Development of polyploid genotypes in *Mentha spicata* using *in vitro* somatic polyploidization

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

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

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

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

Introduction

- *Mentha spicata* L. is a medicinal herb from the Lamiaceae family [1].
- Naturally **triploid** (2n=3x=36) [1].
- The average essential oil yield in *M. spicata* is between **0.04 to 2.1%** (*v/w*) [1].
- Yield is comparatively **low** considering the rising demand[2].
- Recently, *in vitro* polyploidization is being widely utilized to **increase essential oil yield** in medicinal and aromatic plants [3][4].
- Although, **no established protocol** for *in vitro* polyploidization is available in *Mentha spicata.*

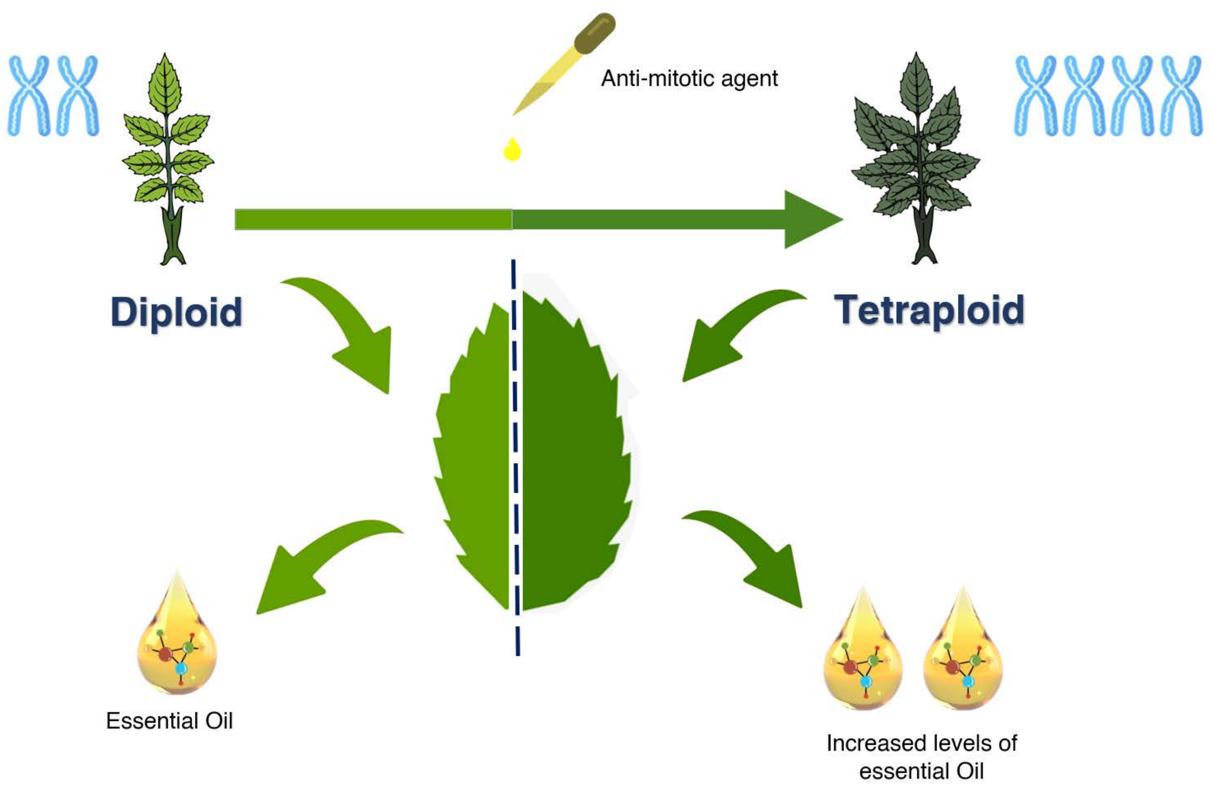

Figure 1: Schematic diagram of the effect of polyploidization on essential oil yield from aromatic and medicinal plants

Methods

***In vitro* micropropagation**: Nodal segments of *Mentha spicata* 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: Micropropagated plants were treated with three concentrations of **Oryzalin (20, 40, and 60 µM)** for two-time treatments **(24 hrs & 48 hrs).** A total of 50 plants were utilized in each treatment.

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 among the newly developed genotypes.

Results

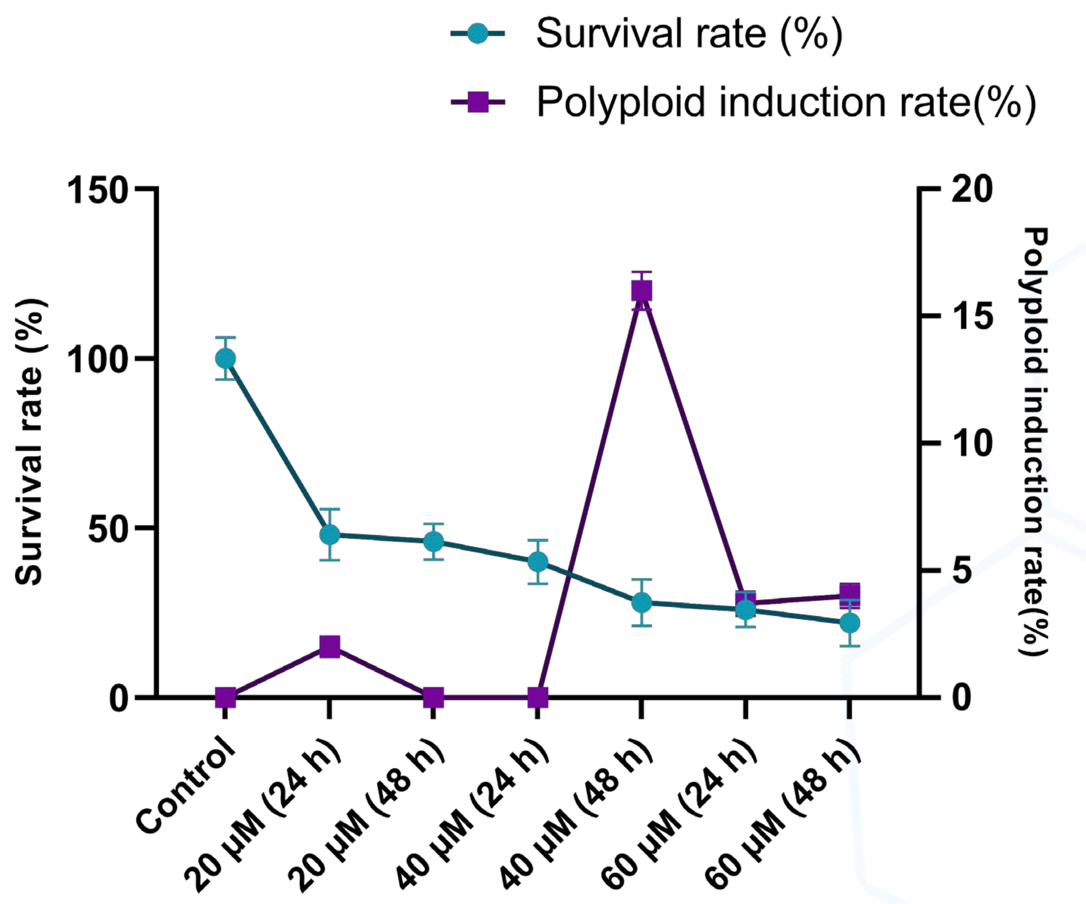

Figure 2. Effect of different concentrations and duration of oryzalin treatment on the survival rate and polyploid induction rate in *Mentha spicata.*

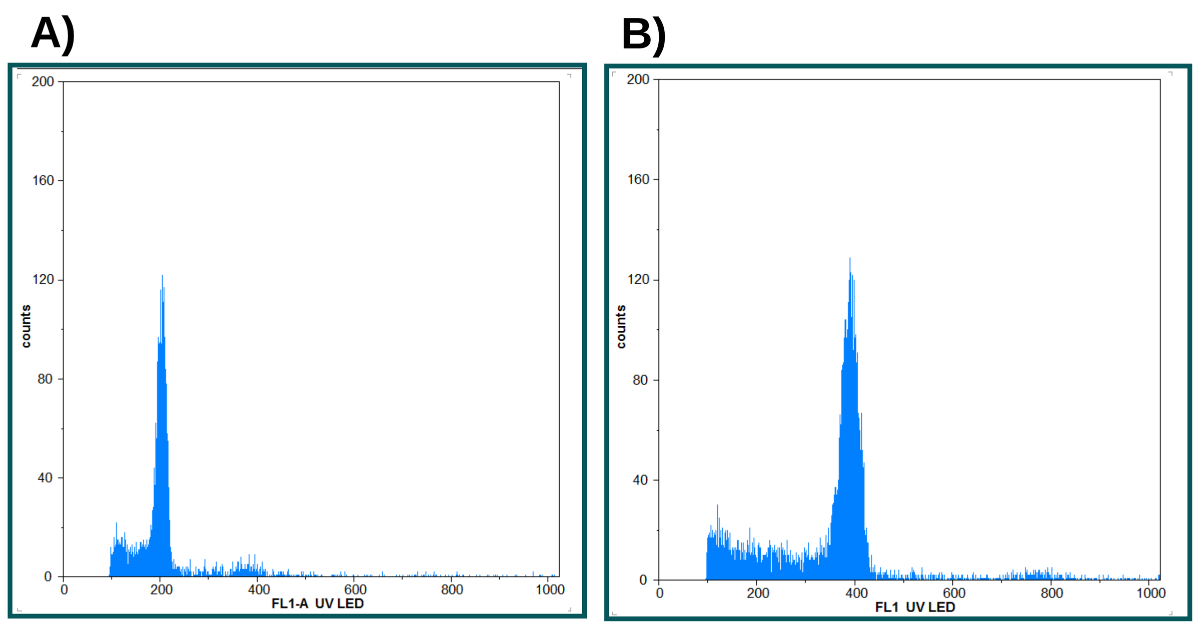

Figure 3. Histogram from flow cytometry for triploid **(A)** and hexaploid **(B)** plants, depicting relative DNA content.

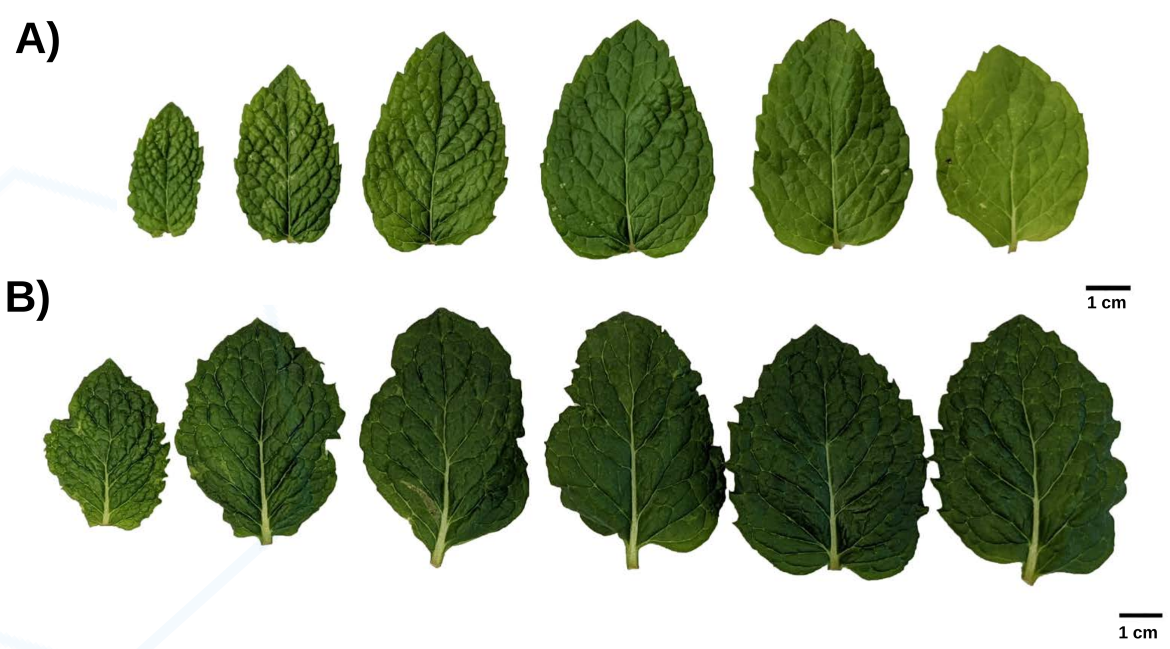

Figure 4. Morphological variations between control triploid **(A)** & induced hexaploid **(B)** leaves of *Mentha spicata.*

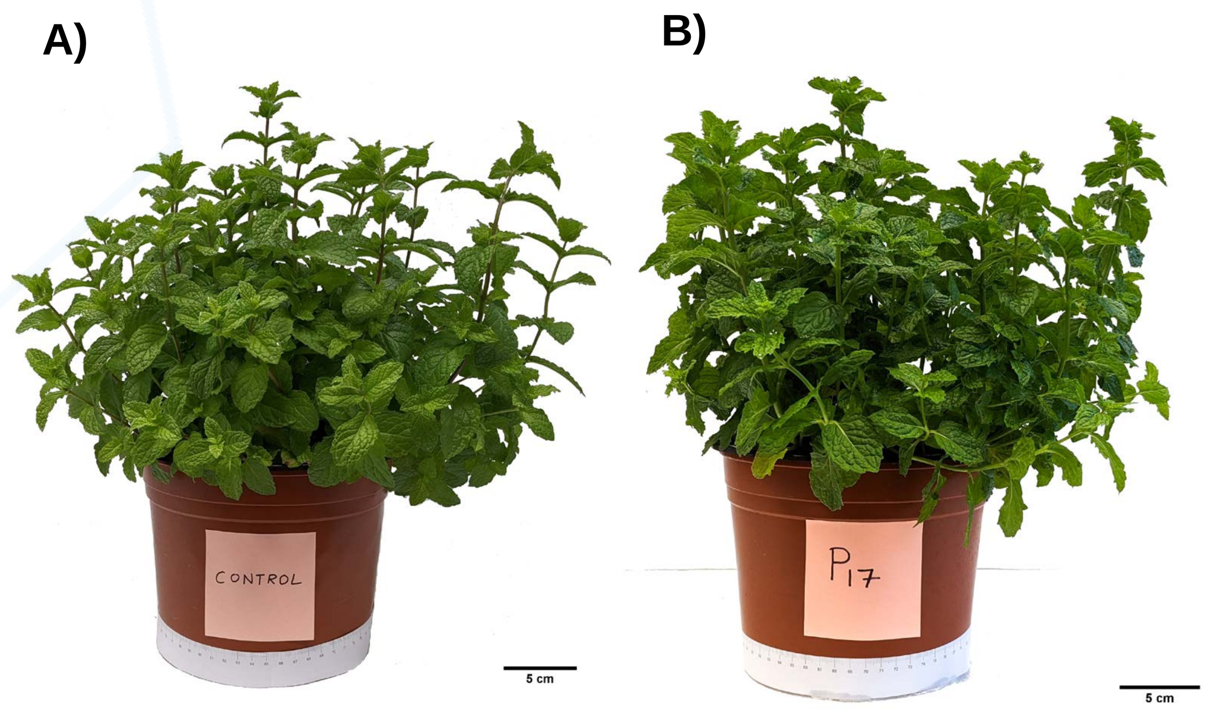

Figure 5. Morphological variation between triploid mother plant **(A)** and Hexaploid plant **(B)**.

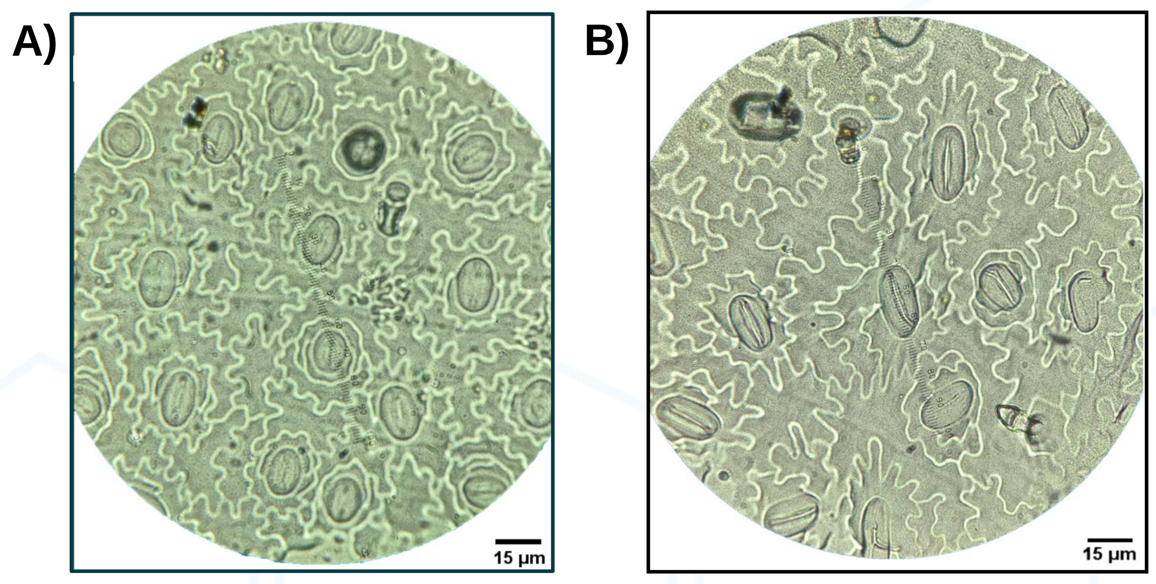

Figure 6. Average stomata size in Hexaploid plants **(B)** significantly increased compared to the **(A)** triploid mother plant.

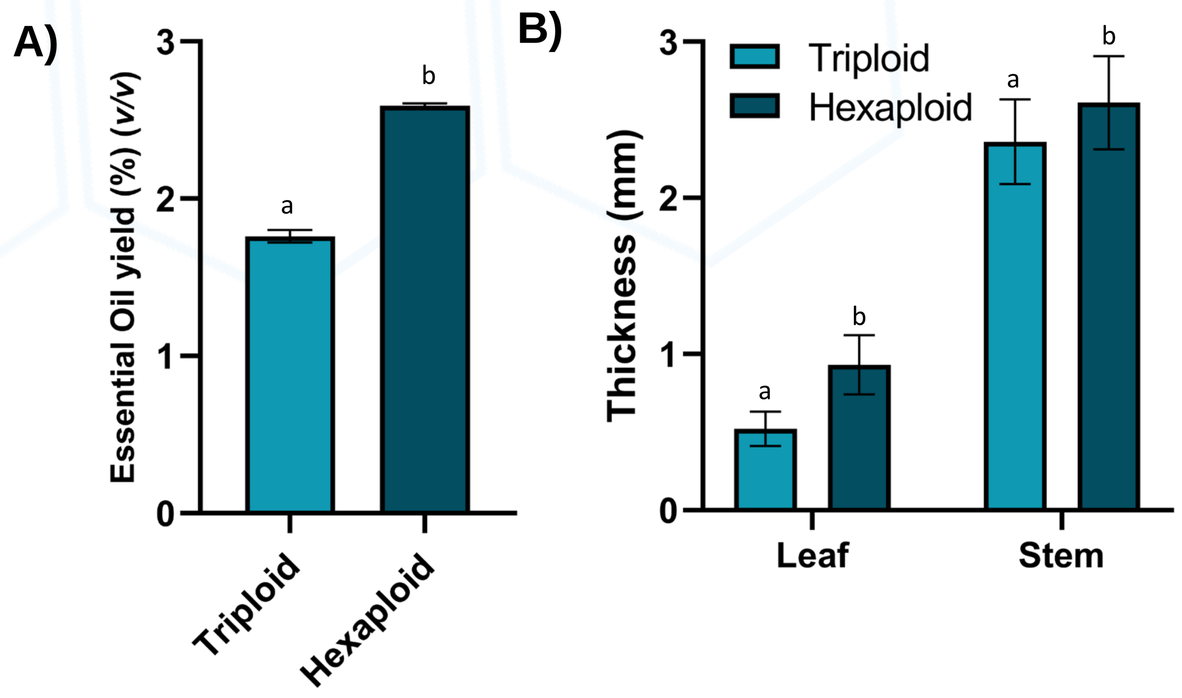

Figure 7. Average essential oil yield in hexaploid plants increased significantly compared to triploid plants **(A);** leaf and stem thickness exhibited a significant increase in hexaploid plants compared to the triploid mother plant **(B).**

Conclusion

- **Oryzalin was effective** in inducing polyploidization in *Mentha spicata*.
- Newly developed polyploids had a significant **increase in essential oil content (47.15%)** 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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