Callus induction and characterization of volatile compounds in *Capparis spinosa*

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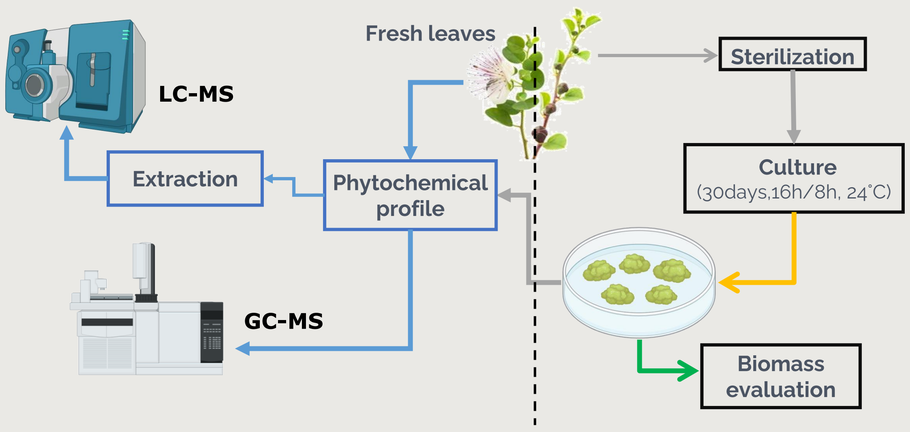
Introduction

Caper (*Capparis spinosa L.*) is a well-established medicinal plant in the Mediterranean region, renowned for its medicinal, pharmacological and culinary properties. Several bioactive phytochemical compounds have been isolated and identified from the various parts of this plant, making it a valuable source of molecules for the pharmaceutical industry (Mohaddab et al., 2024). Plant metabolites are also increasingly used in agronomy, due to their beneficial properties, such as insecticidal and herbicidal activity. However, the increasing use of these metabolites makes it necessary to optimize their production. The integration of biotechnology, in particular *in vitro* culture systems, offers economic and environmental advantages for the sustainable production of these compounds (Mohaddab et., 2022). Callus culture is an effective method for producing biologically active molecules from medicinal plants (Efferth, 2009). Although the production of volatile compounds from *C. spinosa* using callus culture has not yet been studied, this approach could reduce the need to harvest all the plant material, while offering great potential for ensuring the sustainable production of these secondary metabolites.

Methods

1. Induction and secondary metabolite analysis of callus



Induction in *C. spinosa* leaves was performed using MS medium. After thorough disinfection, the leaves were treated with various hormonal combinations: [BAP; ANA], [BAP; 2,4-D], [KIN; ANA], and [KIN; 2,4-D].

We utilized a total of 1,500 explants. For the evaluation of callus induction, and the identification of secondary metabolites, we employed a dual approach, using both GC-MS for volatile components and LC-MS for polyphenols.

3. Emission profiles of volatiles organic compounds

This study examined the response of callus cultures under different experimental conditions and identified volatile metabolites using the GC-MS. The effect of different combinations of PGRs on VOC profiles was analyzed, revealing 72 compounds, classified into terpenes, aldehydes, sulfur compounds, alcohols, ketones, esters, and carboxylic acids. In comparison, fresh *C. spinosa* leaves contained only 18 VOCs, whereas 54 were detected in callus cultures.

PCA (Figures 3a and 3b) demonstrated a significant influence of PGRs treatments on VOCs profiles, with PC1, PC2, and PC3 collectively explaining 47.2% of the total variance. The BAP2,4-D treatment formed a distinct metabolic cluster, while BAPANA and KINANA exhibited similar profiles. In contrast, the KIN2,4-D treatment showed no specific VOCs trend.

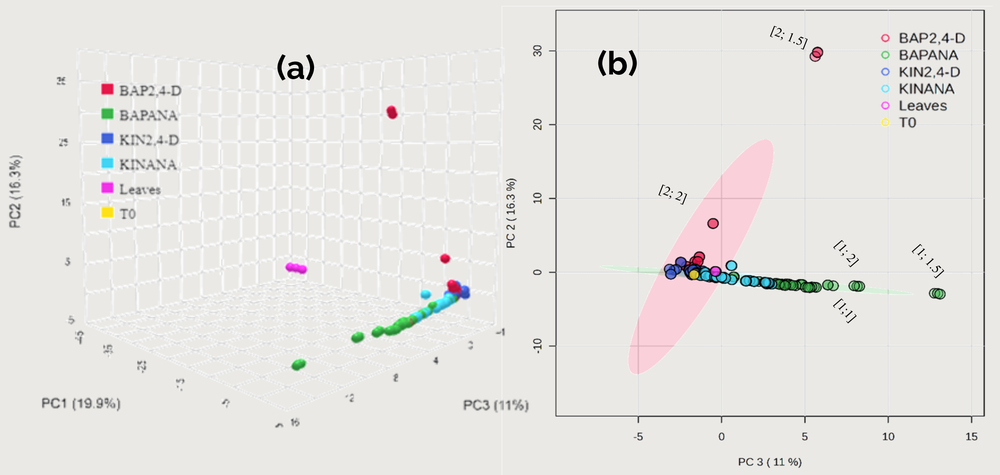


Figure.3. PCA plot comparing the effects of different hormone combinations on the biosynthesis of volatile compounds in callus culture. (a) Three-dimensional PCA plot; (b) Scores plot from the PCA analysis. Red: BAP2,4-D ; green: BAPANA; dark blue: KIN2,4-D; sky blue: KINANA; pink: fresh leaves; yellow: control culture of fresh leaves without hormones.

Results

2. Effects of phytohormones on callus induction, fresh weight gain and dry weight

These results (figure 1a and 1b) highlight the central role of 2,4-D in callus induction and growth, as shown by its significant positive correlation with fresh weight gain (FWG) and dry weight (DW). In contrast, ANA showed a significant negative effect, suggesting a potential inhibition of its action on callus formation. Furthermore, BAP does not appear to have a direct influence on biomass accumulation.

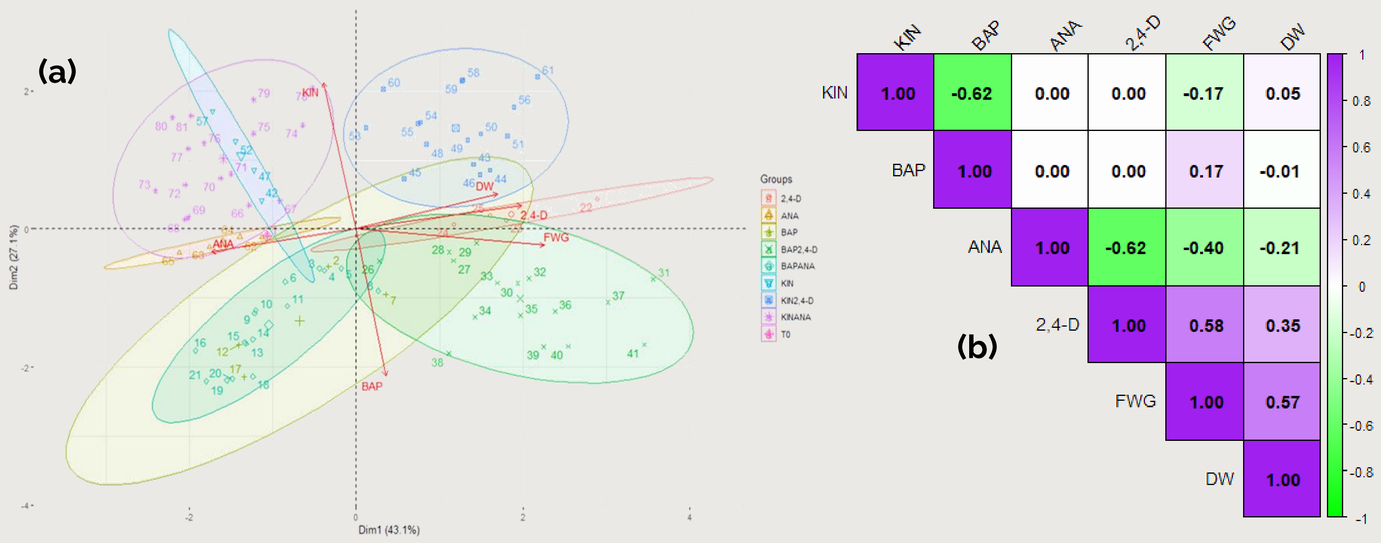


Figure.1. Multivariate Analysis of plant growth regulators (PGRs) effects on Callus Induction in *C. spinosa*. **(a)**: Principal Component Analysis (PCA) and **(b)**: Correlation Matrix. BAP : 6-benzylaminopurine; KIN: kinetin ; ANA : 1-naphthaleneacetic acid; 2,4-D: 2,4-Dichlorophenoxyacetic acid; T0: without hormone.

The analysis highlights non-linear interactions between plant growth regulators (PGRs) in *C. spinosa* callus (figure.2.). Figures A and B show that the combination of BAP and 2,4-D produces an optimal response around 1.5-2 mg/L of 2,4-D and 0.5-1 mg/L of BAP, favoring maximum callus growth. In contrast, figures C and D reveal an antagonistic effect of ANA when combined with BAP. Furthermore, figures E and F show that KIN has a moderate impact when combined with 2,4-D, with growth mainly stimulated by 2,4-D at optimal doses (1.5-2 mg/L). Finally, figure G and H indicate that high concentrations of ANA (>1.5 mg/L) have a negative effect on biomass, while KIN alone does not appear to significantly influence callus formation.

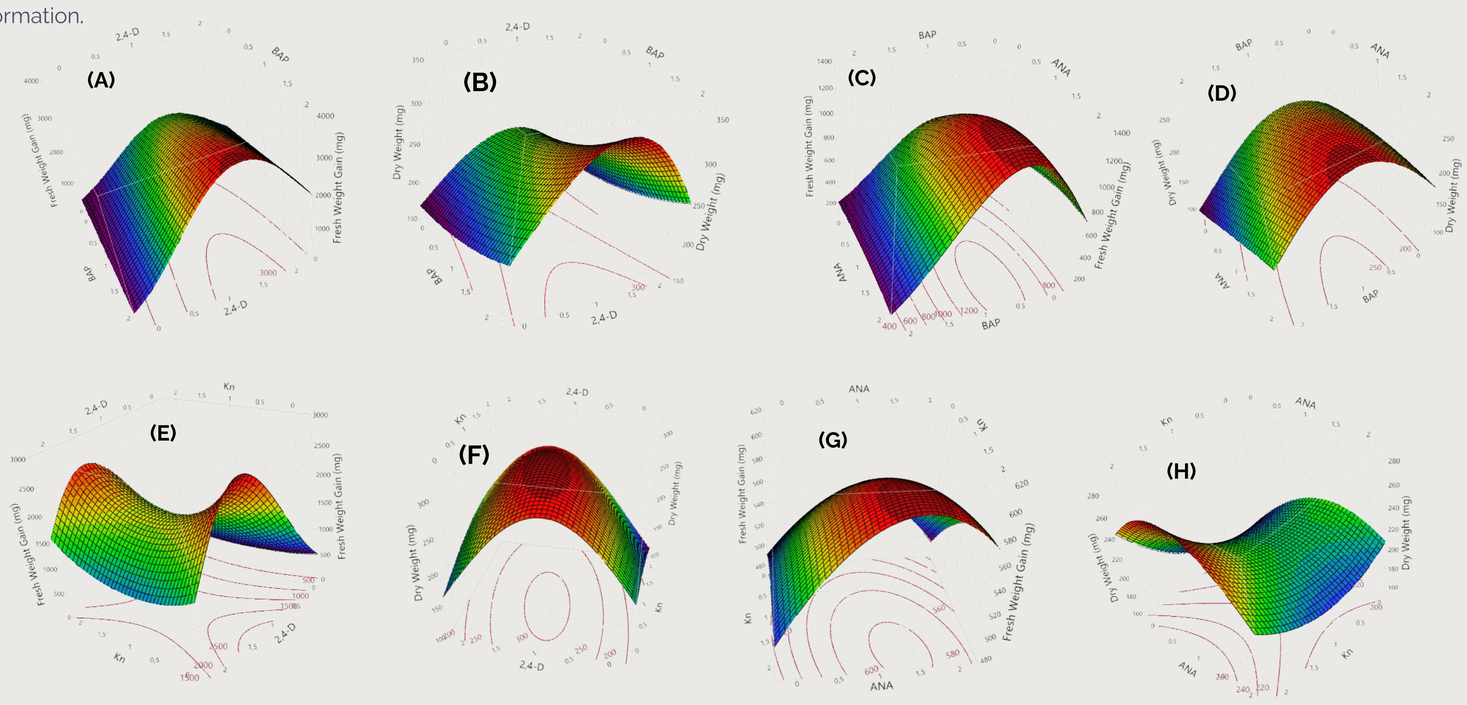


Figure.2. Three-dimensional (3D) response surface plots generated by JMP to evaluate the interactions between varying concentrations of PGRs. **(A, B)**: Combined effect of BAP and 2,4-D on callus FWG and DW; **(C, D)**: Combined effect of BAP and ANA on FWG and DW of callus; **(E, F)**: Combined effect of 2,4-D and KIN on FWG and DW of callus; **(G, H)**: Combined effect of KIN and ANA on FWG and DW of callus.

Correlation analysis (Table 1) identified key VOCs responsible for metabolic differences. PC1 was mainly associated with carotenoid degradation, PC2 with terpenoid accumulation under BAP2,4-D treatment, and PC3 with aldehyde and terpene biosynthesis. Specific metabolites, such as Safranal and β-myrcene, were exclusively detected in callus cultures but were absent in fresh leaves.

Table 1: Top 15 contributions to PC1, PC2, and PC3, with highly significant correlations ($p < 0.001$).

PC1		PC2		PC3	
VOC considered	**Correlation**	**VOC considered**	**Correlation**	**VOC considered**	**Correlation**
Dihydroactinidiolide	0.99	(Z)-Menthone	0.97	Methanethiol	0.77
Verticillol	0.99	(Z)-Rose oxide	0.97	3-Methylbutanal	0.77
(E)-2-Hexenal	0.99	Citronellol	0.96	Methyl isovalerate	0.76
Methyl benzoate	0.99	Benzaldehyde	0.95	(Z)-3-Hexen-1-ol	0.65
(Z, E)-2,4-Heptadienal	0.99	(E)-Rose oxide	0.95	Pentanal	0.64
(E)-Geranyl acetone	0.99	Menthol	0.95	Hexanal	0.64
(E, E)-2,4-Heptadienal	0.99	α-Agarofuran	0.94	Heptanal	0.63
Dihydroedulan I	0.99	Isomenthone	0.94	3-Heptanol	0.62
Butyl isothiocyanate	0.99	Citronellyl formate	0.92	1-Octen-3-ol	0.61
Ethyl benzoate	0.99	(E)-Linalool oxide	0.91	6-methyl-5-Hepten-2-one	0.57
Dihydroedulan II	0.98	2-Isopropyl-5-methyl-3-cyclohexen-1-one	0.91	Dimethyl sulfide	0.54
Methyl isothiocyanate	0.95	Benzene acetaldehyde	0.78	D-Carvone	0.48
(E)-β-Ionone	0.94	Safranal	0.52	(Z)-2-Hexen-1-ol	0.45
β-Cyclocitral	0.89	Nonanal	0.44	Eucalyptol	0.45
6-methyl-5-Hepten-2-one	0.65	Dimethyl trisulfide	0.41	2-Methylbutanal	0.43

Conclusion

This study examines the impact of hormone treatments on VOCs production in *C. spinosa* calli, revealing greater molecular diversity in callus cultures than in fresh leaves. This suggests that *in vitro* culture can optimize the production of valuable compounds. The study also aims to identify non-volatile components in order to understand the relationship between phytohormones and biosynthetic pathways. It lays the foundations for the sustainable and controlled production of secondary metabolites that can be used in pharmaceuticals and agronomy. Future research should focus on biosynthetic and epigenetic mechanisms, while machine learning and modelling could optimize the large-scale production of these molecules.

References

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