Enhancing Essential Oil Yield and Agronomical Traits in *Melissa Officinalis* L. through Synthetic Polyploidization

Rohit Bharati[1], Aayushi Gupta[2] Pavel Novy[3], Eloy Fernández Cusimamani [1]

[1] Department of Crop Sciences and Agroforestry, Faculty of Tropical AgriSciences, Czech University of Life Sciences, Prague, Czech Republic

[2] Department of Botany and Plant Physiology, Faculty of Agrobiology, Food, and Natural Resources, Czech University of Life Sciences Prague, Suchdol, Czech Republic.

[3] Department of Food Science, Faculty of Agrobiology, Food, and Natural Resources, Czech University of Life Sciences, Prague, Czech Republic

Rohit Bharati
bharati@ftz.czu.cz
0000-0001-5989-6153

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Introduction

- *Melissa officinalis* L. (2n=2x=32) is a perennial herb from the Lamiaceae family [1].
- The average essential oil yield in *M. officinalis* is between **0.02% - 0.30%** [1].
- The average essential oil yield is relatively **low,** considering the rising demand[2].
- Recently, synthetic polyploidization has been widely utilized to **increase essential oil yield** in medicinal and aromatic plants [3][4].
- Although **no attempts** have been made to enhance essential oil yield using synthetic polyploidization in *M. officinalis*.

Methods

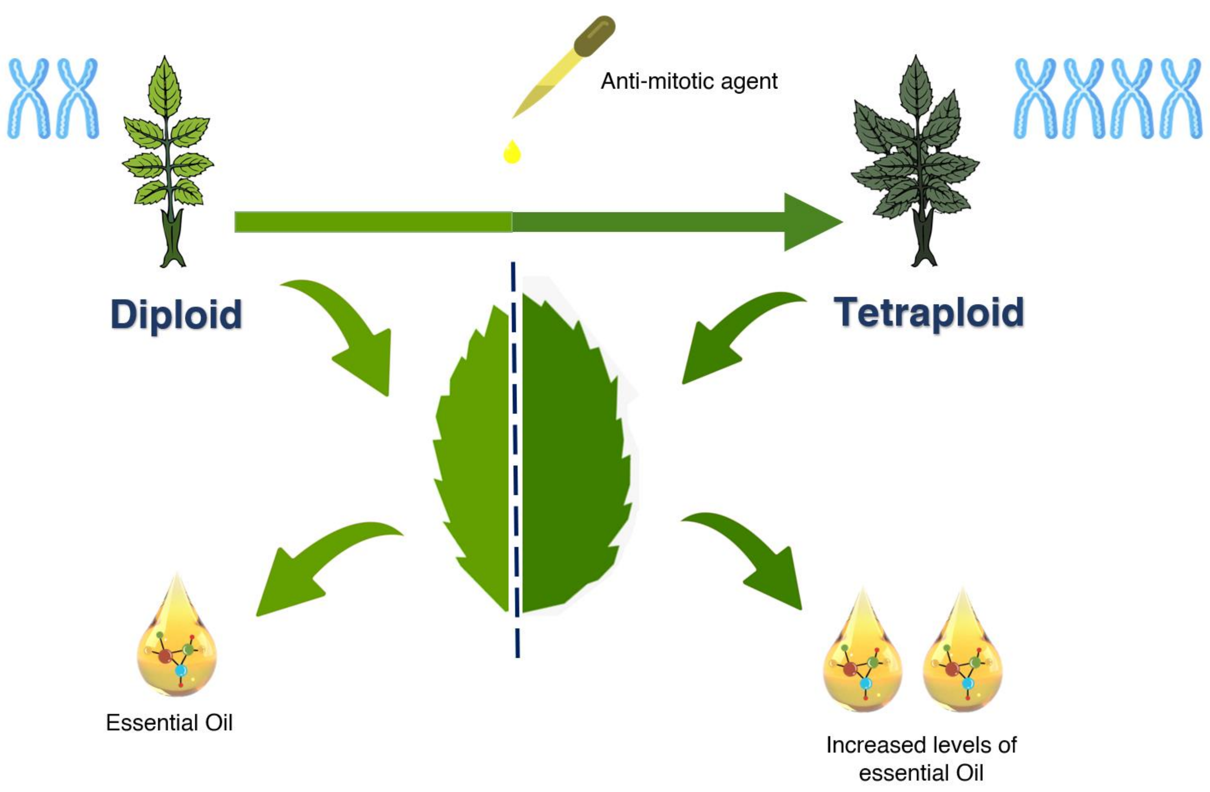
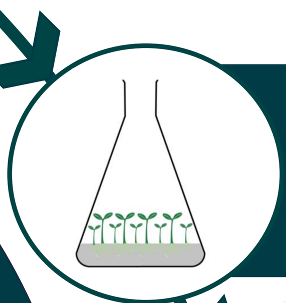
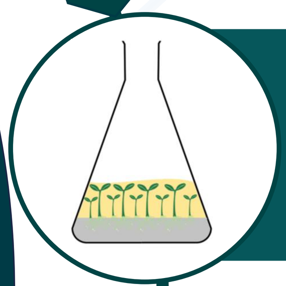


Figure 1: Simplified schematic diagram of the effect of polyploidization on essential oil yield from aromatic and medicinal plants

Basic steps involved



Micropropagation: Nodal segments of *M. officinalis* were surface sterilized and transferred to **MS basal media** (without plant growth regulators). A sufficient number of shoots were generated for anti-mitotic treatment.



Polyploid Induction: A total of six treatments were performed where oryzalin was applied for 24 h and 48 h in increasing concentrations of 20 µM (T1 and T2, respectively), 40 µM (T3 and T4) and 60 µM (T5 and T6).

Polyploid Detection: To detect the polyploids among the treated plants, **flowcytometry** and **chromosome counting** was used.

Novel Genotypes assessment: Morphological, Biochemical (**GC-MS**), and anatomical parameters were assessed to screen for superior agronomical traits compared to the diploid genotype

Results

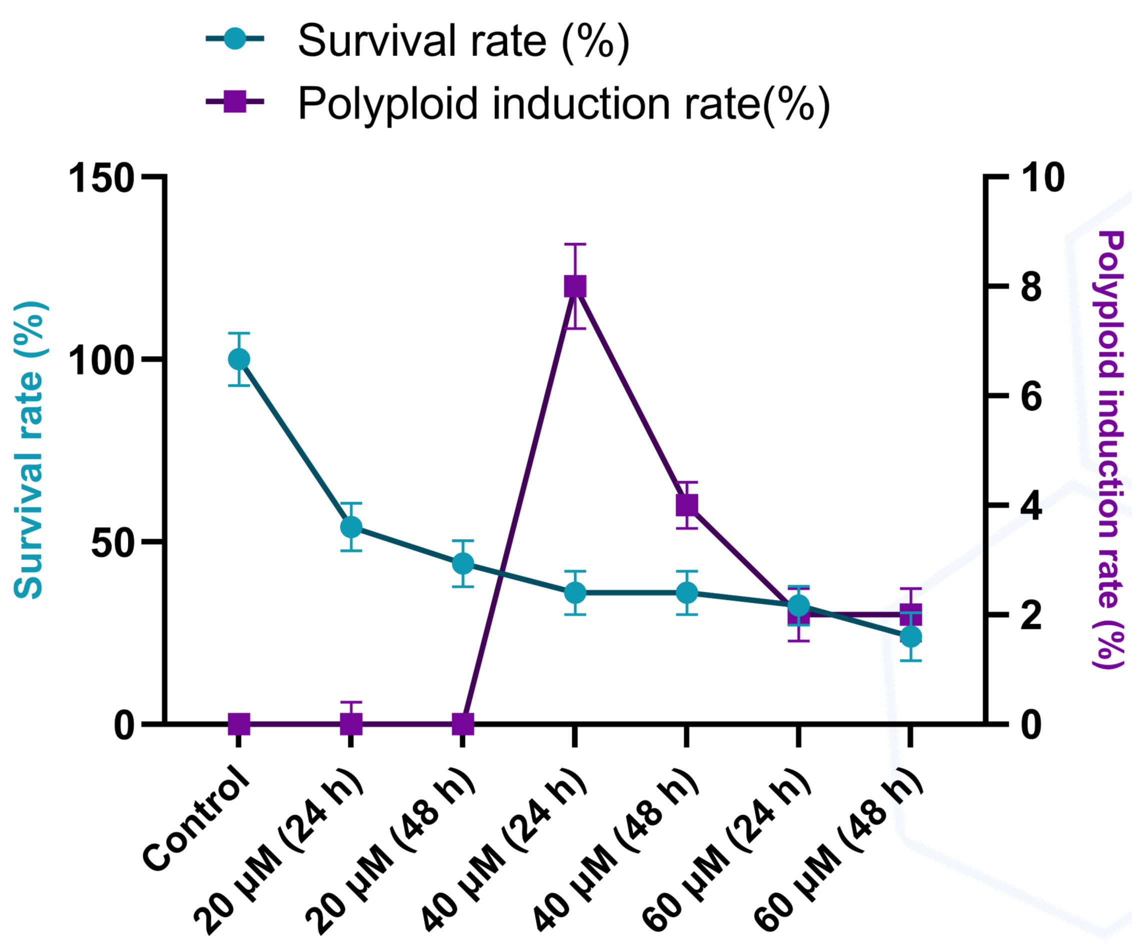


Figure 2. Effect of different concentrations and duration of oryzalin treatment on the survival rate and polyploid induction rate in *M. officinalis*.

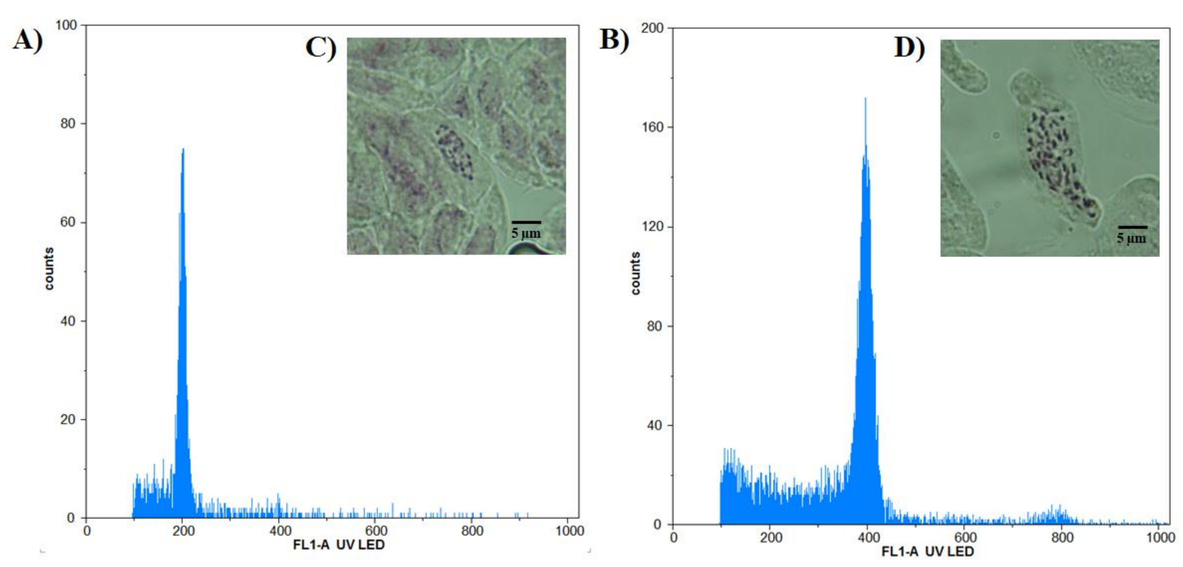


Figure 3: Histogram obtained from flowcytometry analysis for **(A)** diploid and **(B)** tetraploid plants, depicting relative DNA content along with chromosomes under 100x magnification for **(C)** diploid and **(D)** tetraploid plants. Bar = 5 µm.

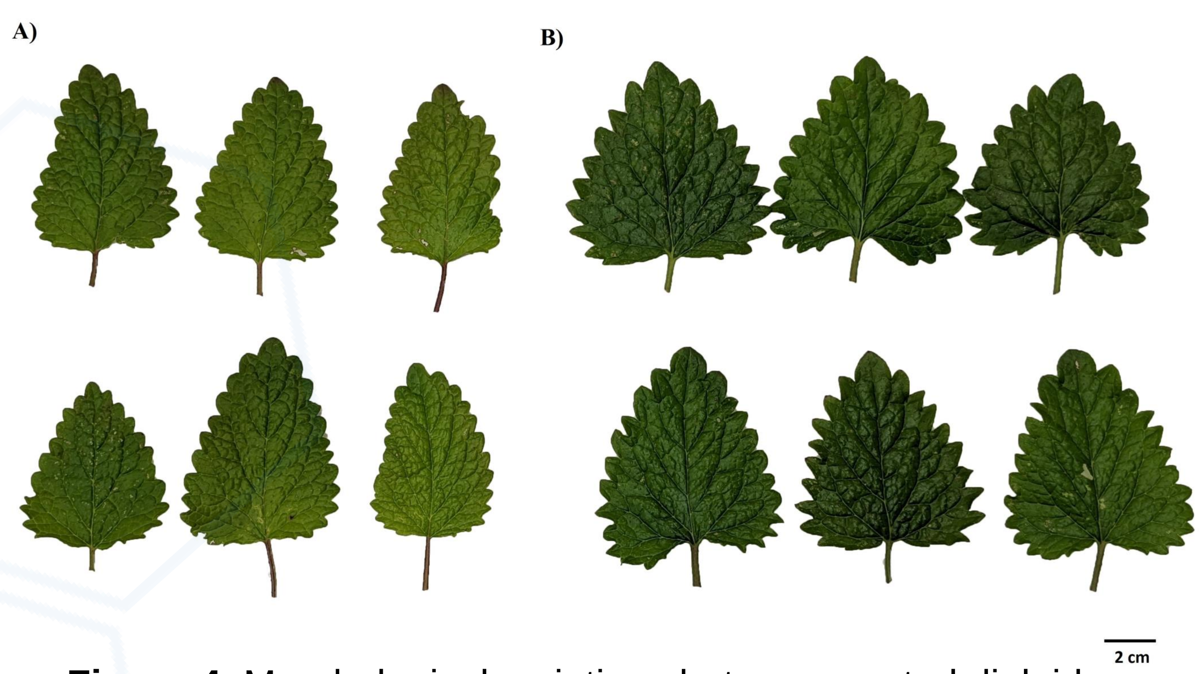


Figure 4. Morphological variations between control diploid, **(A)** induced tetraploid **(B)** leaves of *M. officinalis*.

Figure 5. Morphological variation between diploid mother plant **(A)** and tetraploid plant **(B)** of *M. officinalis*.

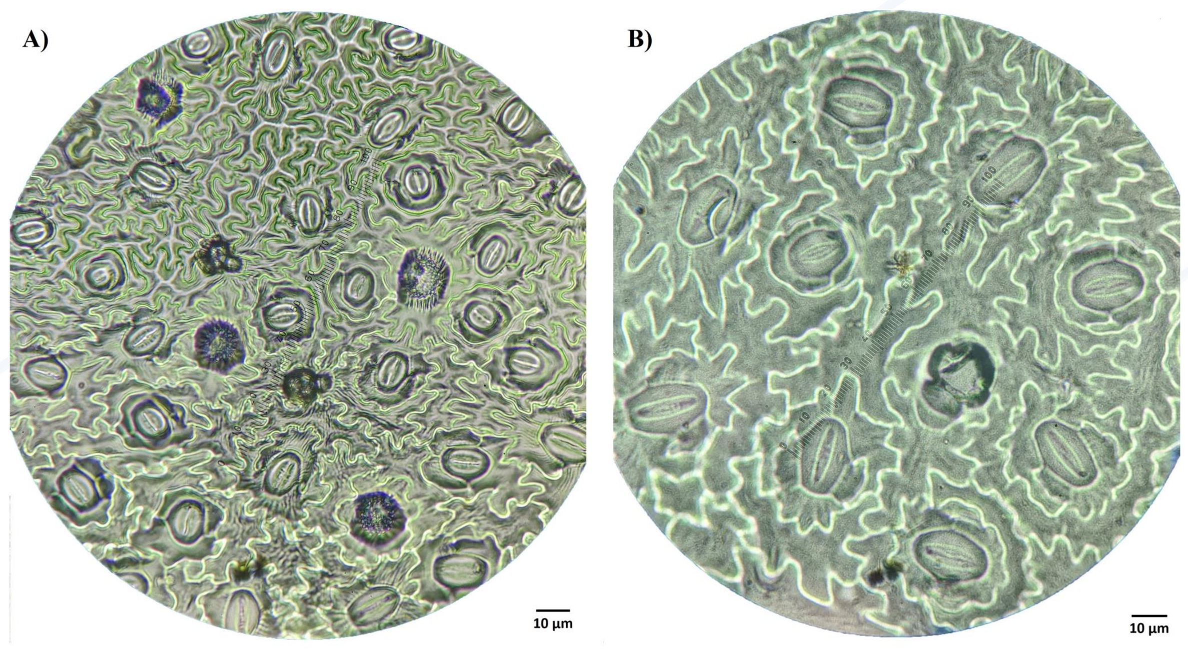


Figure 6. Average stomata size in tetraploid plants **(B)** significantly increased compared to the **(A)** diploid mother plant.

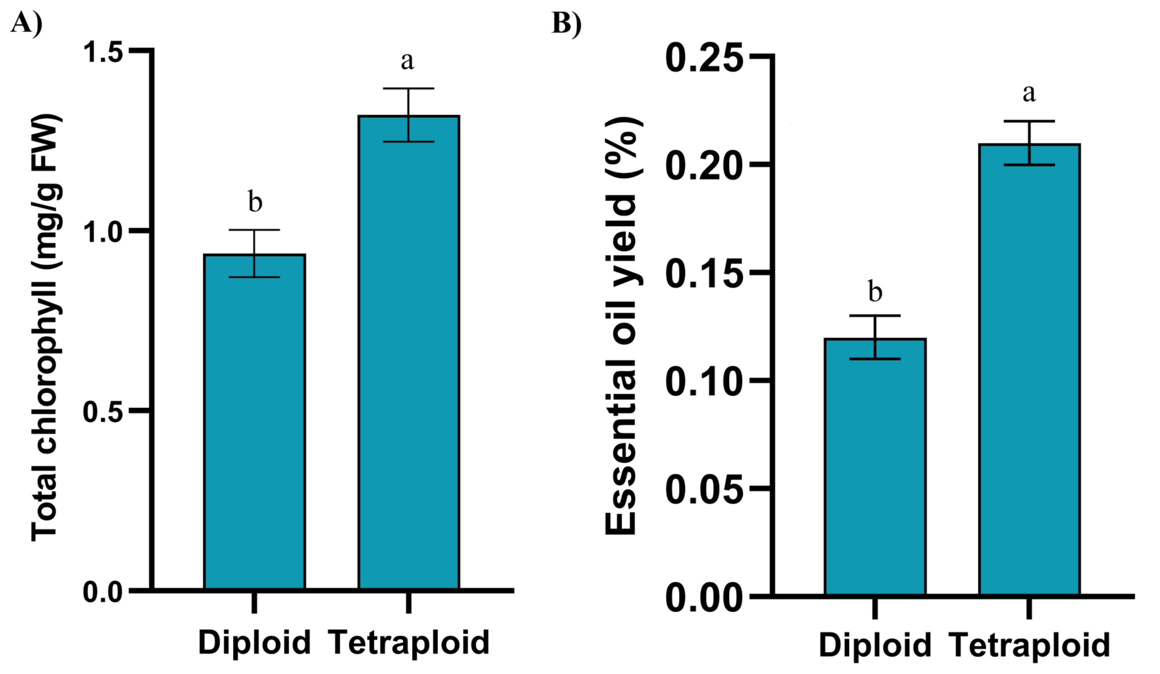


Figure 7. The total chlorophyll content exhibited a significant increase in tetraploid plants compared to the diploid mother plant **(A);** Average essential oil yield in tetraploid plants increased significantly by **75 %** compared to diploid plants **(B).**

Conclusion

- **Oryzalin was effective** in inducing polyploidization in *Melissa officinalis*.
- The newly developed polyploid genotype had a significant **increase in essential oil content (75 %)** and exhibited various **superior agronomical traits**.
- The current study could be a **valuable addition to the breeding attempts** to increase essential oils and other secondary metabolites in this and related species.

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