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CYTOGENETIC ANALYSES ON SEVERAL *IN VITRO* REGENERANTS OF *MELISSA OFFICINALIS* L.

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Key words: *Melissa officinalis, cytogenetic, mitotic index*

INTRODUCTION

Melissa officinalis (lemon balm) is a medicinal plant used by herbal medicine for more than 2000 years for its positive therapeutic actions, mostly on the nervous and digestive systems. Lemon balm is a perennial herb belonging to the Lamiaceae family, that is common in the Mediterranean region. The main action of its active principles, particularly of the volatile oils, is smasmolytic and sedative, recommended in the treatment of gastro-intestinal spasms and cardiac neurosis. Its effects are: antiseptic, carminative, choleretic, stomachic, cicatrisant, galactagogue, pest control. There were cytogenetically analyzed several regenerants of lemon balm that were provided *in vitro* on hormone-free MS medium (control variant), and on its variants enriched with growth regulators (medium variants A2, KN1, BG1).

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 lemon balm roots of 1.5 to 3 cm in length, harvested from vitro plants grown on different nutritive medium variants of MS (Murashige – Skoog, 1962). The original plant was brought from Greece, and cultivated into soil pots 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: MS (control), A_2 (comprising 2 ml/l^{-1} indole acetic acid), KN_1 (enriched with 0.5 ml/l^{-1} naphthylacetic acid, and 1 ml/l^{-1} kinetine), BG_1 (comprising 1 ml/l^{-1} benzylaminopurine and 0.5 ml/l^{-1} giberellic acid).

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

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

The slides were prepared using the *squash* technique.

The fresh material has 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 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 complex cytogenetic analysis on *Melissa officinalis* L. 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 chromosome number of lemon grass is 2n=32 (PÂRVU, 2000); 2n=32, 64 (LAZA and RACZ, 1975, cited by MUNTEAN, 1990).

A large number of hydrolysis and colouring tests were accomplished during our research. Most of them were unsuccessful. The roots were harvested at various ages of the *in vitro* shoots (due to the various growth rates of the roots, depending on the nutritive medium variant). Nevertheless, the roots were fragile, and the meristemetic tips remained in the nutritive solidified medium in the majority of cases.

Due to the fact that there were no references on the mitotic process in the root meristems of the *in vitro* shoots of lemon balm, we depicted (by a great number of tests) the best hydrolysis and colouring of the biological material. Among all the stain-fixatives (Carr, Schiff, carmin-acetic, acetic-orcein), it was ascertained that the nuclear and extranuclear genetic material is best stained in basic carbol-fuchsin solution (Carr), and subsequently microscopically observed 10 days after the root immersion into the stain-fixative.

The colouring was enhanced by using acetic-orcein instead of acetic water to get some microscopic preparates in this species, that could be better microscopically photographed. Of all the tested variants, we obtained some significant results in case of the following variants: MS - control, A_2, KN_1, and BG_1.

Our study reveals the variation of the mitotic index into the meristems of the shoots provided on several variants of the Murashige – Skoog medium, enriched with growth regulators, compared to the control (in vitro shoots provided on hormone free MS medium).

It was ascertained that the cells in the control plants and in the regenerants from various medium formuli are small, rather difficult to be observed and numbered using the 40X microscope objective, considering that the roots need a long period of time for staining. The mitotic activity proved the presence of cells in all the division phases (fig. 1-9).

For the *in vitro* shoots of lemon balm, the cytogenetic tests displayed that the mitotic index (M.I.) in the control was the highest (46.18) compared to the experimental variants, followed by the M.I. of the regenerants provided on the KN_1 variant (43.12). A similar value of the M.I. was registered in case of the BG_1 variant (42.58). The lowest mitotic index characterised the A_2 variant (39.73), (fig. 1).

It was ascertained that the majority of cells are represented by prophases (67.84% in the control, 85.53% in A_2). The cells in telophase represent 6.16-14.10% of all the cells in mitotic division)(excepting the BG_1 variant, in which the metaphases percentage outnumbered the telophases by 10%). The metaphase percentage varies between 5.26-14.39%. The cells in anaphase range between 3.03 and 6.43 of all the mitotic cells.

The lowest percentage of cells in prophase was registered in the control (67.84%). The same variant displayed the highest percentage of cells in telophase (14.10%), of all the analyzed variants.

The cytogenetic studies on the *in vitro* regenerants of lemon balm evinced a normal mitotic activity, with cells in all the mitotic phases, with the highest M.I. in the control (46.18), gradually decreasing in the *in vitro* regenerants from the other medium variants.

We intend to further expand our cytogenetic studies on this species in order to come with other 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 *Melissa officinalis* L. have indicated that the growth regulators disturbed the functioning of the mitotic apparatus, i.e. for the contol variant (MS) the mitotic index was 46.18, lower on the other three analyzed variants (43.12 for KN_1; 42.58 for BG_1; 39.73 for A_2).

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.

ABSTRACT

Melissa officinalis (lemon balm) is a medicinal plant used by herbal medicine for more than 2000 years for its positive therapeutic actions, mostly on the nervous and digestive systems.There were analyzed several regenerants of lemon balm that were provided in vitro on hormone-free MS medium (control variant), and on its variants enriched with growth regulators (medium variants A2, KN1, BG1). Regarding the mitotic activity, it was ascertained that the regenerants registered a mitotic index that ranged between 39.73 to 43.12 for the variants comprising

growth regulators, compared to 46.18 in control. The distribution of cells on various phases of mitotic division does not alter compared to control plants; the highest frequency is held by prophases, followed by metaphases and telophases, and the lowest by anaphases.

The high percentage of cells with chromosomal aberrations depicted in the root meristems of the *in vitro* regenerated plants confirms, once more, the fact that this culture method is accompanied by a high variability at the cytogenetic level, important for the practical valorization.

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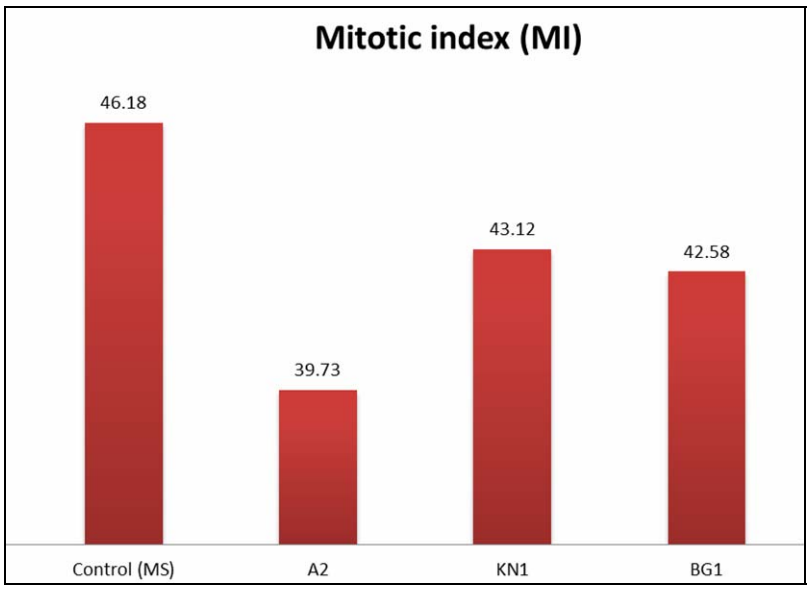

Fig. 1. The mitotic index and the frequency of the cells in mitotic division for the *in vitro* shoots of *Melissa officinalis* L.

Several phases of the mitotic division in *Melissa officinalis* L.

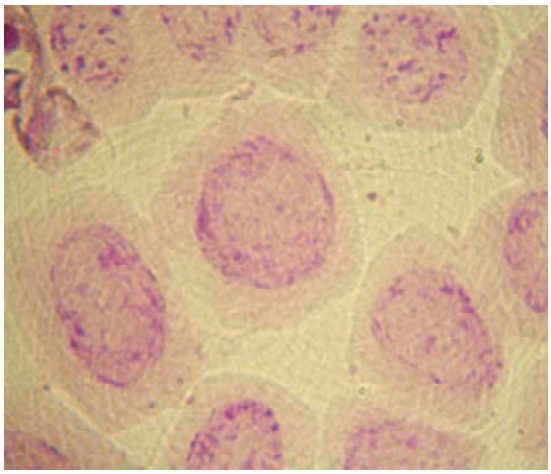

Fig. 2 - Cells in interphase (control variant MS)

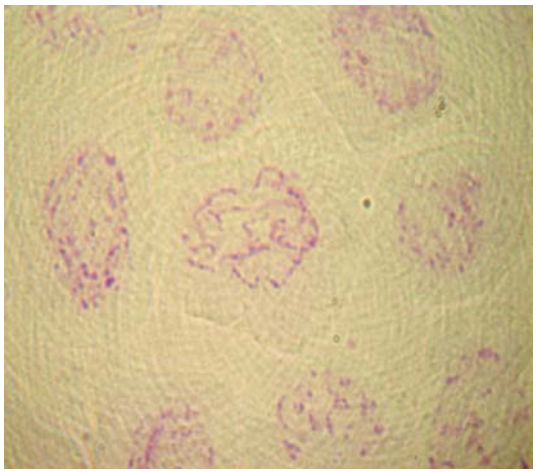

Fig. 3. Prophase (KN_1)

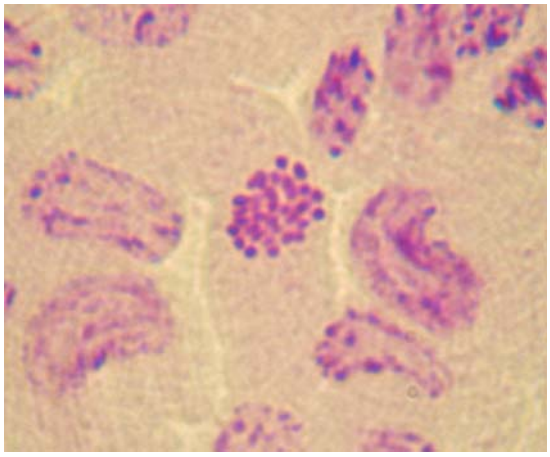

Fig. 4. Cell in late prophase (B_{02})

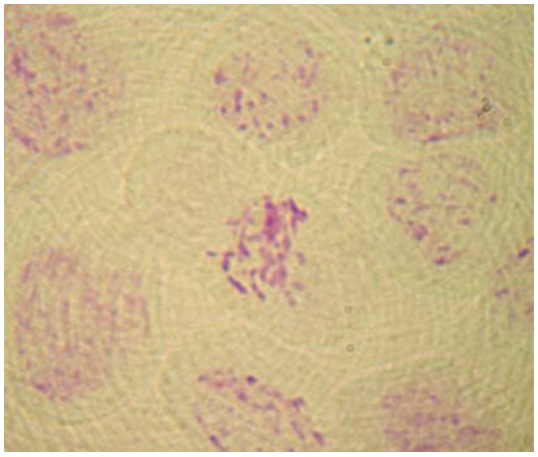

Fig. 5. Prometaphase (MS)

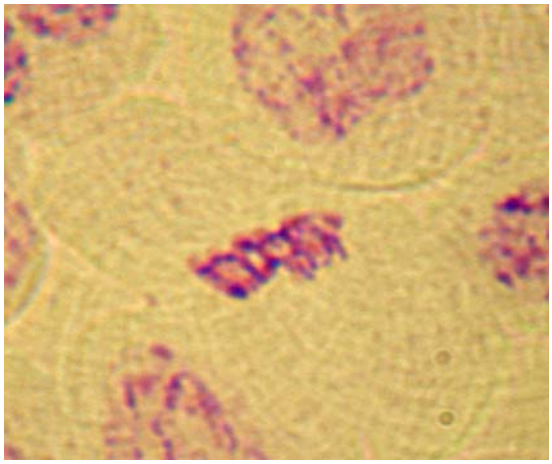

Fig. 6. Early anaphase (KN_1 variant)

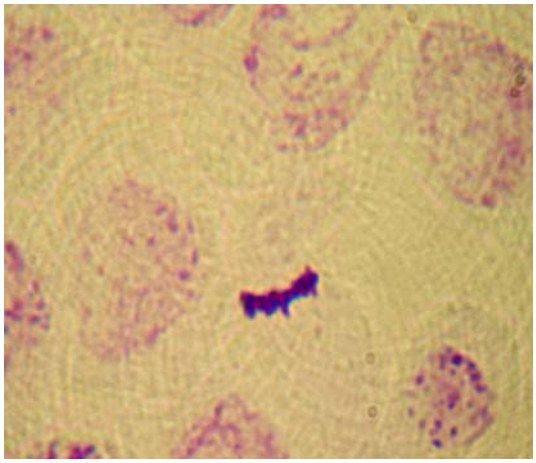

Fig. 7. Cell in metaphase, surrounded by several cells in interphase and prophase (BA_1)

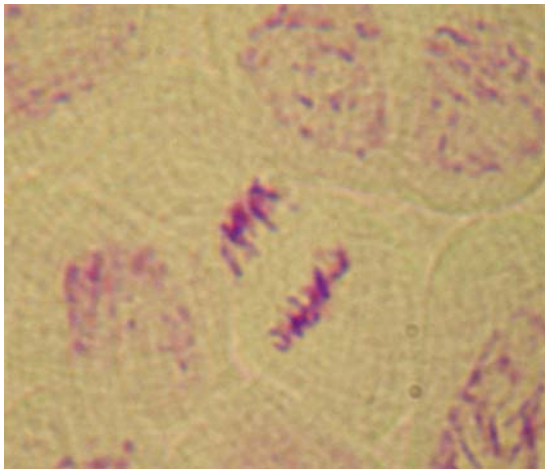

Fig. 8. Ana-telophase (KN_1)

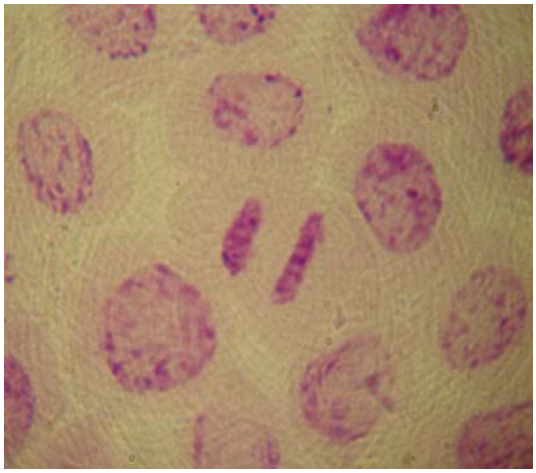

Fig. 9. Telophase (B_{02})