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Synthetic polyploidization a promising tool in crop management: Induces enhanced phytochemical profile and biological activities in *Thymus vulgaris* L.

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Abstract: Consumption of medicinal and aromatic plants is broadening and boosting globally. However, the primary supply of raw materials due to wild harvesting is destroying habitats, resulting in a loss of genetic diversity. This situation makes the development and application of breeding programmes imperative. Polyploidisation has emerged as a promising tool in plant breeding and crop management to generate genotypes with novel genetic combinations that can confer enhanced desirable biochemical, morphological, physiological, and biological and increased resistance to both biotic and abiotic stresses. Polyploidization in medicinally and economically important herbs has a plethora of benefits. Essential oil from *T. vulgaris* has valuable therapeutic potential that is highly desired in the pharmaceutical, food, and cosmetic industries. Considering these advantages and the rising market demand, induced polyploids were obtained using oryzalin to enhance essential oil yield. However, their therapeutic values were unexplored. So, this study aims to assess the phytochemical content and antimicrobial, antioxidant, and anti-inflammatory activities of tetraploid and diploid thyme essential oils. Induced tetraploids were found to have higher essential oil yield with enhanced thymol and γ-terpinene content than diploid. Tetraploids exhibited higher antibacterial activity against respiratory pathogens than the diploid. Similarly, in DPPH radical scavenging assay, tetraploid essential oil was more potent than diploid. Tetraploids exhibited more effective inhibition of in vitro catalytic activity of the pro-inflammatory enzyme cyclooxygenase-2 (COX-2) than diploids. Succintly, these results suggest that synthetic polyploidization using oryzalin could effectively enhance the quality and quantity of secondary metabolites, produce genotypes that are highly tolerant to stress and develop more efficient essential oil-based commercial products using this induced genotype.

Keywords: Crop management, medicinal plants, plant breeding, polyploidization, thyme

Introduction: *Thymus vulgaris* L., popularly known as garden thyme, an aromatic herb belonging to the Lamiaceae family, has immense antimicrobial, antioxidant, and anti-inflammatory properties that help treat cough, bronchitis, sore throat, arthritis, and rheumatism. Apart from these, thyme essential oil is used commercially as a natural preservative in food industries to prevent spoilage as well as for food packaging systems. Keeping in mind that consumers prefer natural products over genetically modified plants, synthetic polyploidization is conducted in which chromosome duplication of the whole genomic constitution of an organism using chemical antimitotic agents like oryzalin, colchicine, trifluralin, etc., results in superior or inferior genotypes with enhanced or reduced morphological, physiological, and biochemical properties. The study aims to extract essential oil from induced tetraploid thyme plants and evaluate how polyploidization affects its chemical composition and biological activities, including antimicrobial, anti-inflammatory, and antioxidant properties. The findings could demonstrate that synthetic polyploidization enhances secondary metabolite production, thereby improving the biological activities of thyme, which is valuable for the pharmaceutical, cosmetic, and food industries (Gupta et al., 2024; Shmeit et al., 2020).

Material and Methods: Tetraploid (2n=4x=60) and diploid *T. vulgaris* plants (2n=2x=30) were obtained and maintained in the field condition at the botanical garden of the Faculty of Tropical Agrisciences (FTA), Czech University of Life Sciences Prague (CZU). A confirmatory test was conducted to verify the stability of tetraploid and diploid samples. 100g of polyploid and diploid thyme dry samples were hydrodistilled separately for 3h to obtain the crude essential oil and stored at 4ºC. Further, GC-MS analysis was conducted to compare the content of various phytochemicals. Broth Microdilution Volatilization (BMV) method depicted the antibacterial activity of EOs at different concentrations in both liquid and vapor phases against *Haemophilus influenzae* ATCC 49247, *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619, and *Streptococcus pyogenes* ATCC 19615. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was conducted using 96 well micro-titre to compare the lipid oxidation action between polyploid and diploid EO (Stastny et al., 2022)**.** Inhibitory activity against cyclooxygenases (COX-2) was determined. Molecular interactions between major volatile of *T. vulgaris* essential oil and various bacterial proteins along with protein human cyclin-dependent kinase 2 complex and cyclooxygenase-2.

Results and Discussion:

Essential oils from *T. vulgaris* were extracted using the hydro-distillation method from both tetraploid and diploid genotypes, yielding 1.2% and 0.85% of essential oil, respectively (Fig 1.). Thymol was identified as the predominant compound, making up 53.5% in the tetraploid genotype and 50.65% in the diploid genotype. The monoterpenes γ-terpinene and p-cymene were the next most abundant compounds, comprising 21.81% and 7.85% in the tetraploid genotype, and 5.55% and 20.40% in the diploid genotype, respectively (Fig 2). The tetraploid essential oil showed an increased amount of DPPH radical scavenging activity which means it is a better hydrogen provider compared to the diploid control genotype (Table 1). The compounds present in *T. vulgaris* essential oils contain conjugated carbon double bonds and hydroxyl groups that readily inhibit free radicals that lead to antioxidant effects. Therefore, a slightly higher amount of thymol in tetraploid contributes to its higher anti-inflammatory activity (Table 2). The docking study showed a higher binding efficacy of thymol with the cyclooxygenase-2 protein (Table 4). The antimicrobial activity on respiratory pathogens such as *H. influenzae*, *S. aureus*, *S. pneumoniae*, and *S. pyogenes* revealed that the induced tetraploid *T. vulgaris* essential oil has higher antibacterial activity in comparison to diploid control, although both genotypes exhibited the best results in the liquid phase (Table 3). The molecular interactions between major volatile compounds like p-cymene, γ –terpinene, and thymol of T. vulgaris essential oil and the vital enzymes involved in biosynthesis and repair of cell walls, nucleic acids, and proteins in bacteria along with protein human cyclin-dependent kinase 2 complex and cyclooxygenase-2 (Table 4).

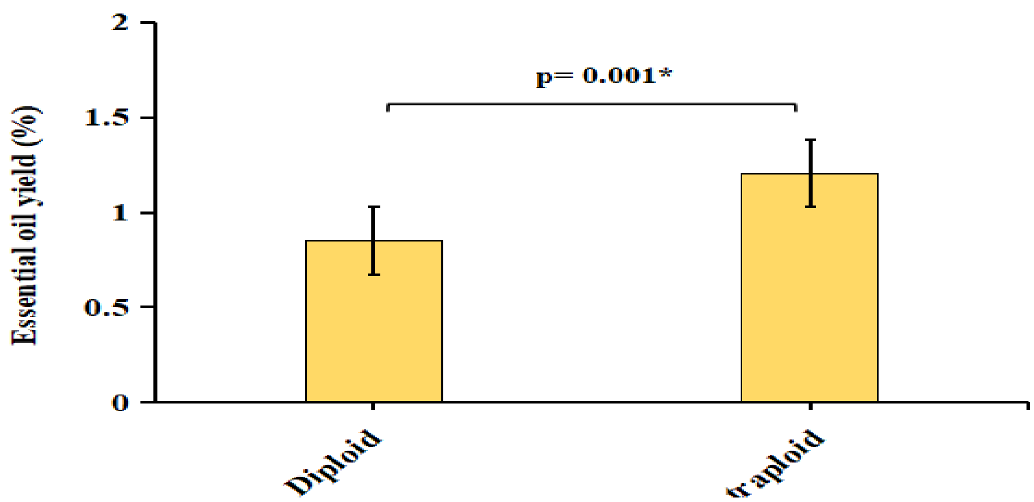

Fig 1: Essential oil yield in control diploid and induced polyploid of *T. vulgaris* "*" expressed a significant difference (Tukey HSD Test, $p < 0.05$).

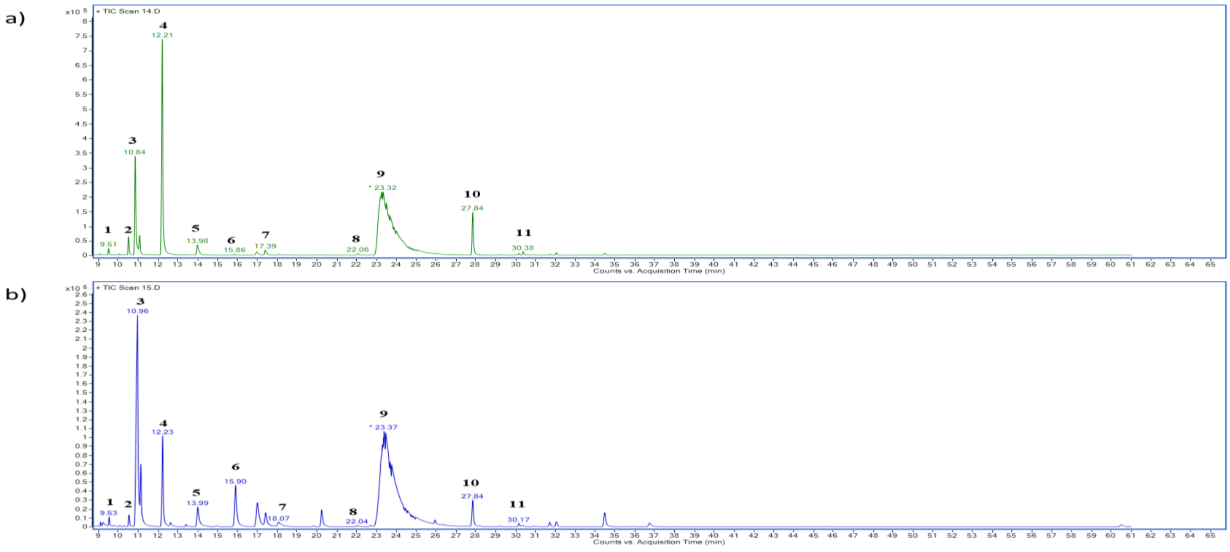

Fig 2: GC-MS chromatograms of *T. vulgaris* essential oil obtained from a) tetraploid and b) diploid genotype. Peak number and compound names: 1. Mycrene, 2. 4-carene, 3. p-cymene, 4. γ-Terpinene, 5. Linalool, 6. d-camphor, 7. 4-Terpineol, 8. Borneol, 9. Thymol, 10. Caryophyllene, 11. D-Germacrene.

Table 1. Antioxidant activity of *T. vulgaris* diploid and polyploid essential oils.

Plant samples	DPPH	
	IC_{50} ±SD[1](μg/mL)	μg TE/mg ± SD
Diploid	> 512	>12.68
Tetraploid	180.03 ± 51.50*	40.05 ± 14.01*
Positive control Trolox	6.49 ± 1.01	-

[1] IC_{50} ± SD: half maximal inhibitory concentration ± standard deviation; TE = Trolox equivalent; *: shows a significant difference between the diploid and tetraploid genotype based on Tukey's test for post hoc analysis at 5% significance level

Table 2. In vitro anti-inflammatory activity of *T. vulgaris* diploid and polyploid essential oils determined by inhibition of COX-2 enzyme.

Samples	Concentration (μg/mL)	Inhibition %
Diploid	500	80.96 ± 7.2
	50	70.53 ± 11.86
	5	2.02 ± 17.13
Tetraploid	500	85.57 ± 7.5
	50	83.74 ± 5.8*
	5	6.74 ± 23.48
Ibuprofen	5	74.52 ± 10.2

The results are expressed as means ± SD for two independent experiments measured in duplicate. The results were compared by Tukey's test for post hoc analysis at 5% significance level. *: shows a significant difference between the diploid and tetraploid genotypes

Table 3. In vitro growth-inhibitory effect of *T. vulgaris* essential oils (control and tetraploid) in liquid and vapor phases against respiratory infection bacteria.

	Bacterium/Growth Medium/Minimum Inhibitory Concentration							
Essential Oil	*Haemophilus influenzae*		*Staphylococcus aureus*		*Streptococcus pneumoniae*		*Streptococcus pyogenes*	
	Agar* (μg/mL)	**Broth (μg/mL)**	**Agar (μg/mL)**	**Broth (μg/mL)**	**Agar (μg/mL)**	**Broth (μg/mL)**	**Agar (μg/mL)**	**Broth (μg/mL)**
Diploid	>1024	256	>1024	1024	1024	512	1024	1024
Tetraploid	>1024	128	1024	512	1024	512	1024	512
	Positive antibiotic control							
Amoxicillin	NT	NT	NT	NT	>2	>2	NT	NT
Ampicillin	>2	0.25	>2	2	NT	NT	>2	2

NT: Not tested; *: If the distribution of volatiles is uniform in liquid and gaseous phase, the concentrations can be expressed as weight of volatile agent per volume unit of a well, whereas their real values will be 256, 128, 64, 32, 16, 8, 4 and 2 μg/cm^3 for 1024, 512, 256, 128, 64, 32, 16, and 8 μg/mL, respectively.

Table 4. Binding free-energy values of major volatile compounds of *T. vulgaris* essential oil.

Ligand	**Binding Free Energy ΔG (kcal/mol)**								
	1JZQ*	1KZN	2VEG	2ZDQ	3RAE	3SRW	3UDI	1CX2	1HCK
γ -terpinene	-5.7	-4.6	-4.5	-7.8	-5.3	-5.6	-5.0	-6.3	-5.2
P-cymene	-5.8	-5.8	-4.6	-7.9	-5.8	-5.6	-5.1	-6.3	-4.5
Thymol	-5.4	-6.3	-4.7	-7.7	-5.6	-5.7	-5.2	-6.5	-6.4
Ascorbic acid**	-	-	-	-	-	-	-	-	-5.0

*Protein PDB ID:1JZQ- isoleucyl-tRNA synthetase, 1KZN- DNA gyrase, 2VEG-dihydropteroate synthase, 2ZDQ-D-alanine:D-alanine ligase, 3RAE-topoisomerase 4, 3SRW-dihydrofolate reductase, 3UDI-penicillin-binding protein 1a, 1CX2- cyclooxygenase-2, and 1HCK-protein human cyclin-dependent kinase 2 complex. **Ascorbic acid: Used as a reference for antioxidant activity.

Conclusions and Outlook: In vitro polyploidization using synthetic antimitotic agents can effectively generate polyploid plants with enhanced biological traits. The current study characterizes the biological activities of oryzalin-induced polyploid thyme essential oil for the first time, demonstrating the effectiveness of artificial polyploidization. The induced polyploid genotype showed a significant increase in essential oil yield (41.11%) and higher concentrations of active compounds like thymol and γ-terpinene. This genotype exhibited enhanced antibacterial, antioxidant, and anti-inflammatory activities compared to the diploid genotype. These improved traits suggest that polyploid *T. vulgaris* can be commercially valuable, especially in the pharmaceutical and food industries, and synthetic polyploidization must be included in producing high-cultivar crops that promote crop management. However, further in vivo studies are needed to confirm its practical applications.

References

- Gupta, N., Bhattacharya, S., Dutta, A., Tauchen, J., Landa, P., Urbanová, K., ... & Leuner, O. (2024). Synthetic polyploidization induces enhanced phytochemical profile and biological activities in Thymus vulgaris L. essential oil. Scientific Reports, 14(1), 5608.
- Stastny, J., Marsik, P., Tauchen, J., Bozik, M., Mascellani, A., Havlik, J., ... & Kloucek, P. (2022). Antioxidant and anti-inflammatory activity of five medicinal mushrooms of the genus Pleurotus. *Antioxidants*, *11*(8), 1569.
- Shmeit, Y. H., Fernandez, E., Novy, P., Kloucek, P., Orosz, M., & Kokoska, L. (2020). Autopolyploidy effect on morphological variation and essential oil content in Thymus vulgaris L. *Scientia Horticulturae*, *263*, 109095.