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OVERVIEW ON THE SPECTRUM OF THE CHROMOSOMAL ABERRATIONS IN LEMON BALM REGENERANTS PROVIDED IN VITRO

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Key words: *Melissa officinalis, cytogenetic, chromosomal aberrations*

INTRODUCTION

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). The cytogenetical observations focussed on the spectrum of chromosomal aberrations.

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. 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 cytogenetical 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. All cells were counted, both in mitosis and in interphase. 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 DISCUSSIONS

The cytogenetical research in lemon balm displayed the presence of chromosomal aberrations during the ana-telophases of the mitotic division, as well as several chromosomal abnormalities during other mitotic phases (both in prophase and in metaphase).

The prophases with one micronucleus were rarely depicted in this species. Analyzing the cells in metaphase for the regenerants provided on the four medium variants, it was acknowledged that there were frequently several chromosomes outside the metaphase chromosomal set (1 up to 6 chromosomes), both in the control (MS), and the other analyzed variants.

Our cytogenetic tests evinced some ana-telophases comprising only one type of aberration (simple or multiple bridges, fragments, micronuclei, and delayed chromosomes), and also several cells displaying more types of abnormalities at the same time (cells with delayed chromosomes and fragments; cells with micronuclei and delayed chromosomes; cells with delayed chromosomes, bridges, and fragments, etc.).

The lab cytogenetic tests on *Melissa officinalis* L. revealed a series of abnormal ana-telophases. There was a lower percentage of chromosomal aberrations in the ana-telophases of the control (12.12%), compared to the other analyzed variants

(19.70% in the KN_1 regenerants; 20.00% in the regenerants provided on the BG_1 culture medium, and 27.18% in the plants provided on the variant A_2).

To what concerns the control variant, the most numerous were the ana-telophases with bridges (38.88%), followed by those with delayed chromosomes (23.07%), fragments (22.22%), and the lowest percentage was represented by the ana-telophases with micronuclei (13.88%), (fig. 138).

There was pointed out a similar distribution for the types of chromosomal aberrations in the control (bridges>delayed chromosomes>fragments>micronuclei) as in the variant BG_1.

The cytogenetical tests ran on the regenerants provided on the other tested variants (A_2 and KN_1) evinced a higher frequency of ana – telophases with delayed chromosomes, followed by those with bridges.

Of all the analyzed experimental variants, only for the KN_1 there were registered some cells comprising multiple chromosomal aberrations: a high percentage of delayed chromosomes and fragments (13.02% of all the aberrant ana – telophases), followed by the delayed delayed chromosomes and micronuclei (1.88%), and by the cells with delayed chromosomes, bridges and fragments (1.88%).

CONCLUSIONS

The cytogenetical observations that were the subject of this scientific paper focussed on the spectrum of chromosomal aberrations.

The cytogenetical research in lemon balm displayed the presence of chromosomal aberrations during the ana-telophases of the mitotic division, as well as several chromosomal abnormalities during other mitotic phases (both in prophase and in metaphase).

Of all the analyzed experimental variants, only for the KN_1 variant there were registered some cells comprising multiple chromosomal abnormalities.

ABSTRACT

Melissa officinalis (lemon balm) is a medicinal plant belonging to the Lamiaceae family. Its therapeutical importance resides in its active principles, mostly on the nervous and digestive systems. 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). The study of the mitotic index in lemon balm was the subject of a previous scientific paper.

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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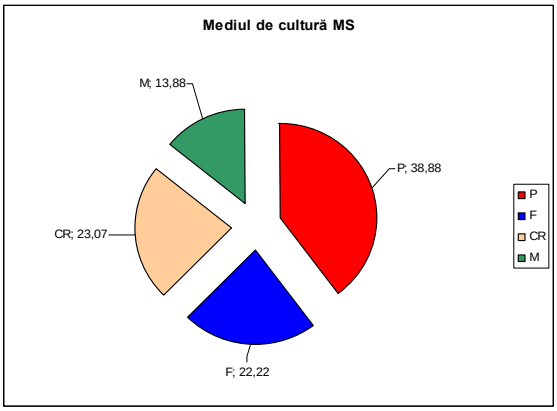

Fig. 1. The share of abnormal ana – telophases during the mitosis within the root meristems of the *in vitro* regenerants of *Melissa officinalis* L. (MS)

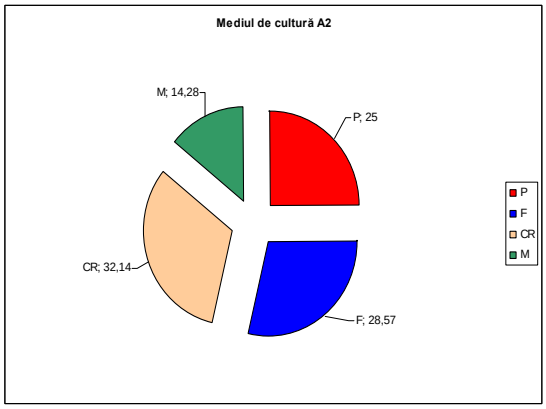

Fig. 2. The share of abnormal ana – telophases during the mitosis within the root meristems of the *in vitro* regenerants of *Melissa officinalis* L. (A2)

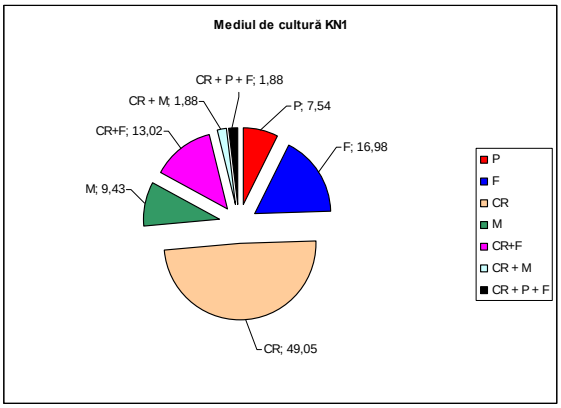

Fig. 3. The share of abnormal ana – telophases during the mitosis within the root meristems of the *in vitro* regenerants of *Melissa officinalis* L. (KN1variant)

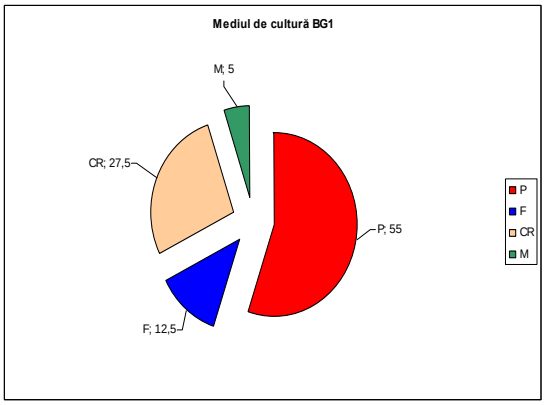

Fig. 4. The share of abnormal ana – telophases during the mitosis within the root meristems of the *in vitro* regenerants of *Melissa officinalis* L. (BG_1)

Several abnormal ana – telophases in the mitosis of the root meristems within the *in vitro* regenerants of *Melissa officinalis* L.

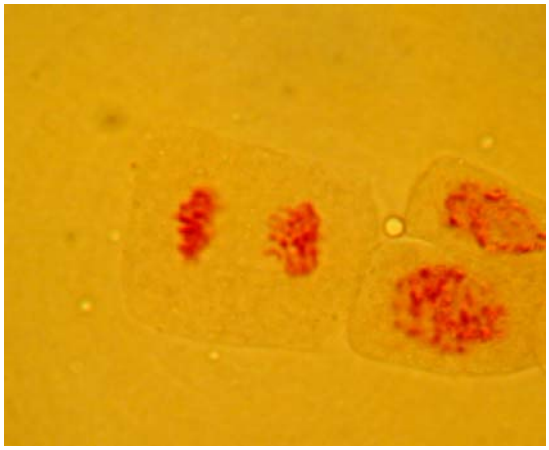

Fig. 5. A-T with delayed chromosomes (B_{02})

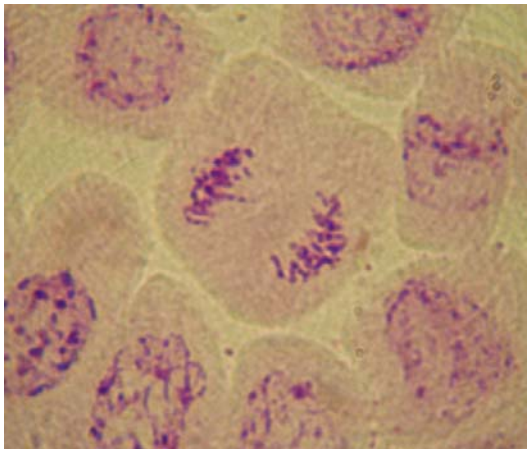

Fig. 6. A-T with fragments and delayed chromosomes (B_{02})

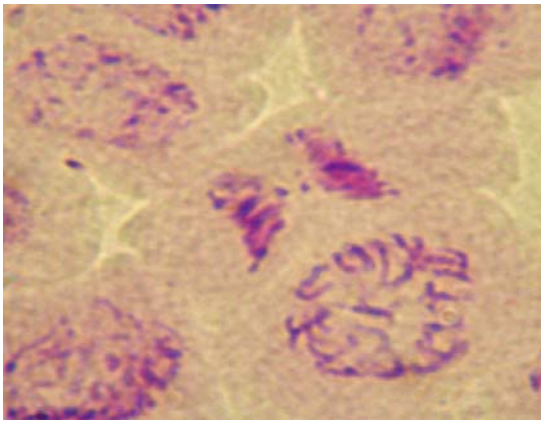

Fig. 7. A-T with fragments
(A_2)

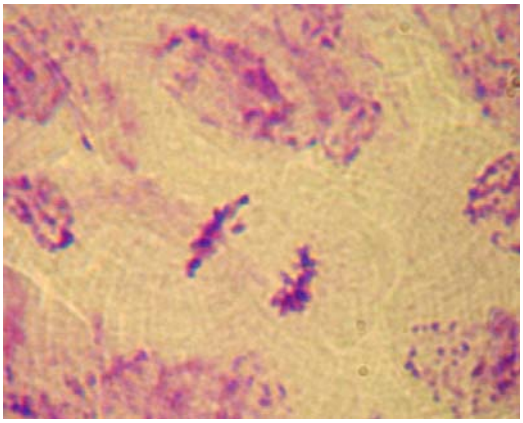

Fig. 8. A-T with a micronucleus and a bridge
(KN_1)

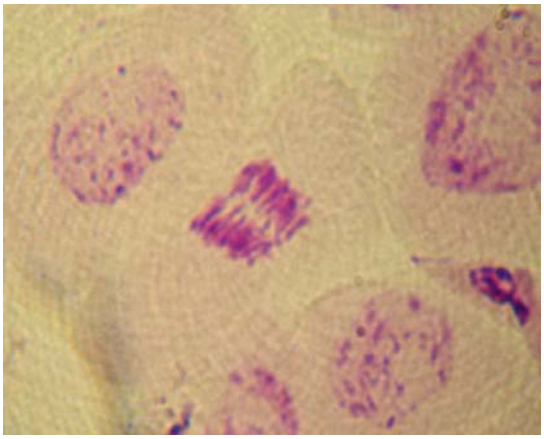

Fig. 9. A-T with bridges and delayed chromosomes
(KN_1)

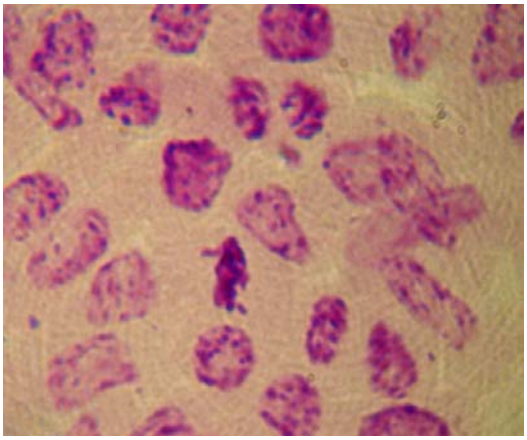

Fig. 10. Ana-telophase with a micronucleus
(KN1)

Chromosomal aberrations during other mitotic phases in *Melissa officinalis* L.

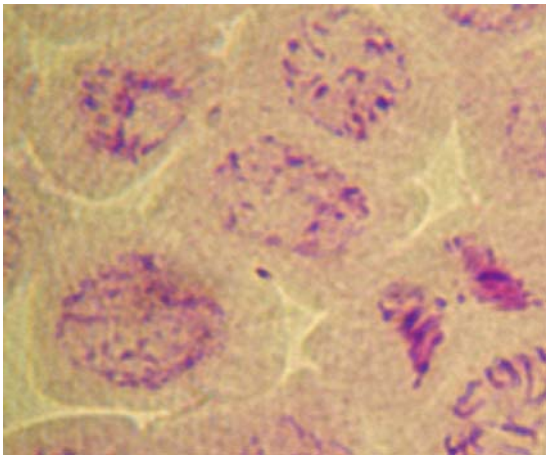

Fig.11. Prophase with one micronucleus
(A_2)

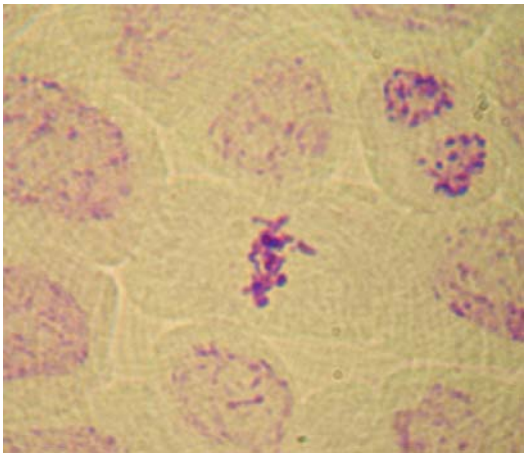

Fig. 12. Metaphase with delayed chromosomes
(KN_1)

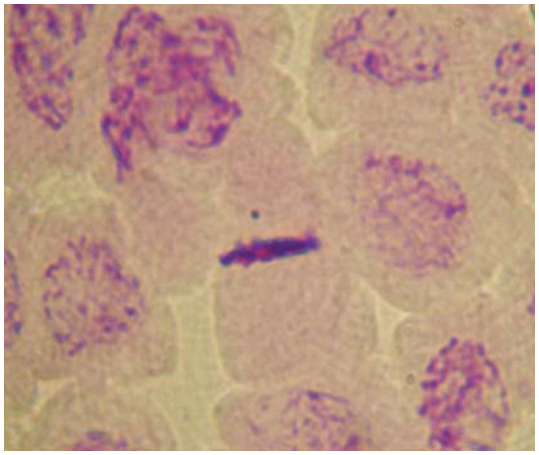

Fig. 13. Metaphase with one micronucleus (MS)

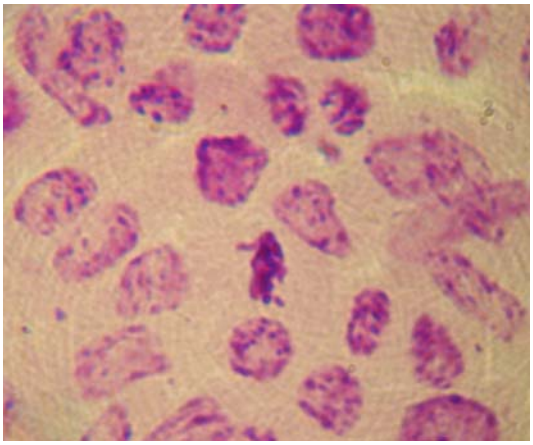

Fig. 14. Metaphase with delayed chromosomes (B_{02})