Genetic and morphological stability of autopolyploid *Thymus vulgaris* L. and changes in its anatomy and physiology

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Introduction

Polyploidization is a plant breeding method which allows us to obtain new genotypes with improved morphological, physiological and biochemical properties. The genomic stability of synthetic polyploids is variable. Several studies confirm the stability of *in vitro* induced polyploids over different periods of time while other studies reclassify polyploids to the ploidy level of their original counterparts from which they were derived (1,2). After two years of growing in field conditions we set out to assess the genetic stability of our *in vitro* induced somatic autotetraploid *Thymus vulgaris* and more. importantly the stability of its morphology and biochemical profile. Moreover, we explored several anatomical and physiological changes in the new genetic material

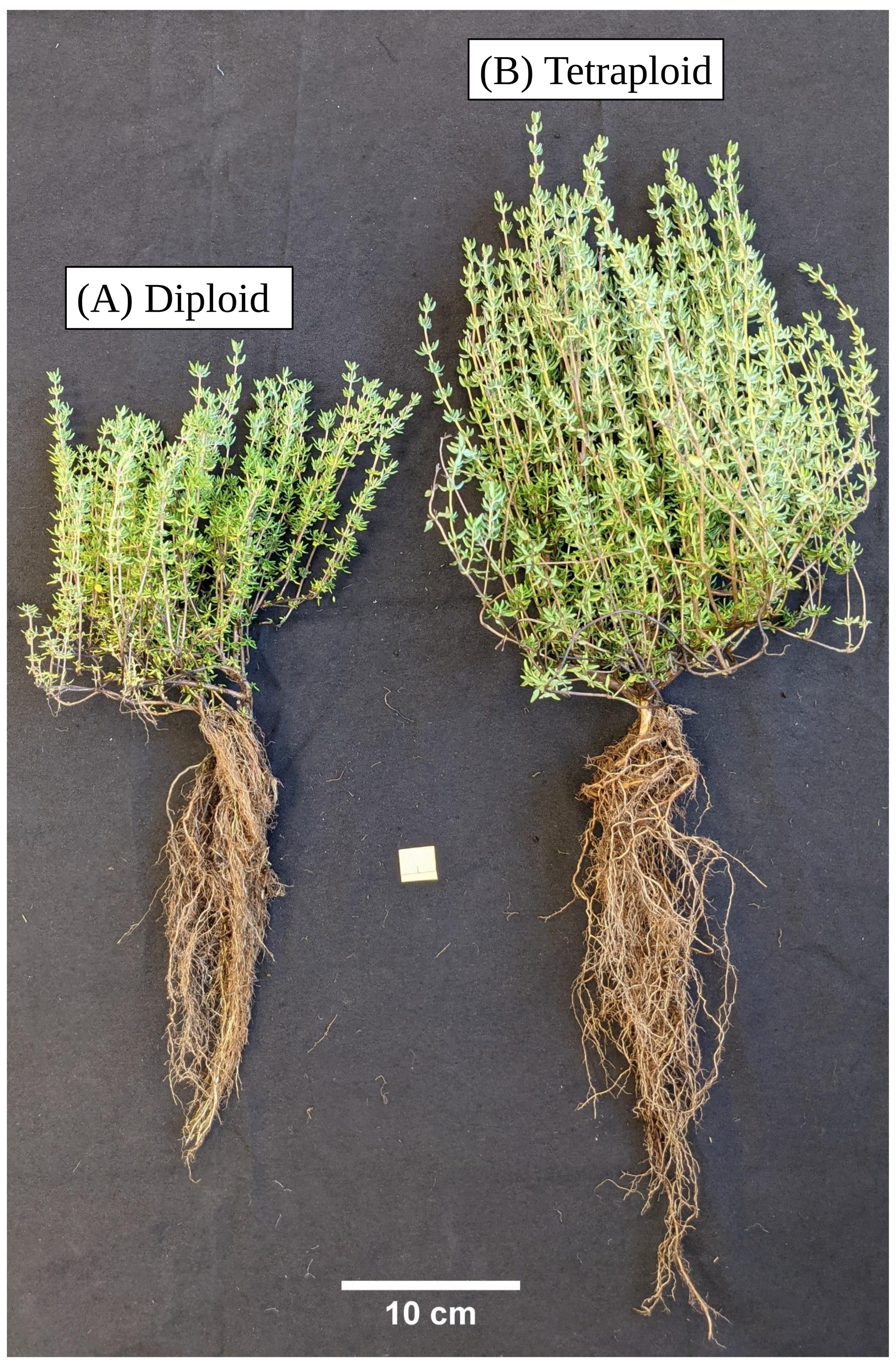

Fig. 1. Diploid *T. vulgaris* on the left side and the new tetraploid genotype on the right.

Materials and Methods

- Diploid (2n = 2x = 30) and tetraploid (2n = 4x = 60) *T. vulgaris* were obtained from the plant collection of Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Czech Republic
- Genetic analysis for ploidy level determination of control and tetraploid plants were reevaluated by flow cytometry analysis (3)
- Morphological analysis repeated for (plant height, number of branches, main plant thickness, branch thickness, length of branches, internodal distances of main stem, internodal distances of branches, leaf length, leaf breadth and leaf thickness)
- Essential oils analysis are still in progress
- Stomata analysis were performed using nail varnish technique (4)
- Chlorophyl analysis was performed using shoot samples. The absorbance was measured using a spectrophotometer at two wavelengths: 663 nm (chlorophyll a) and 645 nm (chlorophyll b), and chlorophyll contents were calculated (5)

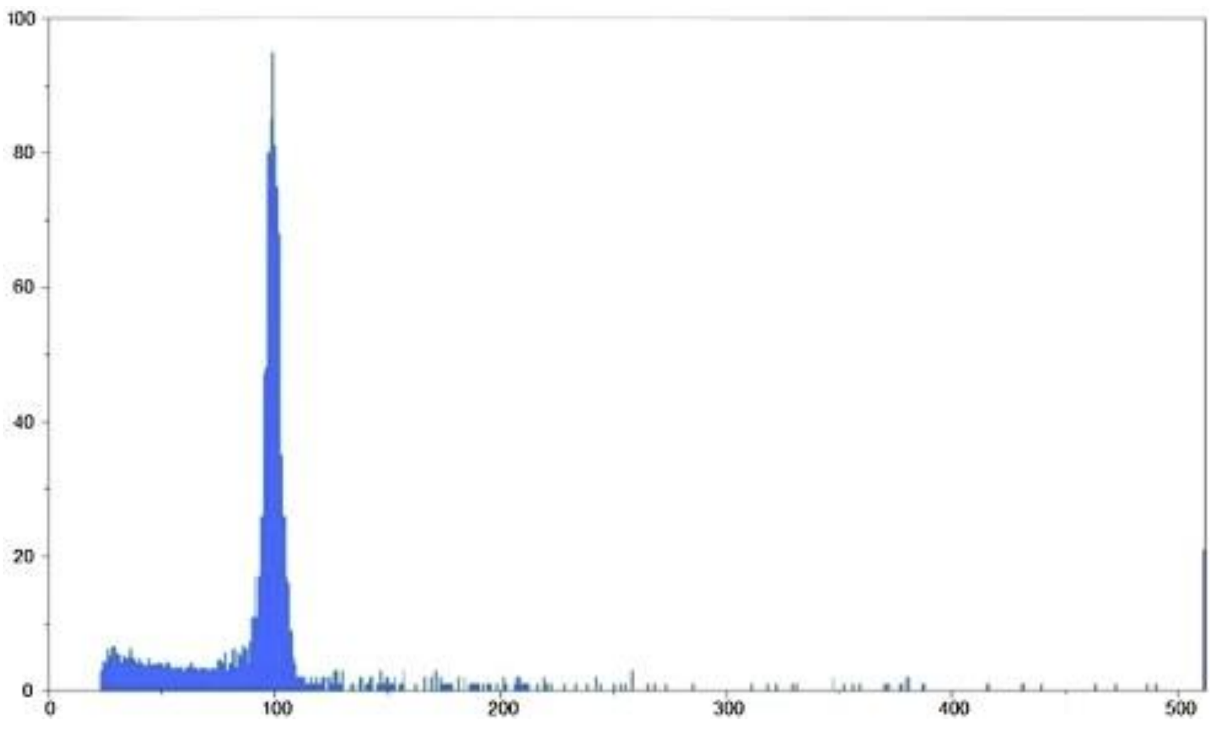

Fig. 2. Histogram of relative DNA content with a peak corresponding to G0/G1 nuclei of the control plant (diploid plant) on Channel 100.

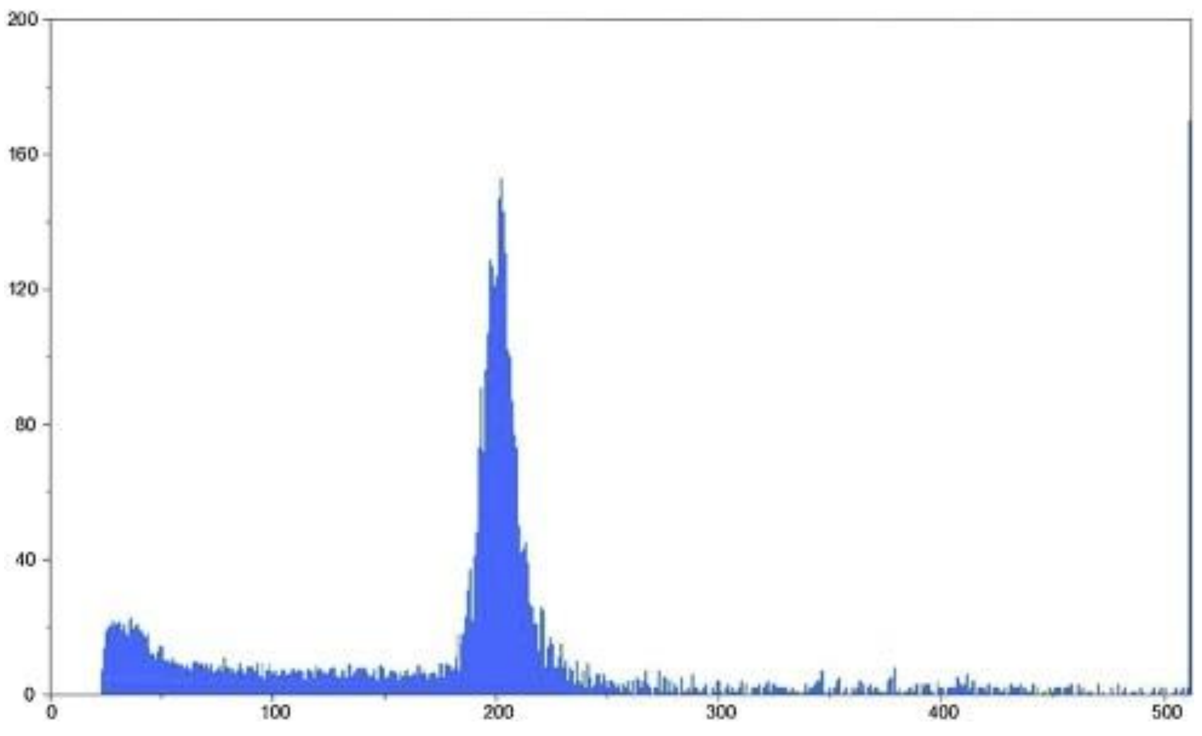

Fig. 3. Histogram of relative DNA content with a peak corresponding to G0/G1 nuclei of tetraploid plant on Channel 200.

Results

- Flow cytometry analysis show the stability in ploidy level for control and tetraploid plants after two years of growing in field conditions (Fig. 2 & 3)
- Tetraploid plants maintained enhanced morphological characteristics. The internodal distance of branches were clearly visible (Fig.1) and other parameters had statistically significant differences
- Stomata cell size and stomata guard cell size were significantly larger in tetraploid leaves. On the other hand, the density of stomata cells was significantly higher in diploid leaves (Fig. 4)
- Chlorophyll analysis revealed notable increased levels in the photosynthetic pigments (Chlorophyll a and b) in the tetraploid shoot tips sample compared to diploid samples (Fig.5; Tab. 1). A healthy range of photosynthesis activity is showed by both plants in (Fig. 6)

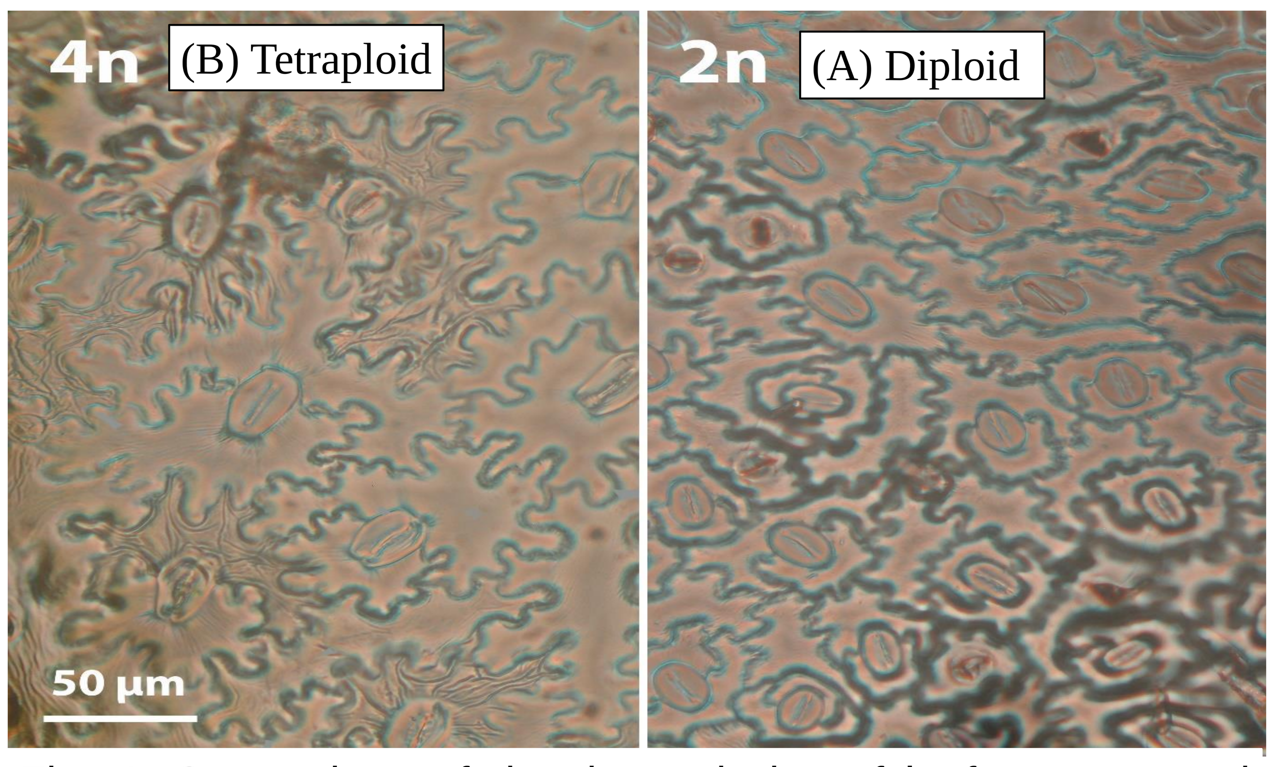

Fig. 4. Comparison of density and size of leaf stomata and stomatal guard cells of tetraploid (4n) *and* diploid (2n) *T. vulgaris* plants.

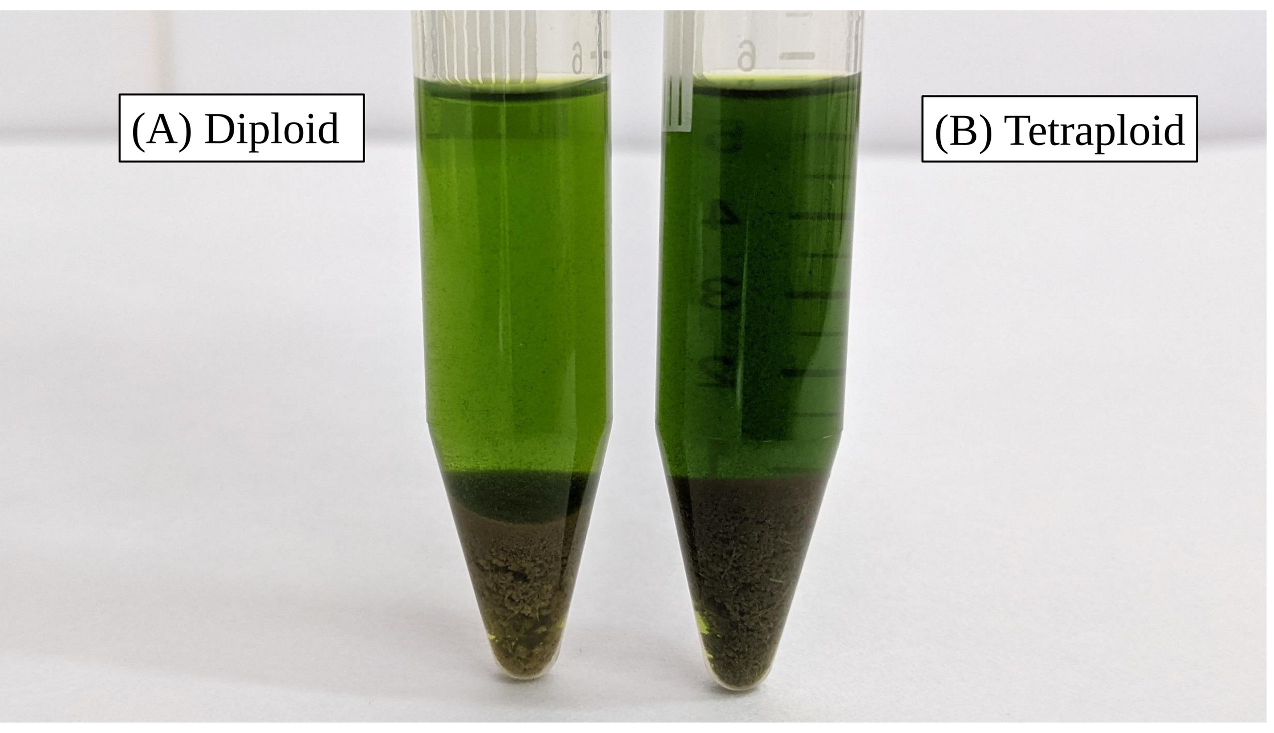

Fig. 5. *T. vulgaris* shoot samples grounded in liquid nitrogen. Diploid sample on the left and tetraploid sample on the right.

Tab. 1. Chlorophyll a & b comparison between diploid and tetraploid *T. vulgaris*

	Chlorophyll A	Chlorophyll B
Diploid	0.44 mg g-1 FW	0.153 mg g-1 FW
Tetraploid	0.76 mg g-1 FW	0.249 mg g-1 FW

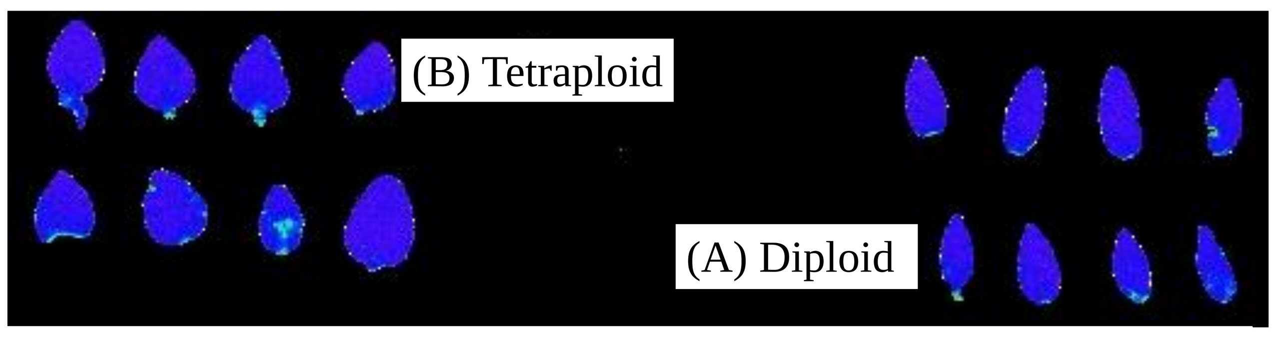

Fig. 6. Photosynthesis intensity of *T. vulgaris* leaves. Tetraploid on the left side and diploid on the right.

Conclusion

- Genetical and morphological analysis repeated after two years of growing on filed conditions proved the stability of the autotetraploid genotype
- New anatomical and physiological experiments varied between control and tetraploid plants. These preliminary results are motivating to further explore different changes in the new genetic material

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