Plant Cell, Tissue and Organ Culture (PCTOC) (2026) 165:73
https://doi.org/10.1007/s11240-026-03493-0

RESEARCH

Biotechnological production of secondary metabolites in *Capparis spinosa* L. through in vitro culture callus induction

Marouane Mohaddab[1,2] · Younes EL Goumi[3] · Manon Genva[1] · Louise Vilain[1] · Clément Burgeon[1] · Mouna Belkessam[1,4] · Malika Fakiri[2] · Marie-Laure Fauconnier[1]

Received: 13 February 2026 / Accepted: 18 May 2026

Abstract

Capparis spinosa L. is a Mediterranean medicinal and aromatic species valued for its flavonoids, glucosinolates, and volatile terpenoids. However, increasing exploitation raises concerns regarding sustainable utilization. An in vitro callus culture system was developed to optimize biomass production and secondary metabolite accumulation. Leaf explants were cultured on Murashige and Skoog (MS) medium supplemented with different combinations of cytokinins (6-benzylaminopurine, BAP; kinetin, KIN) and auxins (2,4-dichlorophenoxyacetic acid, 2,4-D; naphthaleneacetic acid, NAA). Biomass accumulation was evaluated through fresh and dry weight gain. Volatile compounds were analyzed by HS-SPME-GC-MS, whereas non-volatile metabolites were characterized using UPLC-QTOF-MS/MS and semi-quantified by UPLC-TQ-MS/MS. Response surface methodology identified optimal hormonal conditions for callus biomass production after 30 days, corresponding to BAP (1.5-2.0 mg/L) combined with 2,4-D (0.5–1.5 mg/L), yielding fresh and dry weight gains exceeding 3000 mg and 300 mg, respectively. Volatile profiling revealed terpenes and sulfur-containing compounds, with 2,4-D treatments inducing safranal, (E)-rose oxide, and menthol, not detected in leaf tissues. NAA treatments mainly influenced relative volatile abundance. Non-volatile analysis indicated enhanced accumulation of glycosylated flavonoids, including rutin, kaempferol-3-*O*-rutinoside, and quercetin-3-*O*-glucoside, under KIN/2,4-D or NAA conditions, while BAP/2,4-D favored indolic and phenolic derivatives. These results demonstrate that hormonal balance significantly influences biomass production and metabolic profiles in *C. spinosa* callus cultures, highlighting their potential for sustainable phytochemical production.

Key message

PGRs synergistically maximize biomass and secondary metabolite production in *Capparis spinosa* L. in vitro cultures, offering a sustainable biotechnological platform for the production of flavonoids and novel terpenoids.

Keywords *Capparis spinosa* L. · Callus induction · Plant growth regulators · Secondary metabolites · Auxins · Cytokinins

Introduction

Capparis spinosa L., commonly known as the caper bush, is a perennial Mediterranean plant of significant medicinal, culinary, and socio-economic importance (Kdimy et al. 2022). Various organs of *C. spinosa* contain a wide range of bioactive compounds, including flavonoids, phenolic acids, volatile organic compounds (VOCs), and glucosinolates such as glucobrassicin, neo-glucobrassicin, and hydroxyglucobrassicin, which contribute to its diverse biological activities (Yousefi et al. 2025; Hazrati et al. 2025). Due to its high medicinal and economic value, *C. spinosa* is largely collected from wild populations, with limited cultivation practices in Mediterranean regions (Tlili et al. 2017). Overexploitation threatens the sustainability of the species and limits its industrial potential. Moreover, conventional

Communicated by Sergio Ochatt

Extended author information available on the last page of the article

Published online: 29 May 2026

propagation methods, including seed germination after scarification or hormonal treatment, are often slow and inefficient (Sozzi and Chiesa 1995; Labbafi et al. 2018; Nowruzian and Aalami 2023).

In this regard, in vitro micropropagation has been proposed as an alternative to reduce pressure on natural populations, yet its effectiveness remains limited for certain plant species (Sottile et al. 2021; Koufan et al. 2022). Among available plant biotechnology approaches, callus culture is a key technique in plant biotechnology, enabling differentiated cells to revert to an undifferentiated, proliferative state and serving as a basis for organogenesis or somatic embryogenesis (Fauconnier et al. 1993; Mohaddab et al. 2022; Pasternak and Steinmacher 2024). This plasticity is regulated by exogenous application of plant growth regulators (PGRs), which coordinate cell division, differentiation, and the regulation of key metabolic pathways (Zluhan-Martínez et al. 2021). Given their central role in controlling both morphogenesis and secondary metabolism, PGRs constitute critical determinants for optimizing callogenesis and in vitro metabolite accumulation (Bull and Michelmore 2022; Zhiponova et al. 2024). Consequently, callus cultures have become widely recognized as sustainable biotechnological platforms for biomass production and the synthesis of high-value secondary metabolites for pharmaceutical, cosmetic, and food applications, while simultaneously preserving donor plants (Abdulhafiz et al. 2022; Ozyigit et al. 2023).

The successful induction and proliferation of callus tissues largely depend on the type and balance of applied PGRs. The application of cytokinins, such as 6-benzylaminopurine (BAP) and kinetin (KIN), and auxins, such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 1-naphthaleneacetic acid (NAA), have been extensively reported to promote callus formation and stimulate the biosynthesis of bioactive metabolites in diverse plant species (Mastuti et al. 2017; Firoozi et al. 2019; Chacón et al. 2023). Callus cultures may also synthesize metabolites absent from the original plant, including flavonoids, phenolic acids, and terpenoids, highlighting the potential of in vitro systems as sources of novel bioactive compounds (Banaev et al. 2025). Despite the recognized pharmacological significance of *C. spinosa*, relatively few studies have systematically explored its in vitro callus culture and associated secondary metabolite production. The investigations by Wang et al. (2007) and Yin et al. (2014) established that leaf-derived and suspension cell cultures of *C. spinosa* constitute viable systems for biomass accumulation and volatile oil production. Similarly, Al-Safadi and Elias (2011) reported successful callus induction from leaf and shoot explants on MS medium supplemented with BAP and NAA, while Kumari et al. (2015) demonstrated efficient plantlet regeneration through callus-mediated morphogenesis. More recently, Duran and Issah (2022) showed that BAP combined with NAA promoted both callus formation and phenolic compound accumulation, including rutin, quercetin, and chlorogenic acid, in leaf-derived callus cultures. Furthermore, supplementation with the synthetic strigolactone GR24 in combination with NAA and BAP was found to further stimulate rutin and quercetin biosynthesis in callus tissues (Duran and Issah 2022). The relevance of these findings is reinforced by the considerable pharmacological potential of the metabolites produced. Among these, rutin has attracted significant attention due to its antidiabetic, cytoprotective, vasoprotective, anticancer, neuroprotective, and cardioprotective activities (Ganeshpurkar and Saluja 2017; Ullah et al. 2020). Other metabolites, such as isothiocyanates and terpenoids, exhibit anticancer and anti-inflammatory properties and have been commercially exploited as active pharmaceutical ingredients (Brown and Hampton 2011; Kamran et al. 2022; Ludwiczuk et al. 2017). Despite these advances, no study has yet applied a systematic hormonal design based on Response Surface Methodology combined with integrative metabolomic profiling to simultaneously optimize callus biomass and secondary metabolite production in *C. spinosa*.

In this context, the present study employed a systematic hormonal design and integrative metabolomic profiling to maximize callus growth and secondary metabolite production in *C. spinosa*. Specifically, response surface methodology (RSM) was applied to determine the most effective combination of cytokinins (BAP or KIN) and auxins (2,4-D or NAA) for maximum callus growth. In parallel, volatile compounds were analyzed using HS-SPME-GC-MS, whereas non-volatile metabolites were profiled through UPLC-QToF-MS/MS and semi-quantified using UPLC-TQ-MS/MS. This integrative approach provides a unique platform for efficient in vitro propagation and targeted production of bioactive compounds from *C. spinosa.*

Materials and methods

Plant material and disinfection

Fresh leaves of *Capparis spinosa* L. were collected in April 2022 from mature wild plants at the fruiting stage, naturally occurring in the Taounate region (Morocco; latitude: 34°26'31.187" N, longitude: 4°42'11.97" W, altitude: 435 m). Leaves were harvested, sterilized, and cultured on the same day (Fig. 1). The disinfection process consisted of sequential washing under running tap water for 10 min, immersion in 70% ethanol for 2 min, followed by four rinses with sterile distilled water (3 min each). Subsequently, leaves were treated with a 4.5% sodium hypochlorite

Fig. 1 Wild *Capparis spinosa* L. plants collected from the Taounate region, Morocco. (**A**) General view of the natural habitat. (**B**) Mature plant at the fruiting stage, showing the leaves used as the explant source. Yellow arrow: pollinated flowers. Red arrow: fruits

Table 1 Hormonal matrix combinations used for callus induction from *Capparis spinosa* leaf explants

PGRs	BAP or KIN (mg/L)				
2,4-D or NAA (mg/L)	[0; 0]	[0;0.5]	[0; 1]	[0; 1.5]	[0; 2]
	[0.5; 0]	[0.5; 0.5]	[0.5; 1]	[0.5; 1.5]	[0.5; 2]
	[1; 0]	[1; 0.5]	[1; 1]	[1; 1.5]	[1; 2]
	[1.5; 0]	[1.5; 0.5]	[1.5; 1]	[1.5; 1.5]	[1.5; 2]
	[2; 0]	[2; 0.5]	[2; 1]	[2; 1.5]	[2; 2]

BAP: 6-benzylaminopurine, KIN: kinetin, 2,4-D: 2,4-dichlorophenoxyacetic, NAA: 1-naphthaleneacetic acid

solution containing 0.3% Tween-20 for 15 min, and finally rinsed several times with sterile distilled water.

Culture media and growth conditions

Callus induction was performed on Murashige and Skoog (MS) medium (Duchefa, Netherlands) supplemented with 30 g/L sucrose, 8 g/L agar, and MES buffer (M0255, Duchefa). The pH was adjusted to 5.7±0.1 before autoclaving at 121 °C for 15 min under 1 bar pressure. The experimental design (Table 1) was based on RSM, using a full matrix combining five concentrations (0, 0.5, 1, 1.5, and 2 mg/L) for the addition of auxins (2,4-D and NAA) and cytokinins (BAP and KIN). Each combination was tested in triplicate, and callus growth was assessed for each hormone interaction. Leaf explants (1-cm diameter discs) were placed on 20 mL of medium per Petri dish (5 explants per dish). A total of 1500 explants were used. Cultures were incubated in a phytotron (light intensity: 1600 W, Sylvania fluorescent tubes) under a 16 h light: 8 h dark photoperiod at 24±2 °C for 30 days.

Callus biomass assessment

Callus Fresh Weight Gain (FWG) was determined after 30 days of culture by weighing the callus immediately after removal from the medium. Dry Weight Gain (DWG) was determined after oven-drying the callus at 30 °C until a constant weight was achieved. FWG was calculated as follows:

$$\mathrm{FWG} = \text{Final fresh weight} - \text{Initial weight of explant}$$

HS-SPME-GC-MS profiling of volatile organic compounds in callus cultures

Volatile organic components were analyzed using headspace solid-phase microextraction (HS-SPME) coupled with a GC-MS system. Analyses were performed in triplicate on calli for each hormone combination treatment (Table 1), as well as on fresh leaves for comparison. For each analysis, approximately 150 mg of callus or fresh leaf tissue were homogenized using a mortar and pestle and immediately transferred into 2 mL glass vials without the addition of any solvent. The vials were promptly sealed to minimize volatilization losses before analysis. Each sample was incubated for 30 min at 30 °C under shaking (Gerstel, Mülheim an der Ruhr, Germany) at 250 rpm, with a 10 s on/1 s off agitation cycle. VOCs were collected using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 µm) SPME fiber (Supelco, Darmstadt, Germany) exposed to the headspace for 5 min. The injection was performed in splitless mode at 260 °C. GC-MS analysis was conducted using a 7890B-5977CB GC-MS (Agilent Technologies, Santa Clara, CA, USA) equipped with a capillary column HP-5 MS (30 m × 250 µm × 0,25 µm, Agilent Technologies, Santa Clara, CA, USA). Helium was used as a carrier gas at

a flow rate of 1.2 mL/min. The oven temperature program ran as follows: 40 °C for 3 min, followed by a 3 °C min^{-1} increase up to 140 °C, then 10 °C min^{-1} up to 300 °C, temperature held for 5 min. The mass spectrometer was calibrated to maintain an ion source temperature of 230 °C and a quadrupole temperature of 150 °C. The SCAN mode was employed with a scan range of 30 to 400 m/z. The obtained spectra were compared to the NIST 17 reference database for compound identification. Additionally, experimental retention indices (RI) were determined by injecting a series of n-alkanes ranging from C_7 to C_{30} (Sigma Aldrich, Darmstadt, Germany) using the same chromatographic parameters as described above. Experimental RI values were compared with the literature RIs for further identification of the compounds (Burgeon et al. 2021).

LC-MS/MS-based identification and semi-quantification of metabolites in callus cultures

For each treatment, approximately 150 mg of dry callus were placed in a Sorvall centrifuge tube and extracted with 2 mL of MeOH/H_2O (80:20, v/v), containing 5 µL of an internal standard solution. The internal standard consisted of flavone (molecular weight = 220 g/mol) dissolved in DMSO at a concentration of 2 mg/mL. The mixtures were sonicated in an ultrasonic bath for 20 min and centrifuged at 3000 × g for 10 min. The resulting supernatants were filtered through 0.22 µm syringe filters prior to LC-MS/MS analysis.

Metabolite profiling was performed using LC-QTOF for qualitative identification and LC-TQ for semi-quantification as described by Belkessam et al. (2025), with the addition of 4-methoxyglucobrassicin (MRM transition: 477→97). Chromatographic separation was achieved on an Acquity® BEH C18 column (2.1 × 100 mm, 1.8 µm particle size; Waters Co., Milford, MA, USA) thermostated at 30 °C, with an injection volume of 10 µL. The mobile phase consisted of milliQ water with 0.1% formic acid (solvent A) and MS-grade acetonitrile with 0.1% formic acid (solvent B), delivered at a flow rate of 0.450 mL/min. The elution gradient was as follows: 1% B for 1 min, a linear ramp to 100% B over 11 min, an isocratic wash at 100% B for 1.5 min, and re-equilibration at 1% B for 2.5 min (total run time: 16 min). Data were processed using MassHunter® Workstation software (Agilent Technologies). Metabolite content was determined according to the following equation:

$$\begin{aligned} Molecule\,content(A.U./mg\,DW) \\ = (MRM\,peak\,area) \\ \div (MRM\,flavone\,peak\,area \\ \times Sample\,dry\,weight) \end{aligned}$$

Results were expressed in arbitrary units (A.U.) per mg of dry weight.

Statistical analysis

Response Surface Methodology (RSM) was applied to model FWG and DWG responses, allowing the identification of the most favorable hormone concentrations for callus growth. All experiments were performed in triplicate, and the data were standardized using Z-scores before analysis. Data were analyzed by the one-way analysis of variance (ANOVA). When means differed significantly, pairwise comparisons were conducted using Bonferroni-adjusted *p*-values ($p < 0.05$). RSM and ANOVA were performed using JMP Pro 17 (SAS Institute, USA). Principal Component Analysis (PCA) was used to explore variability in VOC profiles across treatments based on peak areas, using MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/). A heatmap was generated in R using the "pheatmap" and "ggplot2" packages, with hierarchical clustering applied to group samples and metabolites based on their profile. Additionally, a bubble plot was constructed in Excel to visualize metabolite concentrations, where bubble size corresponds to the *Z*-score, allowing intuitive comparison of relative abundances among treatments.

Results

Effects of BAP, KIN, 2,4-D, and NAA on callus proliferation and biomass accumulation

The effects of PGRs on callus growth and biomass accumulation were evaluated based on FWG and DWG using four hormone combinations: [BAP; NAA], [BAP; 2,4-D], [KIN; 2,4-D], and [KIN; NAA]. Analysis of variance (Supplementary Table 1) showed that combinations containing 2,4-D or BAP had highly significant effects ($p < 0.001$) on both FWG and DWG. These results confirm that callus growth is strongly dependent on the specific PGR combination (Fig. 2), with 2,4-D primarily influencing FWG, while cytokinins (BAP, KIN) appear to exert a comparatively greater influence on DWG. In contrast, the [KIN; NAA] combination was the least effective, showing no significant effect on FWG ($p > 0.05$) and only a linear effect of KIN on DWG ($p < 0.05$). To further elucidate PGRs interactions, three-dimensional RSM plots were generated to systematically assess the combined effects of varying PGR concentrations on callus development (Fig. 3). The combination of BAP and 2,4-D consistently produced the highest FWG (> 3000 mg) and DWG (> 300 mg) (Fig. 3A, B), corresponding to 1.5–2 mg/L BAP with 1.0–1.5 mg/L 2,4-D for FWG,

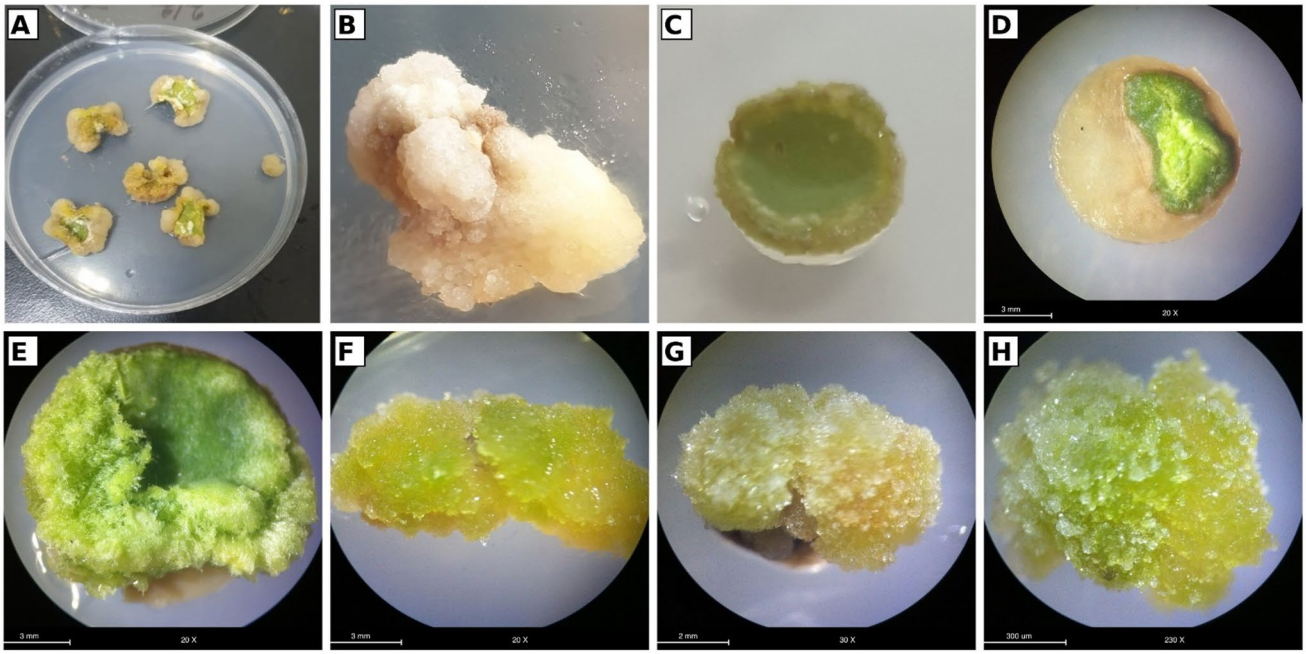

Fig. 2 Morphological observations of *Capparis spinosa* L. callus cultures after 30 days of in vitro culture on MS medium supplemented with different combinations of plant growth regulators. (**A**) Multiple calli in Petri dishes; (**B**) Compact cream-white callus; (**C**) Cup-shaped green callus; (**D** – **H**) Stereomicroscope views illustrating a friable texture and color variation ranging from cream-white to bright green depending on hormonal treatment

and 1.5–2 mg/L BAP with 0.5–1.5 mg/L 2,4-D for DWG. The close proximity of the maximum values of FWG and DWG suggests a potential cooperative interaction between the PGRs used in callus culture.

The combination of BAP and NAA (Fig. 3C and D) resulted in intermediate growth levels, with FWG below 1200 mg and DWG below 250 mg. Optimal concentrations were 0.5-1 mg/L BAP with 1–2 mg/L NAA for FWG and 0.5–1.5 mg/L BAP with 1–2 mg/L NAA for DWG. In Figs. 2, 3E and 4-D alone effectively promoted callus induction, reaching FWG maximum of 2500–3000 mg at concentrations of 0.5-2 mg/L. However, the 3D saddle-shaped response surface suggests that the observed maximum often occurs at the edges of the graph, indicating that the true FWG maximum might lie outside the tested range. The addition of low KIN concentrations (0-0.5 mg/L, Fig. 3F) significantly increased DWG, up to 300 mg, suggesting that FWG increases mainly reflect water uptake, whereas DWG increases correspond to cellular differentiation and solid biomass accumulation. Conversely, the KIN and NAA combination resulted in limited growth, despite a linear effect of KIN on DWG, no significant FWG increase was observed (Fig. 3G, H).

Predictive callogenesis profiles were generated to assess the effect of PGRs on biomass growth (Fig. 4). 2,4-D at 1 mg/L maximized callus formation in *C. spinosa* (Fig. 4A, B, E, and F). The BAP/2,4-D combination showed the highest desirability (60.91% FWG and 54% DWG), corresponding to 3471 mg FWG and 328 mg DWG. Higher 2,4-D (1.29 mg/L) concentration favored FWG, but KIN appeared to negatively affect cell proliferation, whereas low KIN (0.7 mg/L) improved DWG by enhancing biomass accumulation and cellular development. BAP/NAA and KIN/NAA combinations produced moderate or low responses, respectively. ANOVA and RSM confirmed that PGR selection is a critical determinant of callus growth, with BAP/2,4-D yielding the highest FWG and DWG.

Emission profiles of volatile organic compounds

HS-SPME-GC-MS analysis revealed that different PGR combinations markedly influenced the VOCs profile of *C. spinosa* callus. A total of 72 VOCs were identified, including 35 terpenes, 14 aldehydes, 8 sulfur compounds, 7 alcohols, 4 ketones, 3 esters, and 1 carboxylic acid (Supplementary Table 2). Terpenes and sulfur-containing compounds were the predominant classes, highlighting their major contribution to the volatile profile of these in vitro cultures.

To further analyze the data, principal component analysis (PCA) was performed to visualize trends and variations within the dataset. The results of the PCA (Fig. 5A) highlighted that principal component 1 (PC1), 2 (PC2), and 3 (PC3) explain 19.9%, 16.3%, and 11% of the total variance, respectively, accounting for a total of 47.2% of the dataset

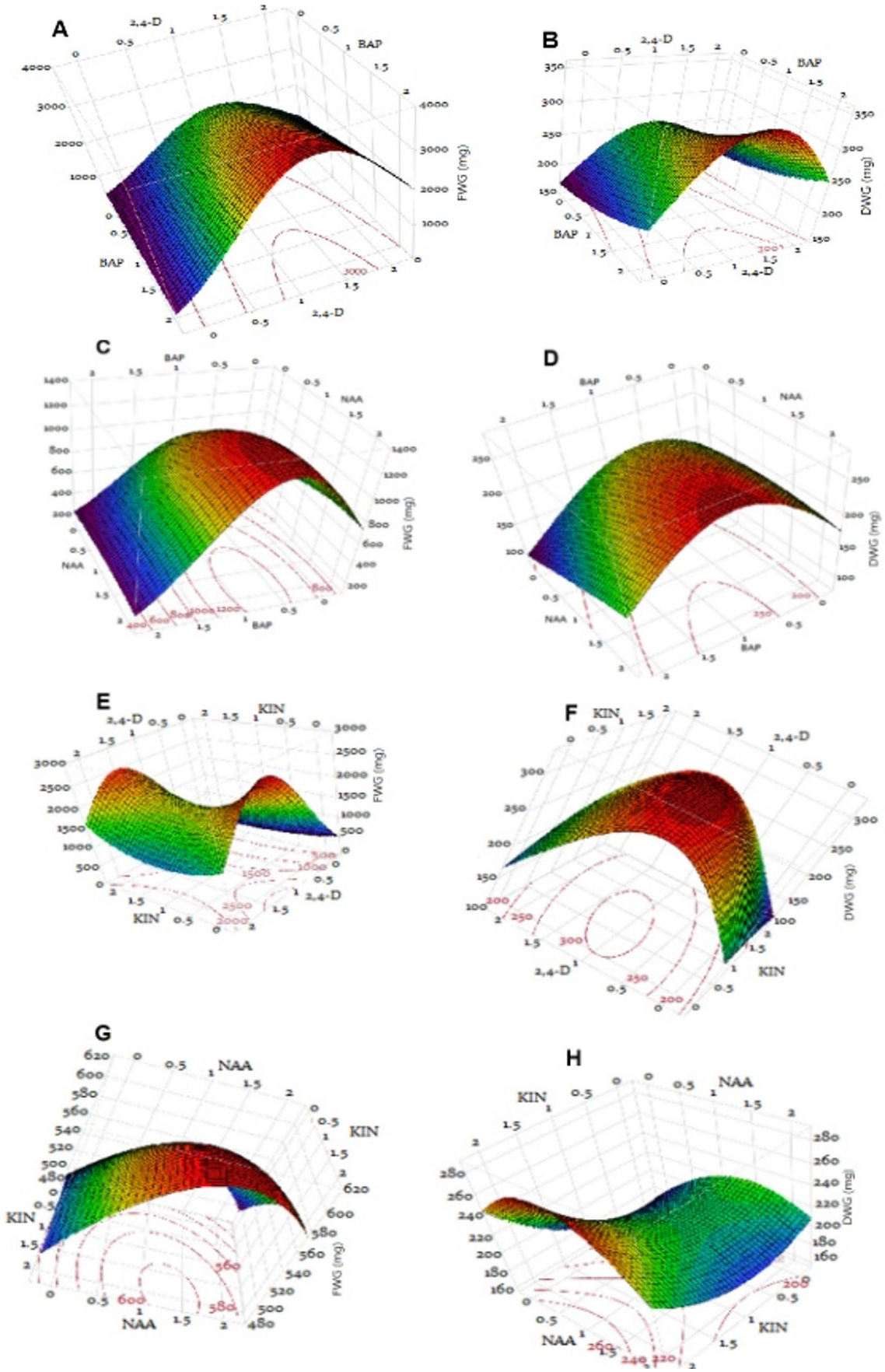
A
2,4-D
BAP
FWG (mg)
B
2,4-D
BAP
DWG (mg)
C
BAP
NAA
FWG (mg)
D
BAP
NAA
DWG (mg)
E
2,4-D
KIN
FWG (mg)
F
KIN
2,4-D
DWG (mg)
G
NAA
KIN
FWG (mg)
H
KIN
NAA
DWG (mg)

Fig. 3 Three-dimensional (3D) response surfaces generated using JMP illustrating the combined effects of different PGR concentrations on callus induction. Panels **A** and **B**: [BAP; 2,4-D]; **C** and **D**: [BAP; NAA]; **E** and **F**: [KIN; 2,4-D]; **G** and **H**: [KIN; NAA]. The surfaces depict a significant nonlinear relationship between PGR concentrations (mg/L) and callus development, measured as fresh weight gain (FWG, mg) or dry weight gain (DWG, mg). Red regions indicate the maximal response ranges. BAP: 6-Benzylaminopurine; 2,4-D: 2,4-Dichlorophenoxyacetic acid; NAA: Naphthaleneacetic acid; KIN: Kinetin

variance. The distribution of samples in the PCA (Fig. 5A) clearly shows that the application of different PGRs significantly affects the VOC composition. A clear distinction is observed between fresh *C. spinosa* leaves and callus cultures subjected to various treatments. Differences along PC2 and PC3 (Fig. 5B) further clarify these patterns and are largely associated with the nature of auxin applied (2,4-D or NAA) during callus culture. For the BAP/2,4-D combination, a gradual increase in BAP and 2,4-D concentrations altered VOC profiles, with a maximum effect observed at 2 mg/L BAP and 1.5 mg/L 2,4-D. Higher 2,4-D concentrations may limit this response, suggesting inhibitory effects and reflecting the nonlinear nature of PGR-induced metabolic regulation. For the remaining combinations (BAP/NAA, KIN/NAA, and KIN/2,4-D), samples clustered together along PC3, with the distribution influenced by cytokinin type and concetration. BAP/NAA and KIN/NAA were positioned in the positive region of PC3, with BAP/NAA exhibiting higher coordinates than KIN/NAA.

Although many VOCs correlated significantly with these principal components, only the top 15 molecules with the highest correlation coefficients were selected for further evaluation (Table 2). PC1 comprises mainly carotenoid-derived compounds, such as *β*-Cyclocitral, (*E*)-*β*-ionone, and dihydroactinidiolide, which are typically linked to both enzymatic and non-enzymatic carotenoids degradation processes (Stutz et al. 2015). PC2 was characterized by terpenoids associated with the BAP/2,4-D treatment, including (*E*)-rose oxide, menthol, and safranal, suggesting a possible hormone-induced modulation of terpene biosynthesis pathways. Notably, safranal was detected for the first time in *C. spinosa*, potentially arising from oxidative cleavage of *β*-carotene. PC3 was primarily composed of aldehydes such as 3-methylbutanal, pentanal, and hexanal, along with sulfur-containing compounds including methanethiol and dimethyl sulfide, reflecting metabolic pathways associated with oxidative stress or other stress-related processes (Zhang et al. 2023). Beyond the top 15 compounds, additional volatile metabolites such as limonene, *α*-pinene, and *p*-cymene were consistently detected across callus culture conditions, indicating a stable baseline metabolic profile. Certain metabolites, notably 3-*δ*-carene, were specific to BAP/NAA treatments, suggesting a treatment-dependent modulation of VOC composition. Treatments containing NAA produced VOC profiles resembling those of fresh leaves, with lower chemical diversity and a predominance of sulfur-containing compounds such as methyl isothiocyanate, suggesting a primarily quantitative effect on existing metabolites (Supplementary Fig. 1). In contrast, 2,4-D treatments induced notable shifts in secondary metabolism, evidenced by the appearance of terpenoids absent in fresh leaves, reflecting a qualitative effect that activates specific metabolic pathways to synthesize additional volatiles.

Overall, these results indicate that NAA, when combined with BAP or KIN, primarily modulates the relative concentrations of existing metabolites. In contrast, 2,4-D in combination with these cytokinins exerts a qualitative effect, promoting the production of novel metabolites not detected in fresh leaves or under NAA treatments, highlighting the distinct regulatory roles of these growth regulators on VOC biosynthesis.

Differential effects of cytokinin-auxin combinations on the accumulation of non-volatile secondary metabolites

Hormonal modulation plays a pivotal role in regulating the biosynthesis of secondary metabolites in *C. spinosa* callus cultures (Li et al. 2025). In this context, the BAP/2,4-D combination was selected for the analysis of non-volatile metabolites, as it exhibited the highest FWG and DWG values (Section "Effects of BAP, KIN, 2,4-D, and NAA on callus proliferation and biomass accumulation"). Additionally, the BAP/NAA, KIN/NAA, and KIN/2,4-D formulations were chosen due to the most significant contributions to the volatile compound profiles (Fig. 5B). Metabolite identification was carried out using UPLC-QTOF-MS/MS, and semi-quantification was achieved via UPLC-TQ-MS/MS in the MRM mode (Supplementary Table 3). Results were compared to control cultures grown in vitro without phytohormones and to extracts from *C. spinosa* leaves.

The metabolic profiles revealed pronounced variations in the accumulation of phenolic and flavonoid compounds depending on the cytokinin/auxin combinations applied to the in vitro cultures (Fig. 6). Eight major metabolites were detected: 4-methoxyglucobrassicin, rutin, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, quercetin-*O*-rhamnosyl-arabinopyranoside, caffeic acid, and myricetin-3-*O*-glucoside. Treatments containing BAP combined with either 2,4-D or NAA led to moderate accumulation of secondary metabolites, whereas control callus and leaves exhibited minimal levels across all compounds analyzed. These low concentrations suggest that the presence of phytohormones is required to effectively sustain secondary metabolite biosynthesis under in vitro conditions. Combinations of BAP and 2,4-D at low to moderate

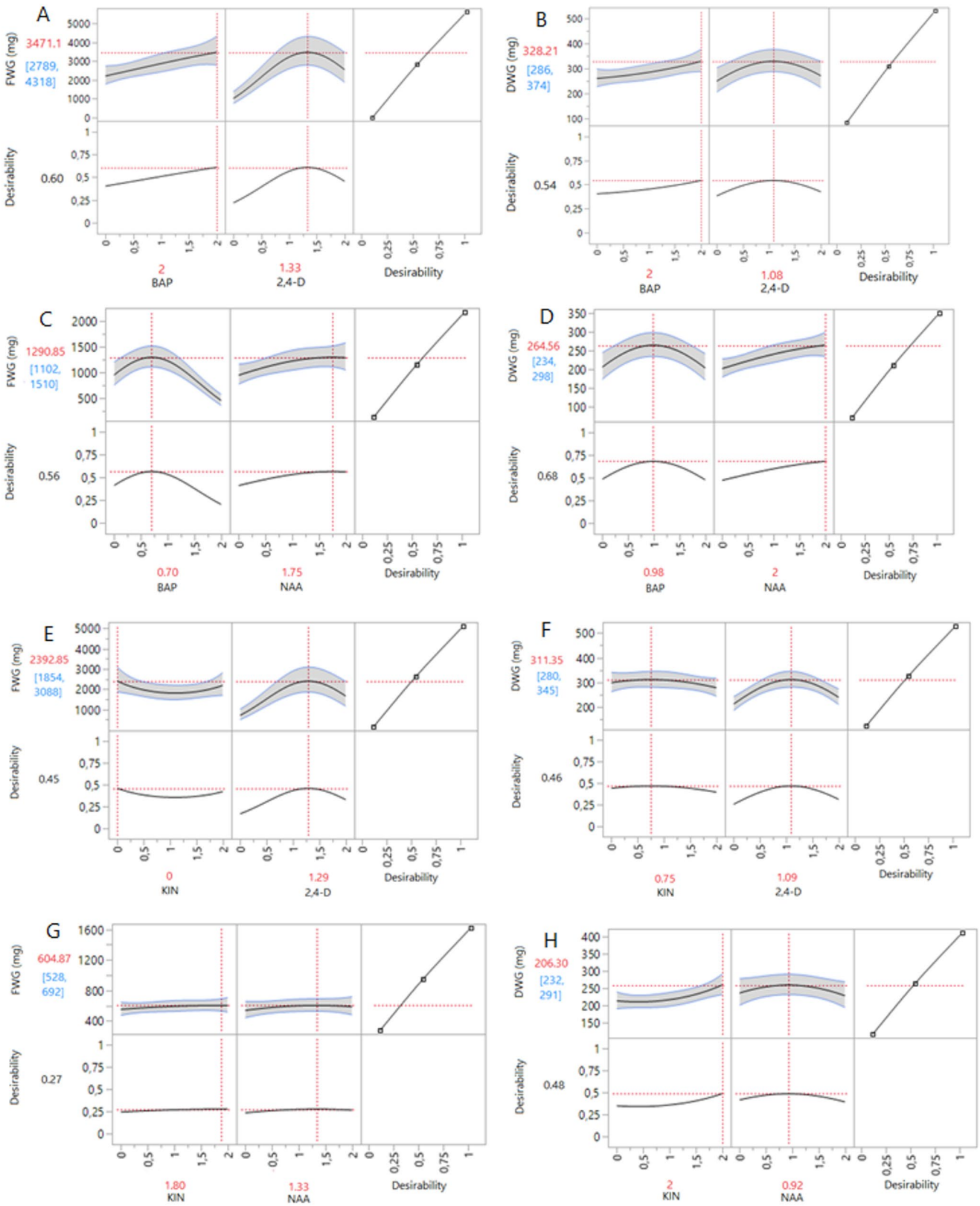
A
FWG (mg)
3471.1
[2789, 4318]
Desirability
0.60
2
BAP
1.33
2,4-D
Desirability
B
DWG (mg)
328.21
[286, 374]
Desirability
0.54
2
BAP
1.08
2,4-D
Desirability
C
FWG (mg)
1290.85
[1102, 1510]
Desirability
0.56
0.70
BAP
1.75
NAA
Desirability
D
DWG (mg)
264.56
[234, 298]
Desirability
0.68
0.98
BAP
2
NAA
Desirability
E
FWG (mg)
2392.85
[1854, 3088]
Desirability
0.45
0
KIN
1.29
2,4-D
Desirability
F
DWG (mg)
311.35
[280, 345]
Desirability
0.46
0.75
KIN
1.09
2,4-D
Desirability
G
FWG (mg)
604.87
[528, 692]
Desirability
0.27
1.80
KIN
1.33
NAA
Desirability
H
DWG (mg)
206.30
[232, 291]
Desirability
0.48
2
KIN
0.92
NAA
Desirability

Fig. 4 Prediction profiler plots generated using JMP software illustrating the effects of plant growth regulators (PGRs) on fresh weight gain (FWG) and dry weight gain (DWG). Panels **A-H** represent different treatment combinations: **A**, **C**, **E**, and **G** for FWG (mg) responses, and **B**, **D**, **F**, and **H** for DWG (mg) responses. In each plot, the X-axis represents the tested PGR combinations, while the Y-axis shows the measured response. Desirability values indicate the optimal range for each parameter. Red numbers denote the predicted response at the optimum factor levels, whereas blue numbers represent the minimum and maximum responses achievable under these conditions. Gray-shaded areas indicate confidence intervals, providing an estimate of the reliability of the predictions. The bottom plot of each panel shows the maximum desirability obtained when the optimal conditions of each factor are applied, allowing identification of the most favorable combinations for maximizing FWG and DWG. BAP: 6-Benzylaminopurine; 2,4-D: 2,4-Dichlorophenoxyacetic acid; NAA: Naphthaleneacetic acid; KIN: Kinetin

concentrations (0.5-1 mg/L) effectively enhanced the flavonoid biosynthesis pathway. Treatment with 0.5 mg/L BAP and 2,4-D resulted in a pronounced accumulation of rutin, reaching relative abundance levels approximately 2.5 and 3.2-fold higher than those observed in the hormone-free control and in the leaves of *C. spinosa*, respectively. In contrast, cultures supplemented with KIN in combination with 2,4-D or NAA exhibited the highest relative accumulation of flavonoid glycosides, particularly rutin, kaempferol-3-*O*-rutinoside, and quercetin-3-*O*-glucoside. Conversely, the production of 4-methoxyglucobrassicin responded weakly to hormonal treatment, reaching its maximum relative abundance at 1 mg/L BAP and 0.5–1.5 mg/L 2,4-D or NAA (Fig. 6). With respect to phenolic compounds, caffeic acid remained low and relatively stable regardless of the treatment, whereas myricetin-3-*O*-glucoside reached its maximum accumulation under higher concentrations of BAP and 2,4-D (1.5-2 mg/L and 1-1.5 mg/L, respectively). These trends were further corroborated by the hierarchical clustering analysis (Supplementary Fig. 2), which revealed two major metabolic clusters: one corresponding to BAP/2,4-D, associated with a higher relative accumulation of quercetin derivatives, and another corresponding to KIN/NAA, characterized by a broader diversity of glycosylated flavonols. The clustering of control and leaf samples within the same branch confirmed their metabolic similarity and highlighted the specificity of responses induced by exogenous hormonal combinations.

Discussion

Callus culture represents an effective biotechnological strategy, supporting both organogenesis and the biosynthesis of bioactive molecules (Kour et al. 2025). Moreover, the transition from callus to suspension culture provides a pathway for the industrial-scale production of secondary metabolites via bioreactors (Anuradha et al. 2025). The success of this approach relies heavily on PGRs, which play an essential role in the proliferation and differentiation of plant cells, thereby modulating specific metabolic pathways (Ikeuchi et al. 2013; Singh et al. 2025).

These findings are consistent with previous reports in *C. spinosa*, where Yin et al. (2014) demonstrated that MS medium supplemented with 3.0 mg/L BAP and 1.5 mg/L 2,4-D effectively promoted callus proliferation and biomass accumulation, while also establishing callus cultures as a promising sustainable source of volatile organic compounds. Likewise, Duran and Issah (2022) reported that BAP combined with NAA yielded a callus fresh weight of approximately 120.8 mg in leaf explants of *C. spinosa*, a value substantially lower than the fresh weight gains exceeding 3000 mg obtained in the present study, highlighting the higher efficiency of 2,4-D compared with NAA in promoting biomass accumulation in this species. Higher concentrations of 2,4-D (> 1.5 mg/L) were found to inhibit callus development, consistent with the well-documented dose-dependent inhibitory effect of supraoptimal auxin concentrations on cell proliferation (Ikeuchi et al. 2013). The enhanced performance of the BAP/2,4-D combination over other PGR combinations is further corroborated by studies in other species (Pérez-Mendoza et al. 2020; Bano et al. 2022; Lee et al. 2024; Chakrane et al. 2025). Lee et al. (2024) reported that the combination of 2.0 mg/L BAP and 0.5 mg/L 2,4-D achieved 100% callus induction and maximum biomass (0.416 g) in *Tetragonia tetragonoides* leaf explants, while Pérez-Mendoza et al. (2020) demonstrated that leaf-derived callus cultures of *Rosmarinus officinalis* induced with BAP and 2,4-D yielded distinct secondary metabolite profiles compared to intact leaves, highlighting the capacity of in vitro systems to both reproduce and diversify the phytochemical repertoire of the source plant. Similarly, other studies confirmed that BAP and 2,4-D consistently outperform BAP and NAA in promoting callogenesis (Jafari and Daneshvar 2024). Overall, these findings highlight that the combination of BAP and 2,4-D provides an efficient and reliable approach for callus induction from leaf explants in *C. spinosa*.

The effect of PGRs is not limited to cell proliferation, as they additionally modulate metabolic pathways responsible for secondary metabolite biosynthesis (Isah et al. 2018). These findings suggest that callus culture could serve as a promising alternative strategy for the production of high-value molecules. Previous studies have demonstrated that PGRs significantly influence the diversity of terpenes and other volatile compounds under in vitro conditions, resulting in aroma profiles distinct from those of field-grown plants (Passinho-Soares et al. 2013; Soumahoro et al. 2025). A detailed investigation of these metabolic shifts could therefore inform the biotechnological production of desirable

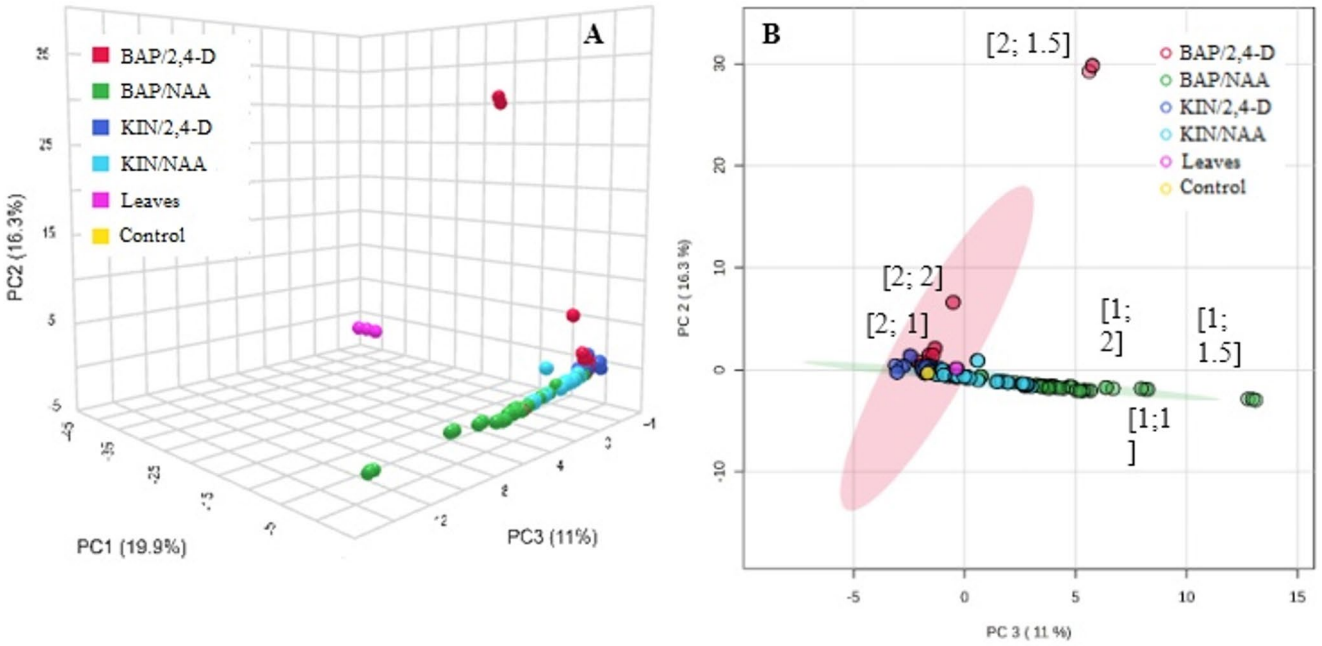

Fig. 5 Principal Component Analysis (PCA) plot comparing the effects of different hormone combinations on callus cultivation. **A**: Three-dimensional PCA plot; **B**: Scores plot from the PCA analysis. Samples are color-coded according to the applied PGRs under experimental conditions. VOCs were extracted from callus cultures grown under specific hormonal treatments: red: BAP/ 2,4-D; green: BAP/NAA; dark blue: KIN/2,4-D; sky blue: KIN/NAA; pink: fresh leaves; yellow: control culture of fresh leaves without hormones

Table 2 Top 15 molecule contributions to PC1, PC2, and PC3 (from left to right)

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

All correlations with PC1, PC2, and PC3 are highly significant ($p < 0.001$)

volatile compounds (Łyczko et al. 2020). The volatile profiles observed in *C. spinosa* callus cultures in the present study also differ markedly from those previously reported in field-grown plant material of this species. (Merlino et al. 2024) reported that the volatile fraction of *C. spinosa* leaf essential oil emulsions was dominated by sulfur compounds, including dimethyl tetrasulfide, dimethyl trisulfide, and methyl isothiocyanate. In the present study, sulfur compounds similarly predominated in callus cultures treated with NAA, whereas 2,4-D-based treatments induced a qualitative shift toward novel terpenoids, including safranal, (*E*)-rose oxide, and menthol, absent in fresh leaves. This qualitative remodeling of the volatile profile under 2,4-D treatment may indicate a modulation of certain terpene biosynthetic pathways in dedifferentiated *C. spinosa* cells, an interpretation consistent with the broader observation that in vitro culture conditions can promote the expression of metabolic pathways that are weakly expressed or not readily in intact

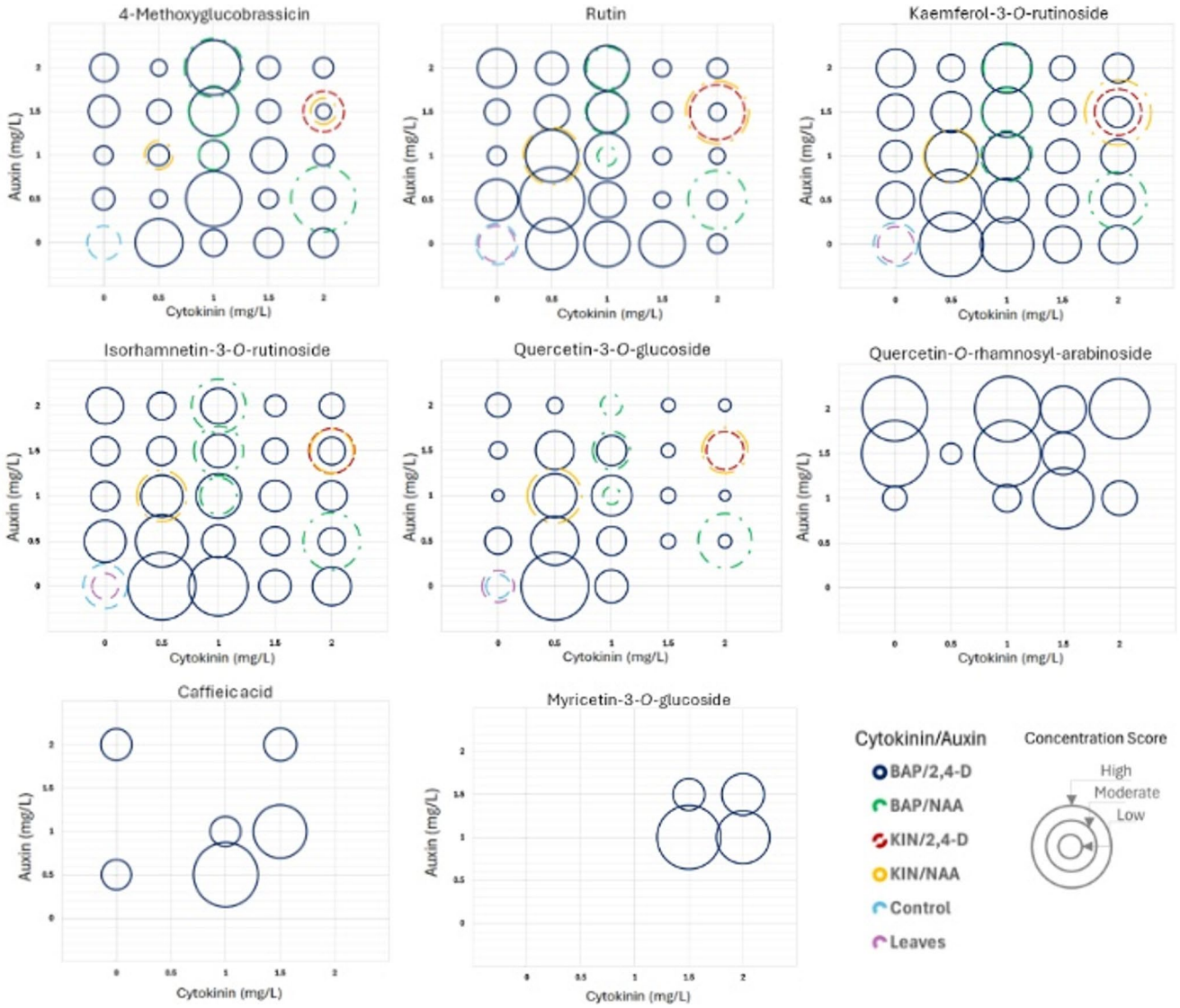

Fig. 6 Effect of cytokinin and auxin treatments on the accumulation of major non-volatile secondary metabolites in *Capparis spinosa* callus cultures. The X- and Y-axes indicate cytokinin and auxin concentrations, respectively. Bubble colors represent each hormonal combination. Dark blue: BAP/2,4-D, green: BAP/NAA, red: KIN/2,4-D, yellow: KIN/NAA, light blue: control culture of fresh leaves without hormones, purple: fresh leaves. Bubble size reflects the relative abundance of each metabolite

plant tissues. Indeed, Jakobina et al. (2024) reported that in vitro cultures of *Coleus scutellarioides* exhibited volatile compound profiles markedly distinct from those of intact plants, with PGR type and concentration determining both the qualitative and quantitative composition of the volatile fraction, underscoring the pivotal role of hormonal conditions in redirecting secondary metabolic pathways during dedifferentiation.

In our study, the compounds most strongly correlated with PC1 (Table 2), representing the leaf metabolites of *C. spinosa*, mainly originate from enzymatic and non-enzymatic carotenoid degradation, including *β*-cyclocitral and (*E*)-*β*-ionone derived from *β*-carotene (Ke et al. 2022). (*E*)-*β*-ionone can further oxidize to dihydroactinidiolide (Sun et al. 2022), while dihydroedulanes I and II likely derive from *β*-ionone (Merlino et al. 2024). The formation of 6-methyl-5-hepten-2-one and geranyl acetone results from *ζ*-carotene degradation (Vogel et al. 2008; Zhou et al. 2022). Among sulfur compounds, methyl isothiocyanate, produced by glucocapparin hydrolysis, predominates in fresh leaves (92.2%), whereas butyl isothiocyanate from glucoerucin remains below 0.1% (Bianco et al. 2012; Sun et al. 2023). Detected aldehydes likely arise from lipoxygenase and lyase activity on oleic and linoleic acids (Zhang et al. 2023; Semmar 2024), and verticillol biosynthesis is associated with enzymatic cyclization of geranylgeranyl diphosphate (Jin et al. 2005).

The VOCs correlated with PC2 are characteristic of callus cultures induced by BAP/2,4-D (BAP = 2 mg/L; 2,4-D = 1.5-2 mg/L), which promotes terpene diversification. Notably, safranal is detected as a volatile compound in callus cultures of *C. spinosa*. Although present in low concentrations, its formation may result from a modification of *β*-carotene oxidative cleavage at position 7,8 rather than 9,10, following the same metabolic pathway as *β*-cyclocitral (Winterhalter and Rouseff 2001; Sergeant et al. 2009; Ke et al. 2022). Safranal, recognized for its antioxidant and aromatic properties, is commonly associated with saffron (*Crocus sativus*) (Elateeq and Sun 2024). Its detection in *C. spinosa* suggests the activation of a latent metabolic pathway or an enzymatic conversion of terpene precursors triggered by hormonal stress (Taherkhani et al. 2021). Geraniol, through enzymatic reduction, generates citronellol, which undergoes hydroxylation and cyclization to produce (*Z*)- and (*E*)-rose oxides (Lin et al. 2019). Limonene, an intermediate in menthol biosynthesis, is converted to (*Z*)-menthone via enzymatic steps; isomenthone, an isomer of (*Z*)-menthone, is synthesized via the pulegone pathway (Afkar and Karimzadeh 2024). Additionally, 2-isopropyl-5-methyl-3-cyclohexen-1-one was identified as a derivative of (*Z*)-menthone (Cohen et al. 2020).

Compounds associated with PC3 mainly originate from the auto-oxidative pathway of unsaturated fatty acids, responsible for aldehydes formation (Noordermeer et al. 2001). These aldehydes can be reduced to fatty alcohols via alcohol dehydrogenases (Munkajohnpong et al. 2020). During this process, methanethiol and dimethyl sulfide can regenerate to form dimethyl disulfide and trisulfide (Zhou et al. 2024). The terpenes *D*-carvone and eucalyptol correlated with PC3 are derived from geranyl pyrophosphate via the monoterpene pathway, with *D*-carvone formed from limonene and eucalyptol derived from *α*-terpineol via cyclic dehydration (El Hassani et al. 2009; Eisenbrand et al. 2021).

Beyond volatile compounds, non-volatile secondary metabolites reflect additional metabolic pathways and the biochemical potential of *C. spinosa* callus cultures. Among these, rutin was identified as the predominant flavonoid in foliar callus tissues and was consistently detected across all tested combinations. In our study, low concentration of BAP and 2,4-D promoted significant accumulation of glycosylated flavonoids, particularly rutin. Similar trends were observed in *Vaccinium corymbosum* callus cultures (Rybin et al. 2024). Duran and Issah (2022) showed that BAP (1 mg/L) and NAA (2 mg/L) promoted rutin production in *C. spinosa* callus cultures, consistent with our observations that BAP 1 mg/L combined with NAA 1.5-2 mg/L enhances rutin content. This effect is mainly attributable to PGR-modulation of key genes in the phenylpropanoid-flavonoid pathway, including phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and UDP-glycosyltransferases (UGTs) (Kianersi et al. 2020a, b; Kumari et al. 2024; Li et al. 2025). Although PAL, CHS, and UGT activity and gene expression levels were not measured in the present study, increased flavonoids accumulation under KIN/2,4-D and KIN/NAA conditions likely results from targeted transcriptional activation, intensifying flavonoid biosynthesis. 4-Methoxyglucobrassicin generally exhibited low accumulation in all treatments. As an indolic glucosinolate, it is synthesized from tryptophan via indole-3-acetaldoxime, also a precursor for indole-3-acetic acid (IAA) (Bak et al. 2001; Bansal et al. 2024). Exogenous auxins (2,4-D or NAA) tend to redirect flux toward IAA, reducing substrate availability for glucosinolate production (Malka and Cheng 2017; Mitreiter and Gigolashvili 2021). Indole glucosinolate pathways appear more sensitive to stress signals and defense responses than to direct transcriptional regulation by cytokinins or auxins (Bielach et al. 2017).

Furthermore, cytokinins, particularly KIN, redirect secondary metabolism toward flavonoid biosynthesis rather than indole derivatives (Bozsó and Barna 2021). This redistribution of carbon and metabolic energy may explain the low accumulation of 4-methoxyglucobrassicin under KIN/2,4-D and KIN/NAA. Similar studies indicate that hormones regulate the balance between glucosinolate and flavonoid synthesis by modulating aldoxime levels and PAL activity, thereby redirecting metabolic flux (Kim et al. 2015, 2020; Shin et al. 2023). Hence, 4-methoxyglucobrassicin appears to be a metabolic marker with low responsiveness to direct hormonal regulation, with production likely depending on stress or defense signals rather than on transcriptional activation by cytokinins or auxins (Bednarek et al. 2009; Liu et al. 2021).

Conclusion

The present study demonstrates that the combination of BAP and 2,4-D effectively enhances callus induction in *C. spinosa*. Using Response Surface Methodology, the concentrations that maximized biomass production were determined to be 2 mg/L BAP and 1-1.5 mg/L 2,4-D, resulting in the highest fresh and dry weight gains.

Analysis of volatile metabolites revealed that callus tissues were dominated by terpenes and sulfur compounds, with the BAP/2,4-D combination inducing safranal, (*E*)-rose oxide, and menthol compounds absent in fresh leaves, suggesting a possible activation of otherwise latent or weakly expressed metabolic pathways under in vitro conditions. For non-volatile metabolites, glycosylated flavonoids, including rutin, kaempferol-3-*O*-rutinoside, and quercetin-3-*O*-glucoside accumulated predominantly under KIN/2,4-D and

KIN/NAA treatments, whereas indole derivatives, such as 4-methoxyglucobrassicin, exhibited low responsiveness to hormonal regulation, suggesting that their biosynthesis is mainly driven by stress-related signaling rather than PGR concentrations.

Overall, these findings demonstrate that precise adjustment of cytokinin/auxin ratios can optimize both callus biomass and secondary metabolite accumulation. *C. spinosa* callus cultures thus represent a viable biotechnological platform for the sustainable production of high-value metabolites, particularly flavonoids, and provide the starting material for the development of cell suspension cultures for large-scale metabolite production. The callus culture can be used as a starting system for micropropagation if there is subsequent plant regeneration. Future research should focus on integrating mechanistic approaches to hormonal regulation with data-driven modeling methods, in order to further optimize callus biomass and secondary metabolite production, as well as to facilitate the transition toward bioreactor-based systems suitable for large-scale industrial applications.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11240-026-03493-0.

Acknowledgements Not applicable.

Author contributions M. M: Writing - review & editing, Writing - original draft, Visualization, Software, Methodology, Investigation, Data curation, Conceptualization. M. G and C. B: Writing - review & editing, Visualization, Methodology, Conceptualization. Y. G: Writing - review & editing, Supervision, Software, Methodology, Conceptualization, Validation. L. V and M. B: Writing - review & editing. M. -L. F and M. F: Writing - review & editing, Validation, Methodology, project administration. All authors read and approved of the final manuscript.

Funding The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data availability The original contributions presented in this study are included in the article. Further inquiries can be directed at the corresponding author.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

References

Abdulhafiz F, Mohammed A, Reduan MFH et al (2022) Plant cell culture technologies: A promising alternative to produce high-value secondary metabolites. Arab J Chem 15:104161

Afkar S, Karimzadeh G (2024) Changes in Physiological Traits, Gene Expression and Phytochemical Profile of *Mentha piperita* in Response to Elicitor. Biochem Genet. https://doi.org/10.1007/s10528-024-10805-6

Al-Safadi B, Elias R (2011) Improvement of caper (*Capparis spinosa* L.) propagation using *in vitro* culture and gamma irradiation. Sci Hortic 127:290–297

Anuradha M, Balasubramanya S, Subbalakshmi G, Shilpa P (2025) Commercialization of i*n vitro* secondary metabolite production: Challenges and opportunities. In: Anuradha M, Balasubramanya S (eds) in vitro Production of Plant Secondary Metabolites. Springer Nature Singapore, Singapore, pp 321–346

Bak S, Tax FE, Feldmann KA et al (2001) CYP83B1, a cytochrome P450 at the metabolic branch point in auxin and indole glucosinolate biosynthesis in *Arabidopsis*. Plant cell 13:101–111

Banaev EV, Erst AA, Khramova EP et al (2025) Comparative analyses of secondary metabolites in microshoot culture, callus culture, and native plants of *Atraphaxis* L. (Polygonaceae). Plant Cell Tiss Organ Cult 160:24. https://doi.org/10.1007/s11240-025-02970-2

Bano AS, Khattak AM, Basit A et al (2022) Callus induction, proliferation, enhanced secondary metabolites production and antioxidants activity of *Salvia moorcroftiana* L. as influenced by combinations of auxin, cytokinin and melatonin. Braz arch biol technol 65:e22210200. https://doi.org/10.1590/1678-4324-2022210200

Bansal S, Lakra N, Mishra S, Ahlawat YK (2024) Unraveling the potential of glucosinolates for nutritional enhancement and stress tolerance in *Brassica* crops. Veg Res. https://doi.org/10.48130/vegres-0024-0016

Bednarek P, Piślewska-Bednarek M, Svatoš A et al (2009) A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. Science 323:101–106. https://doi.org/10.1126/science.1163732

Belkessam M, Genva M, Kouki A et al (2025) Phytochemical profile and cosmeceutical potential of leaf extracts of two species of the Anacardiaceae family from the mediterranean scrubland: *Pistacia lentiscus* L. and *Pistacia atlantica* Desf. Processes 13:3712. https://doi.org/10.3390/pr13113712

Bianco G, Lelario F, Battista FG et al (2012) Identification of glucosinolates in capers by LC-ESI-hybrid linear ion trap with fourier transform ion cyclotron resonance mass spectrometry (LC-ESI-LTQ-FTICR MS) and infrared multiphoton dissociation. J Mass Spectrom 47:1160–1169. https://doi.org/10.1002/jms.2996

Bielach A, Hrtyan M, Tognetti VB (2017) Plants under stress: involvement of auxin and cytokinin. Int J Mol Sci 18:1427

Bozsó Z, Barna B (2021) Diverse effect of two cytokinins, kinetin and benzyladenine, on plant development, biotic stress tolerance, and gene expression. Life 11:1404

Brown KK, Hampton MB (2011) Biological targets of isothiocyanates. BiochimbBiophys Acta 1810:888–894

Bull T, Michelmore R (2022) Molecular determinants of *in vitro* plant regeneration: prospects for enhanced manipulation of lettuce (*Lactuca sativa* L). Front Plant Sci 13. https://doi.org/10.3389/fpls.2022.888425

Burgeon C, Markey A, Debliquy M et al (2021) Comprehensive SPME-GC-MS analysis of VOC profiles obtained following high-temperature heating of pork back fat with varying boar taint intensities. Foods 10:1311. https://doi.org/10.3390/foods10061311

Chacón LMT, Leiva H, Vahos ICZ et al (2023) Influence of plant growth regulators on *in vitro* biomass production and biosynthesis of cytotoxic Amaryllidaceae alkaloids in *Caliphuria tenera* Baker. Biocatal Agric Biotechnol 50:102670

Chakrane I, Chlyah O, Boughribil S et al (2025) Effect of different media and plant hormone concentrations on callus induction and regeneration of red squill (*Squilla maura*) and Peruvian Squill

(*Oncostema peruviana*). Int J Plant Biology 16:4. https://doi.org/10.3390/ijpb16010004

Cohen SM, Eisenbrand G, Fukushima S et al (2020) FEMA GRAS assessment of natural flavor complexes: Mint, buchu, dill and caraway derived flavoring ingredients. Food Cheml toxicol 135:110870

Duran RE, Issah H (2022) The impact of strigolactone GR24 on *Capparis spinosa* L. callus production and phenolic compound content. Plant Cell, Tiss Organ Cult 149:197–204

Eisenbrand G, Cohen SM, Fukushima S et al (2021) FEMA GRAS assessment of natural flavor complexes: Eucalyptus oil and other cyclic ether-containing flavoring ingredients. Food Chem Toxicol 155:112357

El Hassani FZ, Zinedine A, Amraoui MB et al (2009) Characterization of the harmful effect of olive mill wastewater on spearmint. J Hazard Mater 170:779–785

Elateeq AA, Sun Y (2024) Production of bioactive metabolites in *in vitro* cultures of saffron (*Crocus sativus* L). In: Biotechnological Production of Bioactive Phytochemicals of Medicinal Value. Elsevier, pp 17–65

Fauconnier M-L, Jaziri M, Marlier M et al (1993) Essential oil production by *Anthemis nobilis* L. tissue culture. J plant physiol 141:759–761

Firoozi B, Zare N, Sofalian O, Sheikhzade Mosadegh P (2019) *In Vitro* Indirect somatic embryogenesis and secondary metabolites production in the saffron: Emphasis on ultrasound and plant growth regulators. Tarım Bilimleri Dergisi 25:1–10. https://doi.org/10.15832/ankutbd.538973

Ganeshpurkar A, Saluja AK (2017) The pharmacological potential of rutin. Saudi Pharma J 25:149–164. https://doi.org/10.1016/j.jsps.2016.04.025

Hazrati S, Mousavi Z, Mollaei S et al (2025) Effect of phenological variation on the phytochemical composition and antioxidant activity of different organs of *Capparis spinosa* L. Horticulturae 11:702. https://doi.org/10.3390/horticulturae11060702

Ikeuchi M, Sugimoto K, Iwase A (2013) Plant callus: mechanisms of induction and repression. Plant cell 25:3159–3173

Isah T, Umar S, Mujib A et al (2018) Secondary metabolism of pharmaceuticals in the plant *in vitro* cultures: strategies, approaches, and limitations to achieving higher yield. Plant Cell Tiss Organ Cult 132:239–265. https://doi.org/10.1007/s11240-017-1332-2

Jafari M, Daneshvar MH (2024) Machine learning-mediated *Passiflora caerulea* callogenesis optimization. PLoS ONE 19:e0292359. https://doi.org/10.1371/journal.pone.0292359

Jakobina M, Łyczko J, Szumny A, Galek R (2024) Plant *in vitro* cultures of *Coleus scutellarioides* (L.) benth. Electric Lime and possibilities of modification in the biosynthesis of volatile compounds. Molecules 29:2193. https://doi.org/10.3390/molecules29102193

Jin Y, Williams DC, Croteau R, Coates RM (2005) Taxadiene Synthase-catalyzed cyclization of 6-fluorogeranylgeranyl diphosphate to 7-fluoroverticillenes. J Am Chem Soc 127:7834–7842. https://doi.org/10.1021/ja050592r

Kamran S, Sinniah A, Abdulghani MA, Alshawsh MA (2022) Therapeutic potential of certain terpenoids as anticancer agents: a scoping review. Cancers 14:1100

Kdimy A, El Yadini M, Guaadaoui A et al (2022) Phytochemistry, biological activities, therapeutic potential, and socio-economic value of the caper bush (*Capparis Spinosa* L). Chemi Biodivers 19:e202200300

Ke D, Guo J, Li K et al (2022) Carotenoid-derived bioactive metabolites shape plant root architecture to adapt to the rhizospheric environments. Front Plant Sci 13. https://doi.org/10.3389/fpls.2022.986414

Kianersi F, Abdollahi MR, Mirzaie-asl A et al (2020a) Biosynthesis of rutin changes in *Capparis spinosa* due to altered expression of its pathway genes under elicitors' supplementation. Plant Cell, Tissue Organ Cult 141:619–631

Kianersi F, Abdollahi MR, Mirzaie-Asl A et al (2020b) Identification and tissue-specific expression of rutin biosynthetic pathway genes in *Capparis spinosa* elicited with salicylic acid and methyl jasmonate. Sci Rep 10:8884

Kim JI, Dolan WL, Anderson NA, Chapple C (2015) Indole glucosinolate biosynthesis limits phenylpropanoid accumulation in *Arabidopsis thaliana*. Plant Cell 27:1529–1546

Kim JI, Zhang X, Pascuzzi PE et al (2020) Glucosinolate and phenylpropanoid biosynthesis are linked by proteasome-dependent degradation of PAL. New Phytol 225:154–168. https://doi.org/10.1111/nph.16108

Koufan M, Belkoura I, Mazri MA (2022) *In vitro* propagation of caper (*Capparis spinosa* L.): A Review. Horticulturae 8:737. https://doi.org/10.3390/horticulturae8080737

Kour S, Sagar T, Nipunta et al (2025) Role of biotechnology in improvement and regulation of biosynthesis of bioactive compounds. In: Devi J (ed) Biotechnological Intervention in Production of Bioactive Compounds. Springer Nature Switzerland, Cham, pp 43–55

Kumari M, Asthana N, Kumari R (2015) Plantlets regeneration through callus culture in *Capparis spinosa* Linn. IJEK 2:34–40

Kumari S, Nazir F, Maheshwari C et al (2024) Plant hormones and secondary metabolites under environmental stresses: Enlightening defense molecules. Plant Physiol Biochem 206:108238

Labbafi M, Mehrafarin A, Badi H et al (2018) Improve germination of caper (*Capparis spinosa* L.) seeds by different induction treatments of seed dormancy breaking. Trakia J Sci 16:71–74

Lee KY, Nam D-H, Jeon Y et al (2024) Exploring the production of secondary metabolites from a halophyte *Tetragonia tetragonoides through Callus Culture*. Horticulturae 10:244. https://doi.org/10.3390/horticulturae10030244

Li W, Lin S, Wang R et al (2025) Regulation of plant hormones on the secondary metabolism of medicinal plants. M 4:0–0. https://doi.org/10.48130/mpb-0025-0016

Lin J, Massonnet M, Cantu D (2019) The genetic basis of grape and wine aroma. Horticulture Res

Liu Z, Wang H, Xie J et al (2021) The roles of cruciferae glucosinolates in disease and pest resistance. Plants 10:1097. https://doi.org/10.3390/plants10061097

Ludwiczuk A, Skalicka-Woźniak K, Georgiev MI (2017) Terpenoids. In: Pharmacognosy. Elsevier, pp 233–266

Łyczko J, Piotrowski K, Kolasa K et al (2020) *Mentha piperita* L. micropropagation and the potential influence of plant growth regulators on volatile organic compound composition. Molecules 25:2652

Malka SK, Cheng Y (2017) Possible interactions between the biosynthetic pathways of indole glucosinolate and auxin. Front Plant Sci 8:2131

Mastuti R, Munawarti A, Firdiana ER (2017) The combination effect of auxin and cytokinin on in vitro callus formation of Physalis angulata L.–A medicinal plant. In: AIP Conference Proceedings. AIP Publishing LLC, p 040007

Merlino M, Condurso C, Cincotta F et al (2024) Essential oil emulsion from caper (*Capparis spinosa* L.) leaves: Exploration of its antibacterial and antioxidant properties for possible application as a natural food preservative. Antioxidants 13:718

Mitreiter S, Gigolashvili T (2021) Regulation of glucosinolate biosynthesis. J Exp Bot 72:70–91

Mohaddab M, El Goumi Y, Gallo M et al (2022) Biotechnology and *in vitro* culture as an alternative system for secondary metabolite production. Molecules 27:8093. https://doi.org/10.3390/molecules27228093

Munkajohnpong P, Kesornpun C, Buttranon S et al (2020) Fatty alcohol production: an opportunity of bioprocess. Biofuels Bioprod Bioref 14:986–1009. https://doi.org/10.1002/bbb.2112

Noordermeer MA, Veldink GA, Vliegenthart JFG (2001) Fatty acid hydroperoxide lyase: A plant cytochrome P450 enzyme involved in wound healing and pest resistance. Chem Bio Chem 2:494–504. https://doi.org/10.1002/1439-7633(20010803)2:7/8%3C494::AID-CBIC494%3E3.0.CO;2-1.

Nowruzian A, Aalami A (2023) Effect of different treatments on dormancy breaking for seed germination enhancement and metabolite analysis of *Capparis spinosa* L. J Med plants By-Prod 12:387–396

Ozyigit II, Dogan I, Hocaoglu-Ozyigit A et al (2023) Production of secondary metabolites using tissue culture-based biotechnological applications. Front Plant Sci 14. https://doi.org/10.3389/fpls.2023.1132555

Passinho-Soares HC, Meira PR, David JP et al (2013) Volatile organic compounds obtained by *in vitro* callus cultivation of *Plectranthus ornatus* Codd. (Lamiaceae). Molecules 18:10320–10333. https://doi.org/10.3390/molecules180910320

Pasternak TP, Steinmacher D (2024) Plant growth regulation in cell and tissue culture *in vitro*. Plants 13:327. https://doi.org/10.3390/plants13020327

Pérez-Mendoza MB, Llorens-Escobar L, Vanegas-Espinoza PE et al (2020) Chemical characterization of leaves and calli extracts of *Rosmarinus officinalis* by UHPLC-MS. Electrophoresis 41:1776–1783. https://doi.org/10.1002/elps.201900152

Rybin DA, Sukhova AA, Syomin AA et al (2024) Characteristics of callus and cell suspension cultures of highbush blueberry (*Vaccinium corymbosum* L.) cultivated in the presence of different concentrations of 2, 4-D and BAP in a nutrient medium. Plants 13:3279

Semmar N (2024) Overview on structural and functional diversity of secondary metabolites. Secondary Metabolites in Plant Stress Adaptation. Springer International Publishing, Cham pp 23–41

Sergeant MJ, Li J-J, Fox C et al (2009) Selective inhibition of carotenoid cleavage dioxygenases: phenotypic effects on shoot branching. J Biol Chem 284:5257–5264

Shin D, Perez VC, Dickinson GK et al (2023) Altered methionine metabolism impacts phenylpropanoid production and plant development in *Arabidopsis thaliana*. Plant J 116:187–200. https://doi.org/10.1111/tpj.16370

Singh D, Singh N, Dwivedi S, Trivedi PK (2025) Transcriptional regulation of secondary plant product biosynthesis: insights into flavonoid, alkaloid, and terpenoid pathways. Plant Cell Tiss Organ Cult 160:6. https://doi.org/10.1007/s11240-024-02925-z

Sottile F, Caltagirone C, Peano C et al (2021) Can the caper (*Capparis spinosa* L.) still be considered a difficult-to-propagate crop? Horticulturae 7:316

Soumahoro BA, Koné T, Kouakou HT, Koné M (2025) Terpene compounds of essential oils extracted from callus and cell suspension culture of Savannah Tea (*Lippia multiflora* Moldenke, Verbenaceae) cultivated in Côte d'Ivoire. In: Ramawat KG, Mérillon J-M (eds) Natural products. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 1–21

Sozzi GO, Chiesa A (1995) Improvement of caper (*Capparis spinosa* L.) seed germination by breaking seed coat-induced dormancy. Sci Horti 62:255–261

Stutz H, Bresgen N, Eckl PM (2015) Analytical tools for the analysis of β-carotene and its degradation products. Free Radic Res 49:650–680. https://doi.org/10.3109/10715762.2015.1022539

Sun T, Rao S, Zhou X, Li L (2022) Plant carotenoids: recent advances and future perspectives. Mol Hortic 2:3. https://doi.org/10.1186/s43897-022-00023-2

Sun Y, Yang T, Wang C (2023) *Capparis spinosa* L. as a potential source of nutrition and its health benefits in foods: A comprehensive review of its phytochemistry, bioactivities, safety, and application. Food Chem 409:135258

Taherkhani T, Zakaria RA, Omidi M, Taherkhani M (2021) Expression of genes involved in the biosynthesis of Crocin and safranal in cell suspension culture of saffron (*Crocus sativus*). Pharm Chem J 54:1045–1050. https://doi.org/10.1007/s11094-021-02317-z

Tlili N, Feriani A, Saadoui E et al (2017) *Capparis spinosa* leaves extract: Source of bioantioxidants with nephroprotective and hepatoprotective effects. Biomed Pharmacother 87:171–179

Ullah A, Munir S, Badshah SL et al (2020) Important flavonoids and their role as a therapeutic agent. Molecules 25:5243. https://doi.org/10.3390/molecules25225243

Vogel JT, Tan B-C, McCarty DR, Klee HJ (2008) The carotenoid cleavage dioxygenase 1 enzyme has broad substrate specificity, cleaving multiple carotenoids at two different bond positions. J Biol Chem 283:11364–11373

Wang YT, Gan L, Liu W et al (2007) Research on the callus inducing and the cell growth and metabolism characteristics of *Capparis spinosa* L. Coll Life Sci Technol Huazhong Univ Sci Technol Wuhan China 7:1779–1783

Winterhalter P, Rouseff RL (eds) (2001) Carotenoid-derived aroma compounds. American Chemical Society, Washington, DC

Yin Y, He Y, Liu W et al (2014) The durative use of suspension cells and callus for volatile oil by comparative with seeds and fruits in *Capparis spinosa* L. PLoS ONE 9:e113668

Yousefi E, Abedi M, Aghajanzadeh TA, Moreno DA (2025) Caper bush (*Capparis spinosa* L.) bioactive compounds and antioxidant capacity as affected by adaptation to harsh soils. Sci Rep 15:11893. https://doi.org/10.1038/s41598-025-97298-4

Zhang Z, Liang M, Liu B et al (2023) Characterization of the key odorants of ripe *Capparis spinosa* L. fruit and changes in concentrations after storage by means of molecular sensory science. Food Chem 403:134383

Zhiponova M, Yordanova Z, Zaharieva A et al (2024) Cytokinins enhance the metabolic activity of *in vitro*-grown catmint (*Nepeta nuda* L). Plant Physiol Biochem 214:108884. https://doi.org/10.1016/j.plaphy.2024.108884

Zhou Z, Wang X, Cui X, Wang H (2022) Rapid Determination of 6-Methyl-5-hepten-2-one in Fruit with LLE-GC-MS. J Chromatogr Sci 60:280–286

Zhou Q, Zheng C, Wei F, Yang Y (2024) Flavor precursors identification and thermal degradation mechanisms of glucoerucin in fragrant rapeseed oil. Food Chem 435:137484

Zluhan-Martínez E, López-Ruíz BA, García-Gómez ML et al (2021) Integrative roles of phytohormones on cell proliferation, elongation and differentiation in the *Arabidopsis thaliana* primary root. Front Plant Sci 12:659155

Authors and Affiliations

Marouane Mohaddab[1,2] · Younes EL Goumi[3] · Manon Genva[1] · Louise Vilain[1] · Clément Burgeon[1] · Mouna Belkessam[1,4] · Malika Fakiri[2] · Marie-Laure Fauconnier[1]

✉ Marouane Mohaddab
mmohaddab@uliege.be; m.mohaddab@uhp.ac.ma

Younes EL Goumi
y.elgoumi@usms.ma

Manon Genva
M.Genva@uliege.be

Louise Vilain
louise.vilain@uliege.be

Clément Burgeon
cburgeon@uliege.be

Mouna Belkessam
mouna.belkessam@uliege.be

Malika Fakiri
malika.fakiri@uhp.ac.ma

Marie-Laure Fauconnier
marie-laire.fauconnier@uliege.be

1 Laboratory of Chemistry of Natural Molecules, Gembloux Agro-Bio Tech, University of Liege, Gembloux, Belgium

2 Laboratory of Agrifood and Health, Faculty of Sciences and Techniques, Hassan First University, Settat, Morocco

3 Applied Biology for Health, Environment and Sustainable Development Team, Higher School of Technology of Fkih Ben Salah, University of Sultan Moulay Slimane, Beni Mellal, Morocco

4 Laboratory of Environment Biomonitoring (LR01/ES14), Group on "Ecological Risk of Pharmaceuticals & Pesticides", Faculty of Sciences of Bizerta, University of Carthage, Zarzouna, Tunisia