Synthetic Polyploidization a Promising Tool in Crop Management: Induces Enhanced Phytochemical Profile and Biological Activities in *Thymus vulgaris* L.

Neha Gupta[a], Soham Bhattacharya[b], and Eloy Fernandez-Cusimamani[a]

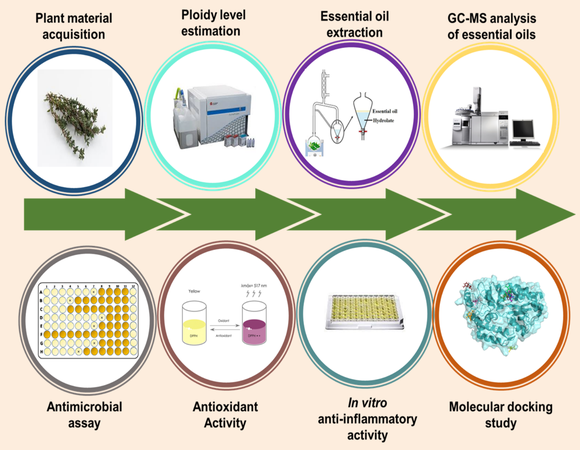
[a] Czech University of Life Sciences, Fac. of Tropical AgriSciences / Dept. of Crop Sciences and Agroforestry, Czech Republic

[b] Czech University of Life Sciences, Fac. of Agrobiology, Food and Natural Resources / Dept. of Agroecology and Plant Production, Czech Republic

Introduction

Polyploidisation has emerged as a promising tool in plant breeding and crop management to generate novel varieties. *Thymus vulgaris* L., an aromatic herb belonging to the Lamiaceae family has immense antimicrobial, antioxidant, and anti-inflammatory properties [1]. However, their essential oil yield and tolerance to stress are naturally low. *In vitro* polyploidization is being widely utilized to increase essential oil yield and induce enhanced biochemical, morphological, and biological properties. The study aims to extract essential oil from induced tetraploid thyme plants and evaluate how polyploidization affects its chemical composition and biological activities. The findings depict that polyploidization enhances secondary metabolite production, thereby improving the biological activities of thyme, which is valuable for the pharmaceutical, cosmetic, and food industries [2, 3].

Methodology



Discussion

In this study, induced polyploidization in *T. vulgaris* increased essential oil yield (1.2% in tetraploids vs. 0.85% in diploids) and altered phytochemical profiles, with more thymol and γ-terpinene but less p-cymene. The tetraploid oil showed enhanced antimicrobial and antioxidant activities, likely due to higher thymol content. Molecular docking revealed thymol's strong binding affinity to bacterial, antioxidant, and inflammatory proteins, suggests it as a key contributor to the increased biological activity.

Conclusion

- 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 the polyploid T. vulgaris L. can be commercially valuable, especially in the pharmaceutical and food industries

References

[1] Salehi, B., Abu-Darwish, M. S., Tarawneh, A. H., Cabral, C., Gadetskaya, A. V., Salgueiro, L., ... & del Mar Contreras, M. (2019). Thymus spp. plants-Food applications and phytopharmacy properties. *Trends in Food Science & Technology*, 85, 287-306.

[2] 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.

[3] 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.

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Results

1

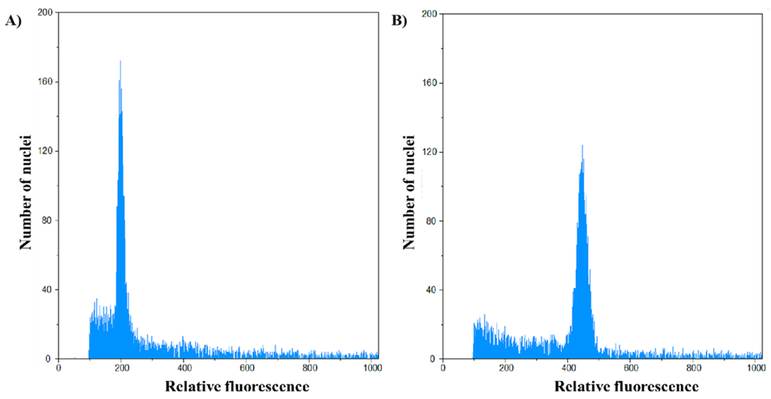

Fig 1: Flowcytometric analysis of *T. vulgaris* (**a**) histogram of relative DNA content of control diploid and (**b**) tetraploid plant.

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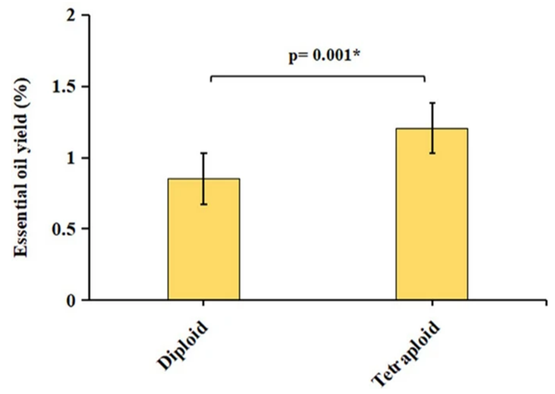

Fig 2: Essential oil yield in control diploid and induced polyploid of *T. vulgaris* "*" expressed a significant difference (Tukey HSD Test, $p < 0.05$)

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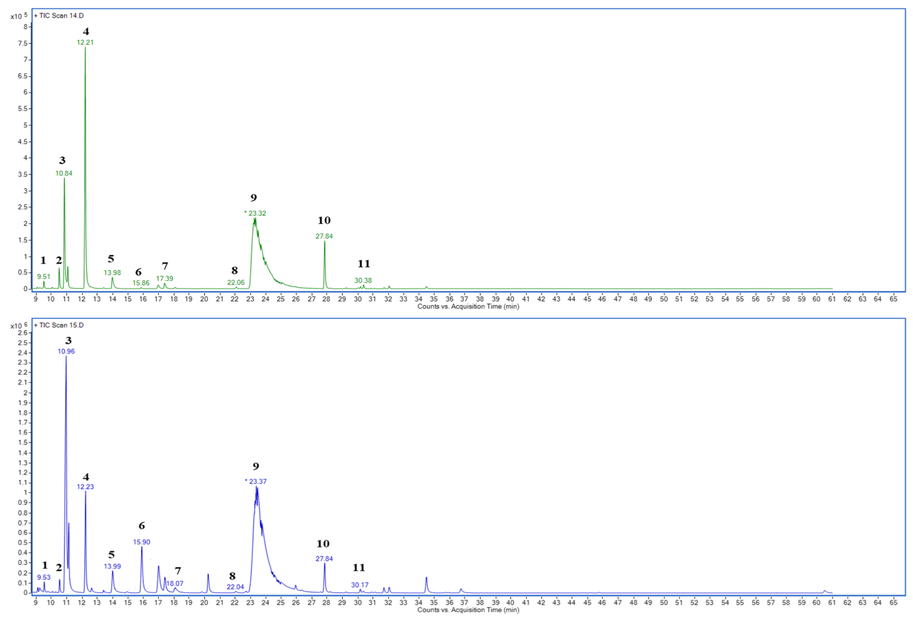


Fig 3: GC-MS chromatograms of *T. vulgaris* essential oil obtained from a) tetraploid and b) diploid genotype. Peak number and compound names: 1. Mycrene, 2. 4-carene, 3. p-cymene, 4. γ-Terpinene, 5. Linalool, 6. d-camphor, 7. 4-Terpineol, 8. Borneol, 9. Thymol, 10. Caryophyllene, 11. D-Germacrene.

Table 1. Major compounds of diploid (Control) and polyploid genotype of *T. vulgaris*.

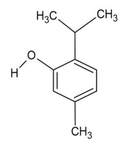
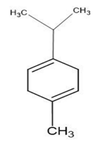
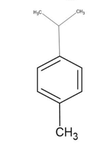
Compound name	Chemical structure	Content [%]	
		Diploid	Tetraploid
Thymol		50.65	53.50
γ-Terpinene		5.55	21.81
p-Cymene		20.40	7.85

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Table 2. *In vitro* growth-inhibitory effect of *T. vulgaris* essential oils (control and tetraploid) in liquid and vapour phases against respiratory infection bacteria.

Essential Oil	*Haemophilus influenzae*		*Staphylococcus aureus*		*Streptococcus pneumoniae*		*Streptococcus pyogenes*	
	Agar (μg/cm^3)	Broth (μg/mL)	Agar (μg/cm^3)	Broth (μg/mL)	Agar (μg/cm^3)	Broth (μg/mL)	Agar (μg/cm^3)	Broth (μg/mL)
Diploid	>256	256	>256	1024	256	512	256	1024
Tetraploid	>256	128	256	512	256	512	256	512
Positive antibiotic control								
Amoxicillin	NT	NT	NT	NT	>2	>2	NT	NT
Ampicillin	>2	0.25	>2	2	NT	NT	>2	2

5

Table 3. Antioxidant activity of *T. vulgaris* diploid and polyploid essential oils

Plant sample	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	-

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Table 4. *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

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Table 5. 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

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.

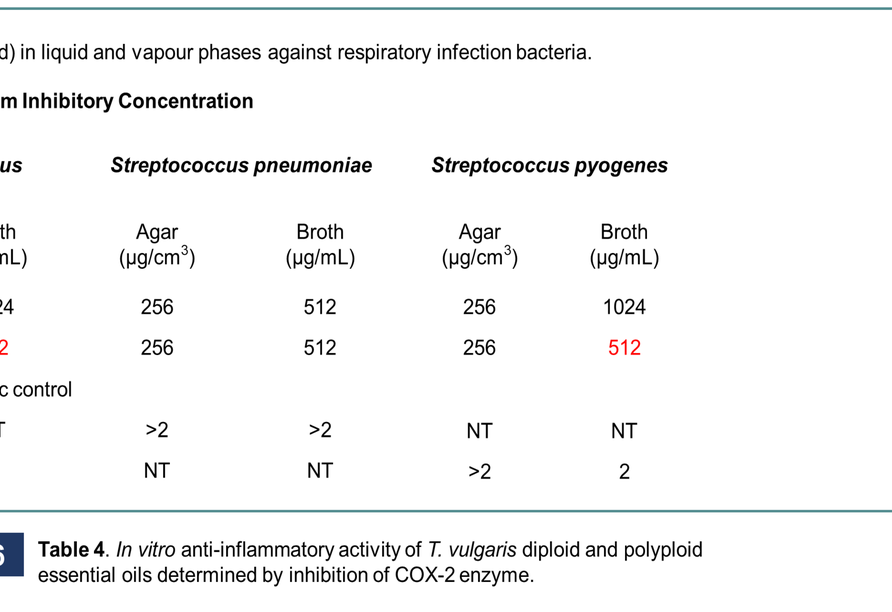

Fig 4: Interactions and docked 3D structures of (A) thymol with 1CX2, (B) thymol with 1KZN, (C) thymol with1HCK

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