley by the treatments with rosemary extract (*Rosmarinus officinalis)*. It was proved that the treatment with this extract upon the barley caryopses had as effect the stimulation of the mitotic division in the radicle apex of the species and did not lead to important cytogenetic changes (chromosome aberrations) in the ana-thelophase of the radicle mitoses.

INTRODUCTION

Rosemary (*Rosmarinus officinalis)*, an evergreen shrub, is one of the herb spices of the family *Labiatae*. It is used for flavoring food and as a beverage, as well as in cosmetics. In folk medicine it is used as an antispasmodic in renal colic and dysmenorrhea, in relieving respiratoty disorders and in stimulating the growth of hair.

The composition of rosemary extracts is quite complex, and many components have been identified, including phenolic acids, diterpenes such as rosmanol and carnosol derivates, and flavonoids, in particular, flavones (Gerhardt et al., 1983; Aeschbach et al., 1986, Brieskorn et al., 1973; Cuvelier et al., 1994; Fieski et al., 1989; Nakatani, 1989).

Rosemary extract relaxes the smooth muscles of the trachea and intestine and has choleretic, hepatoprotective and antitumerogenic activity (Al Sereiti et. al., 1999; Newal et al., 2002; Albu et al., 2004). These effects can be related to its high content of phenolic compounds like caffeic acid derivates such as rosmarinic acid. Phenolic compounds are secondary plant metabolites that have long been associated with flavor and colour characteristics of fruits and vegetables. These compounds attract great interest due to their postulated health-protecting properties, foremost to their antioxidative effect, manifested by the ability to scavenge free radicals or to prevent oxidation of low density lipoprotein (Newal et al., 2002, Miliauskas et al., 2004; Albu et al., 2004).

The antioxidant activity of rosemary extracts depends on their composition. There are many reports that determined their antioxidant capacity by various methods using lipid and aqueous systems. In lipid systems, extracts with higher diterpene content were the most effective (Hopia *et al.*, 1996), while in aqueous systems rosmarinic acid exhibited the highest antioxidant activity (Frankel *et al.*, 1996; Cuvelier *et al.*, 2000). Several reports have been published about the distribution of rosmarinic and/or carnosic acids during growth and vegetative development of rosemary leaves (Hidalgo *et al.*, 1998; Munné-Bosch *et al.*, 1999; Munné-Bosch and Alegre, 2000).

The use of the extract from rosemary leaves as an antioxidant was first reported by Ostric-Matijasevic in 1955 (Rac & Ostric-Matijasevic, 1955). There are several data evince the rosemary extracts action on the delay of the fats oxidation (Berner et al., 1973; Chang et al., 1977, Braco et al., 1981; Tateo et al., 1988; Wu J.W. et al., 1982). Several phenolic compounds with antioxidant activities have been isolated and identified from rosemary leaves: carnosol, rosemanol, carnosic acid and rosemaridiphenol (Brieskorn et al., 1969; Löliger et al., 1989; Schuler et al., 1990; Lamaison et al., 1991).

Starting from the aboved informations, we investigated some cytogenetic effects induced by the treatments with hydroalcoholic rosemary extracts in barley.

MATERIAL AND METHODS

As biological materials, barley caryopses have been used (*Hordeum vulgare* L, 2n=14), *Madalin* cultivar, from S.C.A. Podu Iloaie, 2006 . The seeds have been treated with hydroalcoholic rosemary extract (HRE) of 39%. Various dillutions of this extracts were used (0,10%, 0,25%; 0,50% and 1%) for 6 and 12 hours treatment of the seeds. At each experimental variant 25 caryopses have been treated. The control variant consisted in seed immersion, for the same period, in distilled water. After the treatment, the seeds were washed in sewerage water, and germinated in Petri dishes (covered by filter paper imbued in distilled water) and kept in the thermostate at 22ºC.

Some cytogenetic investigations have been made on radicular meristemes collected from the germinated seeds. These consisted of observations upon the development of the mitotic cycle and of the identification of possible chromosome aberrations. For the cytogenetic observations, roots (lenght: 10-15mm) were detached of the caryopses and fixed by immersion for 20 hours in absolute ethilic alcohol: icy acetic acid (3:1), at the room temperature. Roots were then hydrolized and colored by Feulgen method , (Tudose, 1993).

For the completion of the microscopic preparations the squash technique was used. Microscopic semipermanent (by assembly into glicerine) and permanent preparations (by icluding them into Canada balsam) were used.

The results obtained are presented in Tables 1-4.

RESULTS AND DISCUSSIONS

1. Effects of the 6 hour treatment with hydroalcoholic rosemary extract at barley

1.1. Mitotic index and the frequency of the mitotic division phases

Treating barley caryopses (*Mădălin* cultivar), with alcoholic rosemary extract, for 6 hours, led to changes in frequency of the cells in mitosis. At small concentrations (0.10% and 0.25% HRE) a stimulation of the division took place as compared to the control plants, emphasized by the increase in the mitotic index (MI). According as the increase in the extract concentration, the total amount of the cells in division decreased, the lowest value of the MI being registered at the 0.50% variant, (Table 1).

It seems that the values of the MI mainly influenced by the frequency of the cells in prophase, existing an obvious relationship between these 2 parameters. This way, at the variants (0.10% and 0.20% EHR) wherein MI has high values (7.03 and 8.23%) there is also a higher frequency of the cells in prophase (4.53 and 4.77%), as well as for higher concentrations of HRE (0.50 and 1.0%) where the MI has values of 5.49 and 6.55%, and the frequency of the cells in prophase is lower, of 2.99 and 3.76%. The action of the rosemary extract seems to have been especially on the cells in initial phases of the mitotic division. Though there are some changes in the frequency of the other phases of division too, these are less important. We might observe though only the effect of the 0.25% HRE solution which led to an increase in the frequency of the cells in metaphase (1.41%, as compared to 1.02% at the control) and in the telophase (1.34%, as compared to 0.63% at the control), (Table 1).

Table no. 1. Frequency of the cells in mitotic division from the barley radicle apex after the 6 hour treatment with hydroalcoholic rosemary extract

Variant	Total no of analyzed cells	Total cells in division		Total cells in prophase		Total cells în metaphase		Total cells in anaphase		Total cells in telophase	
	Nr.	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%
M	6363	395	6.21	250	3.93	65	1.02	40	0.63	40	0.63
0.10%	7037	495	7.03	319	4.53	67	0.95	32	0.45	77	1.09
0.25%	5891	485	8.23	281	4.77	93	1.41	42	0.71	79	1.34
0.50%	6916	380	5.49	207	2.99	57	0.82	42	0.61	74	1.07
1.00%	6962	456	6.55	262	3.76	82	1.18	45	0.65	67	0.96

1.2. Frequency of the cells with chromosome aberrations

Treatments with hydroalcoholic rosemary extracts, regardles the concentration, led to the increase in the frequency of the cells with chromosome aberrations (tab. 2). There hasn't been an obvious relationship between the EHR concentration and the frequency of the aberrant cells. This way, while at the 0,10% EHR soncentration there was the highest percentage of aberrant cells (4,24%), at a higher concentration (of 0,25%) we see an obvious decrease of this percentage (only 1,86%). Treatments with 0,50 and 1,0% solutions caused a level of the chromosome aberrations between the two already mentioned (being of 3,16 and 3,51%). As for the spectrum of these chromosome aberrations, the majority was formed of the multiple pole anathelophases (AT), metaphases with expelled chromosomes and C-metaphases (tab. 2).

Table no.2. Frequency and types of aberrant cells in the mytosis of the barley radicle meristemes after the 6 hour treatment with hydroalcoholic rosemary extract

Variant	Cells in division	Cells with aberrations		Aberrant cells with:															
				Multiple bridges		AT with delayed chromosomes		AT with expelled chromosomes		Multiple pole AT		Polar deviation		Metaphases with expelled chromosomes		C-metaphases		Multiple pole AT and multiple bridges	
		Nr.	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	%
M	395	0	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0.00
0.10%	495	21	0	0.00	0	0.00	0	0.00	7	1.41	2	0.40	5	1.01	7	1.41	0	0.00	1.41
0.25%	485	9	2	0.41	0	0.00	0	0.00	1	0.21	3	0.62	0	0.00	2	0.41	1	0.21	0.41
0.50%	380	12	1	0.26	0	0.00	0	0.00	8	2.11	0	0.00	2	0.53	0	0.00	1	0.26	0.00
1.00%	456	16	1	0.22	0	0.00	0	0.00	10	2.19	1	0.22	1	0.22	2	0.44	1	0.22	0.44

2. Effects of the 12 hour treatment with hydroalcoholic rosemary extract at barley

2.1. Mitotic index and the frequency of the mitotic division phases

In the case of prolonging the treatment period with hydroalcoholic rosemary extracts to 12 hours there could also be observed a stimulation of the cell division at the level of the radicle meristemes, and this led to the increase in the MI values. Up to the 0.50% HRE level, this effect was pro ratio to the HRE concentration, and after this, it had a lower influence on the cell division. This way, between 0.10 and 0.50% HRE the MI values were between 8.86 and 10.45%, as compared to 7.62% at the control plants, and then they came back to a value similar to the control one (7.99%), at the concentration of 1.0% HRE. Together with the increase in the treatment period it takes place an intensification of the division process in the barley radicle apex cells, pro ratio to the increase in the extract concentration, except of the 1.0% variant, where this parameter has lower values.

The maximum mythogen effect took place at 0.50% EHR concentrations, (10.45% as compared to 7.62% at the control), (Table 3). The increase in the MI values was completed, at least in the case of two of the HRE concentrations used by the increase of the cell frequency from the prophase (5.39% and 7.10% at the 0.10 and 0.50% EHR concentrations, as compared to 4.47% at the control). On the other hand, at the 0.25% HRE treatment, the increase of the MI value was completed based on the cells from metaphase, whose frequency was of 2.97%, as compared to 1.35% at the control. As for the frequency of the cells from various phases of division, the highest percentage was of the cells in prophase, followed by the ones in metaphase, telophase and then in anaphase (Table 3).

Table no. 3. Frequency of the cells in mitotic division from the barley radicle apex after the 12 hour treatment with alcoholic rosemary extract

Version	Total no of analyzed cells	Total cells in division		Total cells in prophase		Total cells in metaphase		Total cells in anaphase		Total cells in telophase	
	Nr.	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%
M	6312	481	7.62	282	4.47	85	1.35	49	0.78	65	1.03
0.10%	6322	560	8.86	341	5.39	81	1.28	45	0.71	93	1.47
0.25%	6365	627	9.85	274	4.30	198	2.97	70	1.10	94	1.48
0.50%	5933	620	10.45	421	7.10	100	1.61	37	0.62	62	1.05
1.00%	6573	525	7.99	309	4.70	96	1.46	52	0.79	68	1.03

2.2. The frequency of the cells with chromosome aberrations

What is interesting is the fact that the prolongation of the treatment period with EHR solutions did not led to the intensification of the cytogenetic disorders too. As it comes out of Table 4, though at the variants subject to HRE treatment - 12 hours ore a higher frequency of the chromosome aberrations in the mitosis of the radicle meristemes than that of the tests was registered (0,89 – 1,52%, as compared to 0,00% at the test), this frequency is though lower than at the plants coming from seed treated for 6 hours. At the same time, there wasn't observed a certain relationship between the EHR concentration used and the frequency of the aberrant cells.

The main types of aberrant cells observed were: the cells with simple and multiple bridges, anathelophase with expelled chromosomes and/or delayed chromosomes, as well as cells with complex aberrations (with lower frequency), represented by the multiple polar anathelophases and with multiple bridges, (tab. 4).

Table no.4. Frequency and types of aberrant cells in the mytosis of the barley radicle meristemes after the 12 hour treatment with alcoholic rosemary extract

Variant	Cells in division	Cells with aberrations		Aberrant cells with:															
				Simple bridges		Multiple bridges		AT with delayed chromosomes		AT with expelled chromosomes		Multiple pole AT		Polar deviation		C-mitosis		Multiple pole A-T and multiple bridges	
		Nr.	**%**	**Nr.**	**%**	**Nr.**	**%**	**Nr.**	**%**	**Nr.**	**%**	**Nr.**	**%**	**Nr.**	**%**	**Nr.**	**%**	**Nr.**	**%**
M	481	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
0.10%	560	5	0.89	0	0.00	0	0.00	0	0.00	0	0.00	4	0.71	1	0.18	0	0.00	0	0.00
0.25%	627	9	1.44	2	0.32	0	0.00	1	0.16	2	0.32	0	0.00	1	0.16	0	0.00	3	0.48
0.50%	620	6	0.97	1	0.16	2	0.32	1	0.16	0	0.00	2	0.32	0	0.00	0	0.00	0	0.00
1.00%	525	8	1.52	1	0.19	3	0.57	0	0.00	0	0.00	4	0.76	0	0.00	0	0.00	0	0.00

CONCLUSIONS

Our investigations show that treatments with hydroalcoholic rosemary extracts (EHR) treatments, at concentrations of 0,10 – 0,50%, had a contribution to the intensification of the mitotic activity in radicle barley meristemes (*Mădălin* cultivar), regardless the treatment period (6 or 12 hours). The increase of the mitotic index values was especially completed based on the cells in prophase. This effect was expressed best at the 0,50% EHR treatment, for 12 hours. Generally speaking, EHR treatments did not obviously influence the percentage distribution of the cells in various phases of mitosis, the highest frequency having cells in prophase, and the lowest in anaphase.

Treatments with hydroalcoholic rosemary extract did not lead to an important increase of the cells with chromosome aberrations in the mitosis of the barley radicle meristemes. Between aberrations, the most frequent were the anathelophases with delayed and expelled chromosomes, multiple pole anathelophases. The results obteind determine to apreciated that the HRE treatment has mytogen , but non-mutagenic effect.

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