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IN VITRO CYTOGENETIC STUDY OF THE MITOTIC DIVISION IN BASIL (*OCIMUM BASILICUM* L.) PLANTS

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SUMMARY. The cytogenetic studies on the in vitro - derived plants of Ocimum basilicum L. (var. Greek basil) aimed to evince if and to what extent this type of conventional culture altered the mitotic cell division. There were some differences regarding the mitotic index, the distribution of cells during each phase of mitosis, and also regarding the percentage of abnormal ana-telophases. The shoots obtained on a culture medium supplemented with indole butyric acid (IB2 medium variant) displayed the highest mitotic index (33.09), compared to the control (16.14) and to the other studied variants. The lowest mitotic index (7.39) was registered on the A2 variant, enriched with indole acetic acid. Regarding the cell distribution on mitotic phases, the highest percentage was registered by prophases, followed by telophases, metaphases, and anaphases (in all the analyzed variants, including the control).

Keywords: basil, cytogenetic analysis, mitotic index.

Introduction

Basil (*Ocimum basilicum* L.) is a herbaceous, annual plant belonging to the Lamiaceae family. It requires great amounts of light and high temperatures, being resistant to drought (Muntean, 1990; Păun *et al.*, 1988; Pârvu, 2000; Tiță, 2003). Its essential oil comprises estragol, eugenol, linalool, citral, camphor, cineol etc (Stănescu *et al.*, 2002; Tiță, 2003). The main actions of basil volatile oil are: digestive, antispastic, antinauseous, carminative, choleretic, antifungic, stomachic, galactagogue, diuretic (Stănescu, 2002). Taking into account that basil is a very important plant from a pharmaceutical and economical point of view, morphogenetic reactions in *in vitro* culture have been tested, as well as some explants' growth and development on several hormonali formulae, in order to evince possible valuable genotypes and to develop an efficient technology for their micropropagation. The present paper reveals several data on the cytogenetic studies in basil, that were aimed to depict the influence of the *in vitro* culture system and of the growth regulators

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within the culture medium on the mitotic index, on the frequency of the abnormal ana-telophases in the mitosis of root meristems, and the range of chromosomal abnormalities, as well. The chromosomal aberrations, their type and frequency are the subject of another research paper on *Ocimum basilicum* L. It was acknowledged that the *in vitro* culture itself triggers a great genetic (somaclonal) variability, that may be further used in amelioration.

Materials and methods

Ever since the 19th century – the moment chromosomes were discovered (Hertwig, 1875), several methods of analysis were perfected, for their study during both the mitotic and the meiotic cell division. One should consider the following essential aspects:

1. To determine the chromosome number, their shape and size during mitosis, and to prepare the karyotype for the respective species;
2. To study the intra- and interspecific chromosome and gene transfer;
3. To depict ploidy level for the intraspecific, interspecific, and intergeneric hybrids, and of the plants treated with chemical substances that induce polyploidy;
4. The study of aneuploids and gene arrangement on chromosomes;
5. To detect the homology degree of the chromosomes by means of studying metaphase I of meiosis and their splitting during other phases of cell division for the interspecific and intergeneric hybrids;
6. To study the chromosomal alterations (numeric or structural) caused by physical or chemical mutagens (Raicu, 1987).

Cytogenetic studies have been conducted on basil roots of 1.5 to 3 cm in length, harvested from vitroplants grown on different nutritive medium variants of MS (Murashige – Skoog, 1962). The original plant belongs to the variety Greek basil, brought from Greece, and cultivated at the 'Stejarul' Research Centre in Piatra Neamt. The control variant was represented by small roots obtained from vitroplants grown on hormone-free MS. The results were compared to the control sample, represented by roots grown *in vitro* on the basic Murashige-Skoog culture medium (hormone-free). The medium variants were: A_2 (comprising 2 ml/l^{-1} indole acetic acid), N_2 (enriched with 2 ml/l^{-1} naphthylacetic acid), BA_1 (comprising 1 ml/l^{-1} benzylaminopurine and 0.5 ml/l^{-1} indole acetic acid), B_2 (2 ml/l^{-1} benzylaminopurine), B_{02} (0.2 ml/l^{-1} benzylaminopurine), IB_2 (2 ml/l^{-1} indole butyric acid).

The plant material was fixed in Farmer solution and hydrolysed with HCl 18.5 % for 8 -10 minutes.

Colouring was achieved in a basic carbol-fuchsin solution, in concentration of 10%.

The slides were prepared using the *squash* technique.

Fresh materials have been examined under an optical microscope (NOVEX), exposed to intense light using a blue filter to highlight the contrast between chromosomes and cytoplasm. The mitotic index was calculated after the analysis of each 10 microscopic fields/medium variant/preparate. All cells were counted, both in mitosis and in interphase. The 10 microscopic fields were chosen at random on the microscope slide, and the cell density was rather high. The same slides used to calculate the mitotic index were studied to detect the abnormal ana-telophases/ preparate/nutritive medium variant. The latter type of microscopic analysis was possible only using the immersion objective of the microscope (due to the cell size and the large number of chromosomes). The chromosomal aberrations were recorded, as well. The best microscopic preparates were rendered permanent (by means of butanol, xylen, and Canada balm). The photos of different phases of the mitotic division have been taken using the 40x and 100x objectives, with an OLYMPUS digital camera.

Results and discussion

The cytogenetic analysis on *Ocimum basilicum* L. (*Greek basil* variety) was aimed to provide data on the mitotic division, the variation of the mitotic index, the frequency of the abnormal ana-telophases, the types of chromosomal abnormalities (simple or complex) that occur during mitotic division in the root tip meristems of the vitroplants cultured on several medium variants enriched with growth regulators, compared to the control sample (vitroplants regenerated on the hormone-free Murashige – Skoog medium).

The *Ocimum* genus comprises about 160 species and many varieties (Păun *et al*., 1988). The chromosome number is 2n = 48 (x = 12).

The cytogenetic studies during mitotic division revealed that there are still variations of the chromosome number. E.g., for *Ocimum basilicum var. citriodorum* (lemon basil) 2n = 72, and for the *crispum* variety 2n = 52; the basic chromosome number is x = 12. The polyploidy and aneuploidy phenomena lead to variations of the chromosome number in *Ocimum basilicum*, (Mukherjee *et al*, 2005).

Our research was done on *Ocimum basilicum* species, the *Greek basil* variety (the explant-donor plants to start the *in vitro* cultures originated from Greece). It was a difficult task to make the microscope slides using root meristems from the basil vitroplants. Because the roots harvested from the *in vitro* basil regenerants were extremely thin and frail there is a risk that the meristematic tip might remain within the hard culture medium.

There was no record of the mitotic processes within the root meristems of the basil vitroplants in the scientific papers published by other authors. This is the reason for searching and implementing the best techniques regarding the hydrolisis

and the best staining method of the biological material (many lab experiments were performed). In an autochtone study on this species, performed on root meristems from germinated basil seeds, not on roots from vitroplants, a series of modifications issued at the material genetic level of meristematic cells of root tips are presented, as a consequence of the treatment with 4-chlorohydrate-bromo-6-methyl-3-dimethylamino-3-chromanone. The 1/10000 dilution induces the increase of frequency of mitotic dividing cells. The cells with chromosome aberrations are in greater number in treated variants, comparatively with control. The aberration spectrum is enough large. (Axente *et al*., 2006).

The attempts to make the microscope slides using the staining with Schiff reagent and acetic orcein were not successful. The genetic nuclear and extranuclear content can be coloured with Carr solution. The chromosomes were evinced on microscope slides within a fortnight since the roots were immersed in the staining solution (a long period of time, compared to the other tested plant species). It normally takes 24-48 hours of colouring, with certain variations due to the analyzed species and the duration of hydrolysis.

To better highlight the genetic material, the roots were treated with a second staining solution (besides Carr solution): they were coloured with acetic orcein, used instead of acetic water for 10-15 minutes, previous to cell layout on the microscope slide by means of the 'squash' method.

The experimental results for basil include the variation of the mitotic index within the meristems of the vitroplants provided on many variants of the basal Murashige – Skoog medium supplemented with growth regulators; the results were compared to the control variant (shoots provided on the basal MS medium).

In case of the vitroplants that were analyzed cytogenetically, one should take into account that the morphogenetic processes and the cell division are caused by a series of internal and external factors, that influence the explant after inoculation. These factors are: the genotype, the hormonal balance, the physiological stage of the cultivated tissue, eventual pre-treatments applied to the explants or to the donor plants, the plant growth conditions. All these factors act simultaneously and stimulate (more or less) the explant development and the processes within the genome. Nevertheless, it is hard to establish which of these factors has the greatest impact in inducing various types of chromosomal aberrations.

In *Ocimum basilicum*, the meristematic cells of the control plants and of those regenerated on various culture medium variants are small, oblong, rather hard to examine and analyze using the 40X objective of the optic microscope. It was ascertained that the mitotic activity was normal, cells in all phases of division have been registered (Fig. 1-6).

In case of the meristematic cells belonging to the vitroplants grown on B_{02} medium (in which the dividing cells were smaller compared to the other analyzed variants) it was rather frequently noticed the presence of several nucleoli inside the

interphasic nuclei (2-4 nucleoli/nucleus), a phenomenon observed in the BA_1 and IB_2 variants. The cytogenetic tests pointed out that the regenerants from the control variant registered a mitotic index (M.I.) of 16.14. The medium variants enriched with indole butyric acid (IB_2) and with cytokinin+auxin (BA_1) displayed a M.I. far superior, of 33.09 and of 29.41, respectively (Fig. 7).

The vitroplants provided on the A_2 variant (supplemented with indole acetic acid) registered a M.I. lower than the witness: 7.39; similar cases were found for three other variants: N_2 (M.I. = 8.88), B_{02} (M.I. = 9.25) and B_2 (M.I. = 9.26) (Fig. 7).

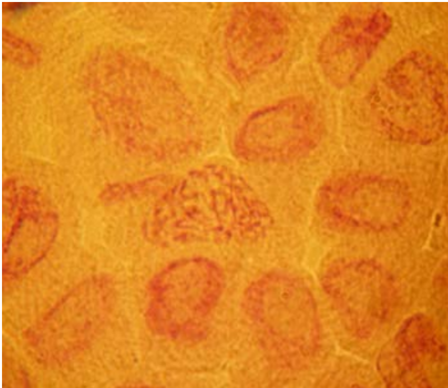

Figure 1. Prophase surrounded by cells in interphase (BA_1 variant)

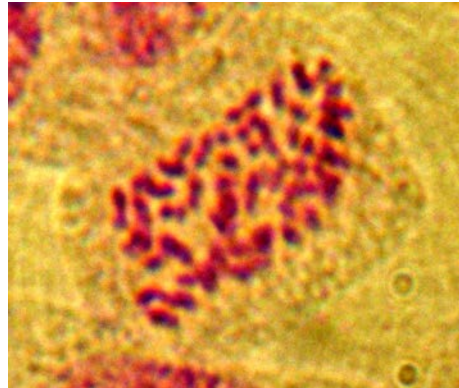

Figure 2. Late prophase (BA_1 variant)

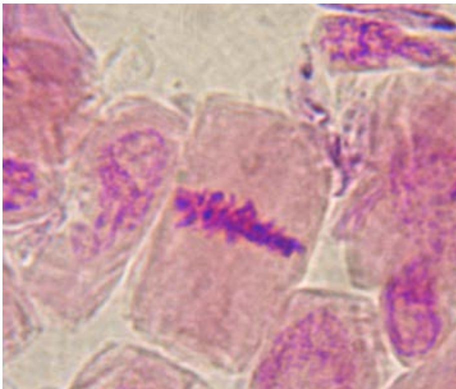

Figure 3. Metaphase (control variant MS)

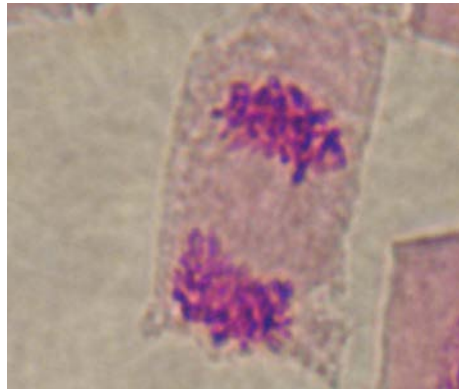

Figure 4. Ana-telophase (BA_1 variant)

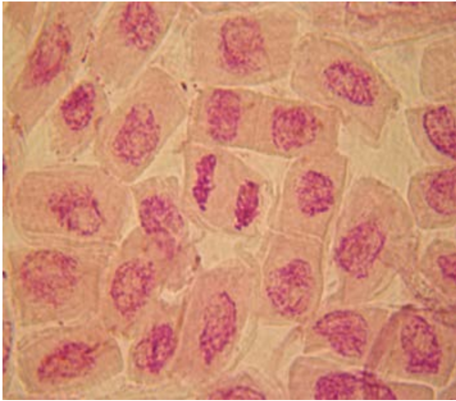

Figure 5. Telophase (BA_1 variant)

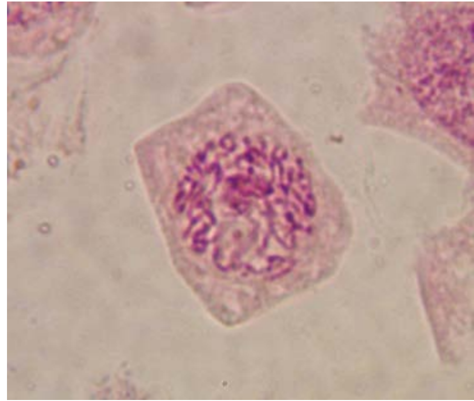

Figure 6. Prophase (BA_1 variant)

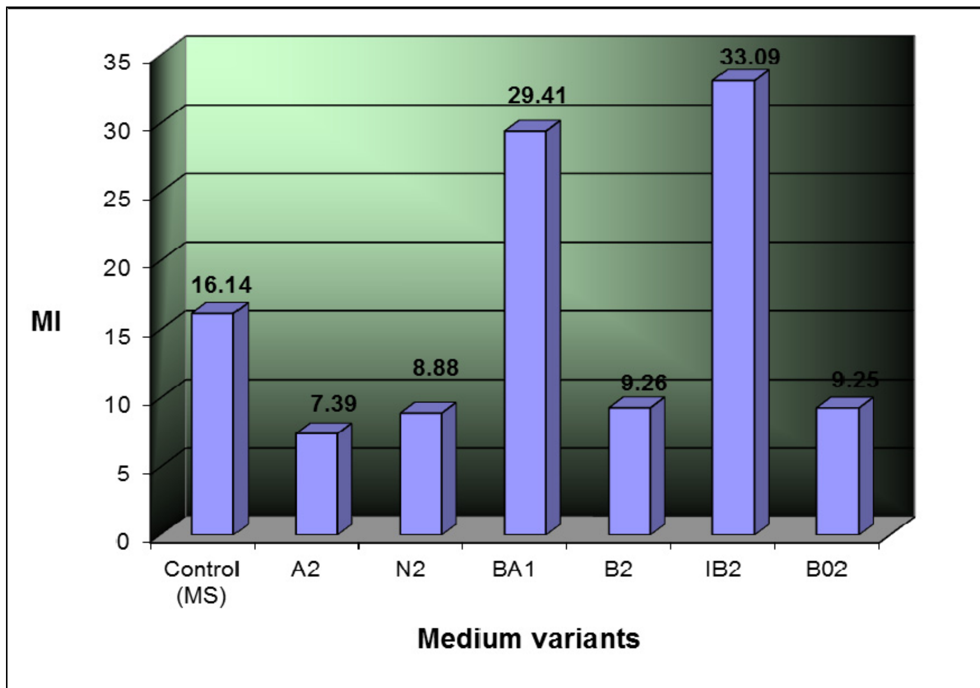

Figure 7. Mitotic index (M.I.) for the vitroplants of *Ocimum basilicum* L.

Table 1.

Mitotic index and number of cells in mitosis in the vitroplants of *Ocimum basilicum* L.

Culture medium	No. of analyzed cells	No. of cells in mitosis	Mitotic index (M.I.)	Cell distribution on mitotic phases							
				PROPHASE		METAPHASE		ANAPHASE		TELOPHASE	
				No. cells	%	No. cells	%	No. cells	%	No. cells	%
MS	5469	883	16.14	513	58.09	160	18.12	45	5.09	165	18.68
A_2	3462	256	7.39	116	45.31	66	25.78	2	0.78	72	28.12
N_2	3940	350	8.88	180	51.42	100	28.57	10	1.00	60	17.14
BA_1	4633	1363	29.41	1180	86.57	74	5.42	16	1.17	93	6.82
B_2	4017	372	9.26	249	66.93	48	12.90	9	2.41	66	17.74
IB_2	2828	936	33.09	828	88.46	36	3.84	4	0.42	68	7.26
B_{02}	3200	296	9.25	212	71.62	40	13.51	4	1.35	40	13.51

Regarding the cell distribution on mitotic phases, the highest percentage was registered by prophases, followed by telophases, metaphases, and anaphases (in all the analyzed variants, including the control). There was one exception: the vitroplants from N_2 medium, in which the percentage of metaphases (28.57%) was higher than the one of the telophases (17.14%).

The number of cells in anaphase decreased in the vitroplants regenerated on several medium variants, compared to the control, excepting the variants A_2 and N_2, where a low percentage of cells in prophase was registered for the control variant (57.93%), compared to 66.93% (B_2 variant), 71.62% (B_{02}), 86.57% (BA_1) and 88.46% (IB_2).

The cytogenetic study on vitroplants of *Ocimum basilicum* L. evinced a normal mitotic activity. Cells in all phases of mitotic division been registered, with a rather low M.I. in the control (16.14), more diminished in the variants A_2 (7.39), N_2 (8.88), B_{02} (9.25) and B_2 (9.26) and higher in the vitroplants regenerated on BA_1 (29.41) and IB_2 (33.09) medium variants (Table 1).

We intend to further expand our cytogenetic studies on this species in order to come with solid conclusions about the influence of the growth regulators on the mitotic division and about the range, frequency and cause of the chromosomal aberrations.

Conclusions

The cytogenetic observations made on root tip meristems of the regenerants obtained by means of *in vitro* cultivation of *Ocimum basilicum* L. have indicated that the growth regulators disturbed the functioning of the mitotic apparatus, i.e.the

mitotic index (M.I.) of the vitroplants was diminished on certain hormonic variants (A_2, N_2, B_{02}, B_2) or augmented on other medium variants (BA_1, IB_2), compared to control plants.

Cells in all phases of mitotic division have been registered.

Further studies should be carried out in order to gain more knowledge about the effect of the growth regulators on the molecular metabolism of the cell division and of the cell cycle.

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