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Review Article

Non-Genetic Engineering Methods to Enhance Cannabinoid Production in *Cannabis* sp.: A Systematic Review

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ABSTRACT

Non-genetic engineering of metabolites has become a key focus in enhancing cannabinoid production in *Cannabis*. With the increasing demand for cannabinoid-rich products, rapid and affordable yields improvements can be achieved through non-genetic methods. These methods avoid genetic engineering and regulatory challenges in *Cannabis* development. Although there are limited studies in the field due to historical restrictions on *Cannabis*, existing literature has been summarised to aid further exploration. Therefore, this study aims to explore non-genetic engineering methods for enhancing cannabinoid yields in *Cannabis* sp. A total of 30 selected articles from 1980 to 2024 on cannabinoid metabolites engineering were collected from Scopus, PubMed, and Science Direct, then analyzed and visualized using VOSviewer. The articles reviewed various non-genetic engineering methods, including biotic and abiotic elicitors, environmental stress manipulation, and nutrient management, to provide insights into practical approaches for enhancing cannabinoid production to meet industrial and pharmaceutical needs.

Keywords: Non-genetic engineering, Cannabinoid metabolites, *Cannabis*, Cannabinoid production, Enhancing cannabinoid

Introduction

The increasing demand for cannabinoid-rich products, specifically those containing cannabidiol (CBD),[1] has prompted extensive studies focused on optimizing their production from *Cannabis* sp. However, the variability of cannabinoid content among *Cannabis* sp varieties, caused by environmental differences and other factors, poses challenges in production.[2,3] To address these challenges, non-genetic engineering methods have emerged as a promising alternative. The methods focus on manipulating environmental factors, nutrient availability, and stress responses, offering controlled ways of improving yields through both *in vitro* and *in vivo* culture systems. *Cannabis* sp production in developing countries is considered more efficient with non-genetic engineering methods than genetic engineering. Non-genetic engineering methods are regarded as cheaper and faster because the plant's genetic structure is not directly altered. Meanwhile, genetic engineering involves altering deoxyribonucleic acid (DNA) into ribonucleic acid (RNA) and protein.[4] This process requires more time, higher costs, and may conflict with safety issues and regulations. Several studies have shown that non-genetic engineering methods, such as biotic and abiotic elicitors, can enhance cannabinoid production in *Cannabis*. Compounds, such as salicylic acid (SA), acid (GABA), and methyl jasmonate (MeJA), as well as light treatments, have been proven to trigger stress responses that increase cannabinoid yields.[5,6,7] In addition, manipulation of stress factors, such as mechanical wounding or nutrient management, can elicit physiological responses that support the synthesis of the compound.[8,9,10] Recent studies have shown that tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), the immediate precursors of THC and CBD, can be semi-synthesized by mixing cannabigerolic acid (CBGA) with trichome exudates isolated from *Cannabis* flowers. The synthesis process is often carried out in an *in vitro* hydrophobic-mimicking environment, showing new opportunities for increased yields.[11] Therefore, this study aims to explore non-genetic engineering methods for enhancing cannabinoid yields in *Cannabis* sp. Each method was analyzed to understand its impact on cannabinoid production. The results are expected to provide insights for future studies and industrial-scale production.

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Materials and Methods

Bibliometric Analysis

This bibliometric analysis summarized study developments on this topic and identified potential collaboration opportunities and relevant scientific journals for publishing related study results.[12] Data was collected from the Scopus (https://www.scopus.com/), ScienceDirect (https://www.sciencedirect.com/), and PubMed (https://pubmed.ncbi.nlm.nih.gov/) databases. All sources were accessed on 21 July 2024, and the search covered publications from 1980 to 2024, as shown in Figure 1.

Data Analysis

Articles focusing primarily on "*Cannabis*" were systematically searched based on specific criteria to ensure the relevance of collected data in Zotero for the study on *Cannabis*. The significance of each article was assessed through the presence of terms such as "cannabinoid", "elicitation", "elicitor", "nutrition feeding", "cell cultures", "precursor addition", and "hairy root".

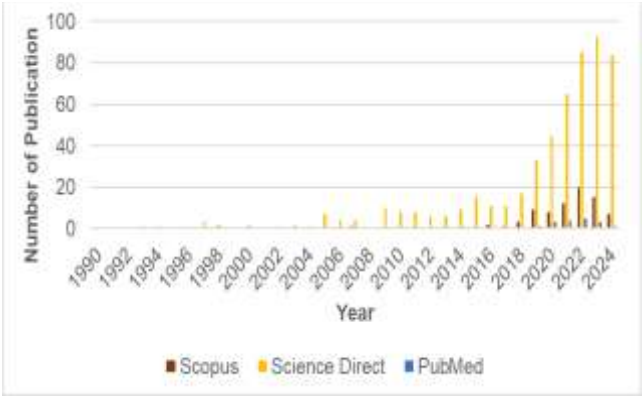

Figure 1: Annual of publications

Articles discussing unrelated issues, such as the clinical uses of *Cannabis*, the extraction of cannabinoid secondary metabolites, and the identification of secondary metabolite compounds, were excluded from the selection. Others included hydrolysis of cannabidiol (CBD) oil with various solvents, phytochemical characterization and cytotoxicity of *Cannabis* parts, studies on *Cannabis* for phytopathogen control in other plant species, the use of various compounds to increase terpene and antioxidant content in *Cannabis*, and instances where *Cannabis* was not the primary focus. Furthermore, selected publications were written in English and published between 1980 and 2023. Only study articles were included, while books, notes, reviews, conference papers, and bibliographies were excluded. Articles meeting these stringent criteria were exported in the study information system (.ris) for further analysis and integration into the study's dataset.

Bibliometric Computational Mapping

Bibliometric mapping was conducted using the VOSviewer software (version 1.6.10). VOSviewer was used to extract and analyze words that appeared in abstracts, author, co-author citations, institutional co-author network maps, journal citations, and keyword occurrences alongside visually qualified titles.[13,14,15,16] Different colors represented clusters in the network map, and connection paths showed collaboration or shared citations. The circle size represented the number of documents, references, or keywords, while the thickness of the connection line showed the strength of the link.[16]

Results and Discussion

The Literature Search

The literature search identified 632 articles relevant to the topic from 3 databases (Scopus, PubMed, and Science Direct). In this study, the flowchart was divided into 4 parts, namely article searching and collection, removal of redundant articles, classification, and selection of targeted topics. Duplicate articles were eliminated from each database, totalling 32 articles. Based on screening, articles whose main topic was not non-genetic engineering to enhance cannabinoids in *Cannabis* were excluded, as were book chapters, article reviews, and conference papers, totalling 570 articles. A total of 30 relevant articles were thoroughly reviewed and summarized in Table 1. The data analysis was shown in the flow chart diagram presented in Figure 2.

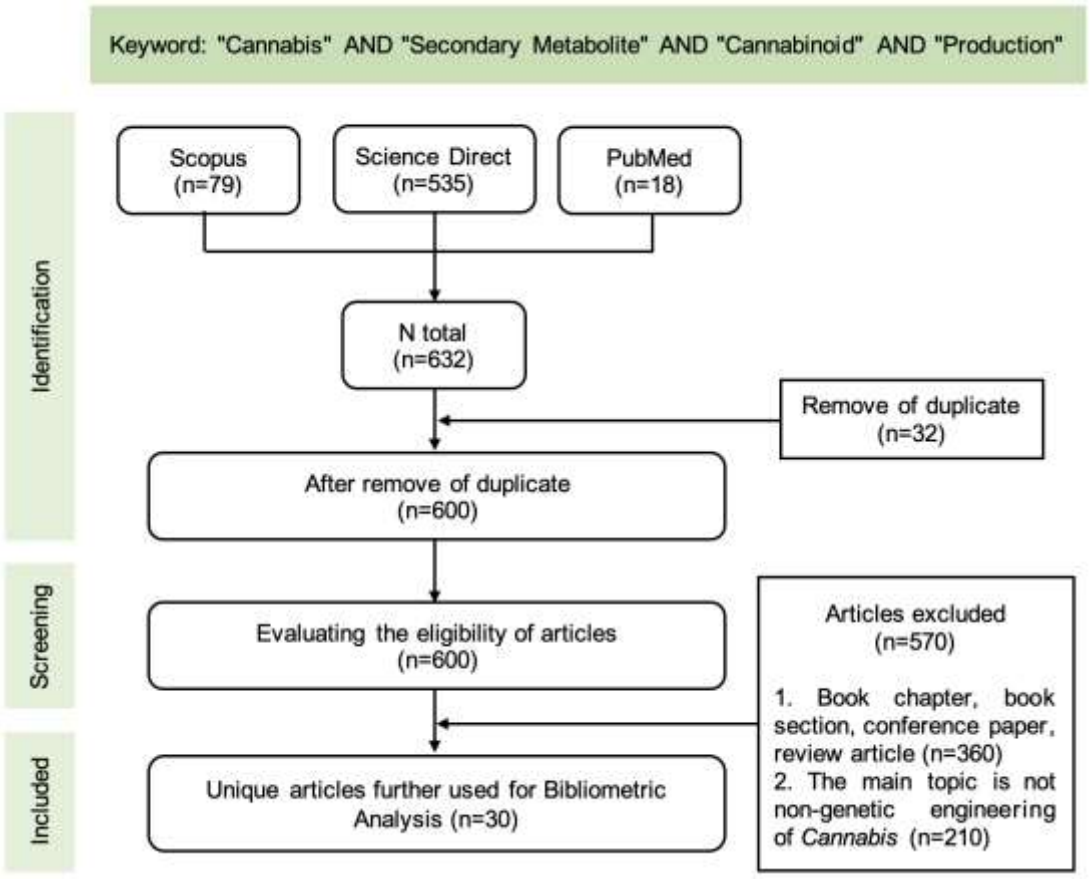

Figure 2: Flow chart identification and screening for the literature search strategy

Bibliometric Analysis of Computational Mapping

The co-occurrence of keywords across the 30 articles was counted, represented by the size of the nodes, as showed in the bibliometric map (Figure 3). The largest node representing *Cannabis* was identified as the central focus in the analyzed literature, while smaller nodes, such as α-(phenylethyl-2-oxo)-IAA (PEO-IAA), were identified. Keywords such as PEO-IAA, microgravity, shoot propagation, dynamic lighting, eustress, nitrate, dwarfism, slow-release fertilizer, triazole, fertigation, mineral fertilization, organic fertilization, soilless cultivation, deoxyribonucleic acid (DNA) methylation, and hairy root were found in only a single article each.

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Table 1: Summary of non-genetic engineering methods to enhance cannabinoid production in *Cannabis*.

Type of Engineering	Treatment Forms	Tested Conditions	Result(s)	DAT	Treatments details	Variety	Reference
(a) Chemical elicitation	(1) SA	0.1 mM (equivalent to 100 µM)	CBDA: ↑ 57.31% THCA: ↑ 52.68%	56	100 µM SA is sprayed on plant leaves until the solution began to drip	Beatriz (THC:CBD= 1:1)	Garrido *et al*.[6]
			THC: ↑ 93.13% CBD: ↓ 33.87%	3	1000 µM SA is sprayed as an elicitor in the flowering female plant	Saghez (drug-type)	Jalali *et al*.[5]
		1 mM (equivalent to 1000 µM)	Did not show an increase in cannabinoid yield in cell cultures under the tested conditions	2,4, and 7	1 mM was added to the culture media	Skunk (drug type)	Flores-Sanchez *et al*.[17]
		1 M (equivalent to 1000000 µM)	THC: ↑ 127.69% CBD: ↑ 89.94%	30	1000000 µM SA were applied as sprays in two stages: first, when the plants had 10 to 12 leaves, and second, at the beginning of bud flowering.	The specific variety used has not been mentioned	Mirzamohammad *et al*.[7]
		0.3 mM (equivalent to 300 µM), and 0.5 mM (equivalent to 500 µM)	Did not show an increase in cannabinoid yield in cell cultures under the tested conditions	2,4, and 7	300 µM and 500 µM were added to the culture media	Skunk (drug type)	Flores-Sanchez *et al*.[17]
	(2) GABA	0.1 mM (which is equal to 100 µM)	THC: ↑ 390.3% CBD: ↓ 94.83%	3	100 µM GABA is sprayed as an elicitor in the flowering female plant	Saghez (drug-type)	Jalali *et al*.[5]
		10 mM (equivalent to 10000 µM)	THC: ↑ 245.45% CBD: ↑ 15%	3	10000 µM GABA is sprayed as an elicitor in the flowering female plant	Saghez (drug-type)	

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	(3) MeJA	0.1 mM (equivalent to 100 µM)	CBDA: ↑ 15.6% THCA: ↑ 11%	56	100 µM MeJA is sprayed on plant inflorescences until the solution began to drip	Beatriz (THC:CBD= 1:1)	Garrido *et al*.[6]
		0.3 mM (equivalent to 300 µM)	No effect in cell culture	0, 6,12, 24, 72 h	300 µM was added to the culture media		
	(4) Jasmonic acid	100 µM		Every 2 days	100 µM was added to the culture media		
	(5) Pectin	0.1 mg/mL		Every 2 days	0.1 mg/mL was added to the culture media		
	(6) Sodium alginate	150 µg/mL	No effect in cell culture	2 and 4	150 µg/mL was added to the culture media		
	(7) $AgNO_3$	50 and 100 µM		2 and 4	50 and 100 µM were added to the culture media		
	(8) $CoCl_2.6H_2O$	50 and 100 µM		2 and 4	50 and 100 µM were added to the culture media		
	(9) $NiSO_4.6H_2O$	50 and 100 µM		2 and 4	50 and 100 µM were added to the culture media	Skunk (drug type)	Flores-Sanchez *et al*.[17]
(b) Biological elicitation	(1) Yeast extract	10 mg/mL		2,4, and 7	Yeast extract as an elicitor		
	(2) Dry fungal suspensions	4 and 8 g/mL	No effect in cell culture	2,4, and 7	*Pythium aphanidermatum* as elicitor		
	(3) Dry fungal suspensions	4 and 8 g/mL	No effect in cell culture	1,2, and 4	*Botrytis cinerea* Pers as elicitor		
(c) Physical elicitation	(1) UV 302 nm	30 s	No effect in cell culture	2 and 4	*Cannabis* cell cultures were irradiated under UV lamps at 302 nm		
	(2) UV 366 nm	30 min		2 and 4	*Cannabis* cell cultures were irradiated under UV lamps at 366 nm		

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(3) UVA	315-400 nm	CBDA: ↑ 17.86% CBD: ↓ 3.57% CBDVA: ↑ 50% THCA: ↓ 66.67% THCVA: ↑ 11.11%	50	*Cannabis* plants were grown in a controlled environment with LED treatment	Finola variety	Kotiranta *et al*.[24]
(4) UVB	280-315 nm	CBDA: ↑ 14.29% CBD: ↓ 21.43% CBDVA: ↑ 30% THCA: ↓ 16.67% THCVA: ↑ 61.11% CBG: ↑ 21.31%				
(5) UVA+UVB	UVA: 385 nm (PFD 50 μmol m^{-2} s^{-1}) UVB: 280-400 nm (PFD 3 μmol m^{-2} s^{-1})	CBGA: No increase THCA: ↓ 1.33% THC: No increase CBC: ↑ 5.26% CBGA: ↑18.18% THCA: ↑ 6.67% THC: ↑ 32.14%	45	*Cannabis* plants were irradiated with UVA for 45 full days, with a duration of 12 hours per day. In the last 20 days of the flowering cycle, the plants were irradiated with UVA + UVB, with UVA provided for 12 hours per day and UVB provided for 5 hours per day.	Meridian genotype (high THC) in inflorescences	Llewellyn *et al*.[19]
(6) LED	MW 240 (Red 35% + Blue 25% + Green 40% FS 240-UV (Red 40% + UVA and Blue 26% + Green 29% + Far red 5%) MB 240 (Red 85% + Blue 15%)	CBD: ↑ 63.2% CBDA: ↑ 89.9% THCA: ↑ 83.4% CBD: ↑ 526.81% CBDA: ↑ 67% THC: ↑ 4033.3% THCA: ↑ 108.32% CBD: ↑ 42.9% CBDA: ↑ 16.7 %	90	The plants received LED treatment, and the automated system injected nutrient water into the root zone for twenty seconds every two minutes. Data were collected at 30, 60, 90 DAT.	Alpine star CBD (strain KHV 1)	Islam *et al*.[18]

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	THC: ↑ 672.29% THCA: ↓ 11.1%				
PF 240 (Red 70% + Blue 30%)	CBD: ↑ 708.57% CBDA: ↑ 28.73% THC: ↑ 1090.14 % THCA: ↑ 25.58%				
Fluorescent tubes (180-200 ˜µmol/m^2/s) LED blue and red light (blue-to-red ratio of 4:1) (˜90 µmol/m^2/s)	Cannabinoid: ↑ 58.9%	35	The plants were grown using fluorescent tube lights during the vegetative period and were illuminated with LED lights during flowering	CS12 dan CS14 (high THC)	Namdar *et al*.[21]
Dynamic spectrum LED	THC: ↑ 26% CBD: ↑ 0.79%	112	*Cannabis* plants were grown in a controlled environment with varying light spectral treatments at different growth stages	Cultivar Gorilla Glue (feminized)	Arora and Yun.[20]
Rose (430 + 630 nm, Blue: Red (1:10))	CBD: ↓ 20.45 % THC: ↓ 22.05%				
Red (630 nm)	CBD: ↓ 37.5% THC: ↓ 37%	56	*Cannabis* plants were grown in a controlled environment with LED treatment	The specific variety used has not been mentioned	Morello *et al*.[22]
Amber (595 nm)	CBD: ↓ 52.27% THC: ↓ 53.54%				
Purple (430 + 630 nm, Blue:Red (2:1))	CBD: ↓ 34.09% THC: ↓ 28.74%				
Blue (430 nm)	CBD: ↓ 51.14%				

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	THC: ↓ 43.31%				
	CBG: ↑ 200% CBDA + CBD: ↓ 27.59% THCA + THC: ↑ 25% CBCA + CBC: ↓ 4%			C14 (high THC) variety	
White LED with ~1:1 (blue:red)	CBG: ↑ 92.5% CBDA + CBD: ↑ 28.57% THCA + THC: ↑ 28.57% CBCA + CBC: ↑ 22.22%	58	*Cannabis* plants were grown in a controlled environment with LED treatment	C12 (THC=CBD) variety	Danziger and Bernstein.[23]
	CBG: ↑ 102.67% CBDA + CBD: ↓ 10.34% THCA + THC: ↓ 5.88% CBCA + CBC: ↓ 6.67%			C10 (high CBD) variety	
1:1 (blue:red)	CBG: ↑ 250% CBDA + CBD: ↓ 29.31% THCA + THC: ↑ 18.75% CBCA + CBC: ↑ 4%			C14 (high THC) variety	

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	CBG: ↑ 87.5% CBDA + CBD: ↑ 14.29% THCA + THC: ↑ 14.29% CBCA + CBC: ↑ 8.89%	C12 (THC=CBD) variety
	CBG: ↑ 94.67% CBDA + CBD: ↓ 13.79% THCA + THC: ↓ 11.76% CBCA + CBC: ↓ 6.67%	C10 (high CBD) variety
	CBG: ↑ 150% CBDA + CBD: ↓ 29.31% THCA + THC: ↑ 9.38% CBCA + CBC: ↓ 8%	C14 (high THC) variety
1:4 (blue:red)	CBG: ↑ 42.5% CBDA + CBD: ↑ 12.86% THCA + THC: ↑ 12.86% CBCA + CBC: ↑ 4.44%	C12 (THC=CBD) variety
	CBG: 6.67% CBDA + CBD: ↓ 17.24%	C10 (high CBD) variety

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	THCA + THC: ↑ 17.65% CBCA + CBC: ↓ 22.67%			
High Red (600-700 nm):Far Red (700-800 nm)	CBDA: ↑ 260.71% CBD: ↓ 66.43% CBDVA: ↑ 15% THCA: ↓ 18.33% THCVA: ↑ 361.1%	48	*Cannabis* plants were grown in a controlled environment with LED treatment	Finola variety	Kotiranta *et al*.[24]
Low Red (600-700 nm):Far Red (700-800 nm)	CBDA: ↑ 200% CBD: ↓ 78.93% CBDVA: ↓ 30% THCA: ↓ 35% THCVA: ↑ 177.78%				

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		Blue	CBDA: ↑ 42.86% CBD: ↓ 17.86% CBDVA: ↓ 80% THCA: ↓ 33.33% THCVA: ↑ 116.67%	50		
(7) Daylength and temperatures, genotype, and nutrients	12.5 h daylength/high temperature (27°C day-24°C night)	The highest content of each cannabinoid was found in this treatment	From 10 days, weekly data were collected	The treatment involved simulating tropical environmental conditions with varying day length, temperature, and nitrogen application variations, to evaluate the growth response of varieties	Morphet Late, ECO-YP16, ECO-GH15 and ECO-MC16	De Prato *et al*.[73]
(8) Enviromental stress	Drought treatment	CBGA:↓ 87.17% CBG: ↑ 85.12% CBDA: :↓ 80.08% CBD: ↓ 74.87% THCA: ↑ 20.24% THC: ↓ 85.23% CBDVA: ↑ 21.74% THCA: ↓ 8% THCVA: ↓ 6%	7	The water-deficient condition was simulated by irrigating twice a week with 200 ml of water, while the control plants were grown under well-watered conditions	Green-Thunder	Park *et al*.[8]
(9) Modifying plant architecture	Single prune	THC: ↑ 8.7% CBDA: ↓ 2.27% CBGA: ↓ 3.33% CBCA: ↓ 4.35%	21	Plant decapitation, keeping the six bottom branches	Himalaya	Danziger and Bernstein.[9]

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		BBLR + Defoliation	CBC: ↑ 8.33% CBDVA: ↑ 26.09% THCA: ↑ 12% THCVA: ↑ 14% THC: ↑ 0.87% CBDA: ↑ 13.64% CBGA: ↑ 13.33% CBCA: ↑ 13.04% CBC: ↑ 12.5%		Removal of all leaves and secondary branches from the bottom 1/3 of the plant + Removal of 85% of the leaves (top leaves were kept)		
		Double prune	CBDVA: ↓ 14.35% THCA: ↑ 4% THCVA: ↑ 4% THC: ↑ 13.04% CBDA: ↑ 4.54% CBGA: ↑ 3.33% CBCA: ↑ 4.35% CBC: ↑ 8.33%		Plant decapitation, keeping the six bottom branches, followed by a second pruning (removal of 5 cm from each of the six branches)	Fuji cultivar (high THC)	
	(10) Microgravity	3 days of microgravity treatment	CBD: ↑ 14400%, CBG: ↑ 290%, CBC: ↑ 13.04% THC: ↓ 64.52% CBN: ↓ 13.04%	49	Leaf explants were cultured in the culture medium and subjected to clinorotation for 3 days. The centrifugal acceleration was calculated from zero at the center to 1.12×10^{-3} g at the edge of the callus ring	"Afghan Kush" strain (THC 17.6% in female flower)	Darigh *et al*.[10]
(d) Nutrient Feeding	(1) N, P, K solution	Organic fertilization: Phytosolution Bio NPK 525, Carbon Eco, Epsom salt, Organic stock solution (240), Organic diluted solution (160) [2],	CBD: ↑ 2775%.	99	N, P, and K were added to the growth media	*Cannabis* with CBD-rich chemotype III	Massuela *et al*.[50]

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	Organic diluted solution (80) [3] 100 mg/L N, 32 mg/L P, and 125 mg/L, with further details provided in the article.	Total THC: ↑ 50.7%	63	N, P, and K were added to the growth media	McLove variety (high THC)	Velechovský *et al*.[51]
(2) K	15 mg/L K	The highest total content of THC, THCA, THCVA, CBD, CBDA, CBCA, CBGA, and CBDVA was observed in this treatment compared to the other treatments	74 and 51	K was a added to the growth media	Royal medic (THC:CBD=5% and Desert Queen (high THC)	Saloner and Bernstein.[49]
	131 mg/L K	The highest concentration of Δ^8-THC (4300 ppm), Δ^9-THC (4100 ppm), THCA (1010 ppm), and CBGA (14 ppm) was found in this treatment	98	K was added to the growth media	ECO-GH15 variety	De Prato *et al*.[56]
	129 mg/L K	The highest concentration of CBD (5200 ppm), CBDV (13000 ppm) was found in this treatment		K was added to the growth media		
(3) N	210 mg/L N	The highest total content of THC (0.17%) and CBD (3.7%) was observed in this treatment compared to the other treatments	360	N were added to the growth media	*Cannabis* Clone 97. Varieties with high CBD and low THC (<0.2%)	Dilena *et al*.[54]

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		30 mg/L N	Cannabinoids: 24%				
		80-320 mg/L N	The cannabinoid percentage decreased as the concentration of N increased	56	N were added to the growth media	Annapurna genotype	Song *et al*.[53]
		10% N-NH_4	THCA: ↑ 18.75% THCVA: ↑ 22.22%				
		50% N-NH_4	CBGA: ↑ 25%				
		100% N-NH_4	CBCA: ↑ 75%	59	N were added to the growth media	Annapurna 2 (High CBD)	Saloner and Bernstein.[48]
		0, 10, 30, 50, and 100% N-NH_4	As the concentration of N-NH4+ increases, the levels of CBDA, CBD, and THC decrease				
		160 mg/L N	THC: ↑ 57.14% CBD: ↑ 60% CBGA: ↑ 22.22% CBN: ↑ 25%		N were added to the growth media		
		30-320 mg/L N	As the concentration of N increases, the levels of THCA, THCVA, CBDA, and CBCA levels decrease	56	N were added to the growth media	Annapurna	Saloner and Bernstein.[47]
	(1) DIN	DIN 25 mg/L (equivalent to 76.63 µM)	Total CBD: ↑ 11.41% Total THC: ↑ 3.62%	35	DIN at 76.63 µM was applied as a root-soaking treatment until adventitious root induction	'Hot blonde'	Hahm *et al*.[62]
(e) PGR	(2) Ethephon	Ethephon 1 µM	Total THC: ↑ 127.27% CBD: ↑ 60%	1	Ethephon treatment was carried out by spraying the entire plant with a 1 µM ethephon solution until the solution began to drip	Male *Cannabis sativa* L. plant Female *Cannabis sativa* L. plant	Mansouri *et al*.[59]

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		Ethephon 1240 µM	CBD: ↑ 30%		Ethephon treatment was carried out by spraying from the earliest flowering with a 1240 µM ethephon solution until the solution began to drip	Felina 32 (dominant CBD) variety	
		Ethephon 420 µM	CBG: ↑ 30.96%	2	Ethephon treatment was carried out by spraying the plants from the earliest flowering with a 420 µM ethephon solution until the solution began to drip	Santhica 27 (dominan CBG) variety	Ruzgas *et al*.[60]
		Ethephon 1660 µM	THC: ↑ 32.14%		Ethephon treatment was carried out by spraying the plants from the earliest flowering with a 1660 µM ethephon solution until the solution began to drip	Kompolti (dominant CBD) variety	
	(3) PEO-IAA	PEO-IAA 1 µM	CBDA: ↓ 42.27% CBC: ↓ 5.66% CBD: ~ <0.05 CBDA: ↑ 36.13% CBD: ↑ 14.44% CBC: ↑ 34.8%	28	PEO-IAA 1 µM were a added to the growth media	USO-31 Tatanka Pure CBD	Šenkyřík *et al*.[61]
	(1) *Mucilaginibacter* sp.		THC: ↑ 11.13% CBD: ↑ 11.6%		0.1 OD *Mucilaginibacter* sp. was added to 25 mL of sterile King's B medium		
(f) PGPR	(2) *Pseudomonas* sp.	0.1 OD (1 x 10^8 CFU mL^{-1}	THC: ↑ 5.9% CBD: ↑ 7.2%	70	0.1 OD *Pseudomonas* sp. was added to 25 mL of sterile King's B medium	CBD Kush	Lyu *et al*.[65]
	(3) *Bacillus* sp.		THC: ↑ 4.8%		0.1 OD *Bacillus* sp. was added in 25 mL of sterile King's B medium		
(g) Precursor addition	(1) +CBGA in glandular trichome	100 µM CBGA	THC: ↑ 125% CBD: ↑ 171.43%	56	The treatments involved collecting exudates from	Female plants of *Cannabis sativa* L.	Rodziewicz *et al*.[11]

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					glandular trichomes of *Cannabis sativa*, incubating them with CBGA in hexane to simulate a hydrophobic environment, and analyzing the bioconversion of CBGA into cannabinoid products using GC-MS	strain Euphoria (Royal Queen Seeds, UK) and variety Finola (Finola Oy, Finland)	
(h) HRC	(1) NAA, IBA, and IAA on solid media	0.5 mg/L NAA (equivalent to 2685.19 µM), 0.5 mg/L IBA (equivalent to 2460.18 µM), 0.5 mg/L IAA (equivalent to 2854.14 µM)	Low cannabinoid production and difficulties in scaling up	35	Hairy roots were produced from callus cultures in B5 medium (containing 4 mg/L NAA), subcultured every 30 days, and transferred to medium with various auxins for further optimization, then maintained in liquid culture for three years	The specific variety used has not been mentioned	Farag and Kayser.[57]

Abbreviations: ↑ - increase; ↓ - decrease; BBLR - Bottom branches & leaves removal; CBC - cannabichromene; CBD - cannabidiol; CBDA; cannabidiolic acid; CBGA - cannabigerolic acid; CBG - cannabigerol; DAT - Days after treatment; FS - full spectrum; HRC - hairy root culture; HPS - high-pressure sodium; IAA - Indole-3-acetate acid; IBA - Indole-3-butyric acid; K - potassium; LED - Light emitting diode; MB - mixed blue; MW - mixed white; N - nitrogen; NAA - α-naphthaleneacetic acid; NH_4 - ammonium; NO_3 - nitrate; OD - Optical Density; PEO-IAA - α-(phenylethyl-2-oxo)-IAA; PF - photon flux; PFD - photon flux density; PGPR - plant growth promoting *Rhizobacteria*; PGR - plant growth regulator; T5 - fluorescent tubes; THC - tetrahydrocannabinol; THCA - tetrahydrocannabinolic acid; UV - ultraviolet.

Others included transcription DNA, 2-chloroethyl phosphonic acid, feminization, sexual expression, osmolytes, cellular stress, reactive oxygen species, microbial growth medium, and plant growth-promoting rhizobacteria (PGPR). Although rarely addressed, these topics could serve as potential directions for future studies when their relevance and demand increase. The map differentiated 17 clusters, with different network colors used to show the thematic differences of each cluster, as summarized in Table 2.

Chemical and Physical Elicitation Techniques to Enhance Cannabinoids

The success of increasing cannabinoid content was greatly influenced by the compatibility between the treatment and the tissue's age. This compatibility determined the effectiveness of the treatment in stimulating cannabinoid biosynthesis. Consequently, combining the appropriate treatment with a particular culture type and tissue age was essential for significantly increasing in cannabinoid yield.

Chemical elicitation techniques had enhanced cannabinoid production, with more significant results observed in mature plants than in undifferentiated cells. Chemical elicitation using salicylic acid (SA), gamma-aminobutyric acid (GABA), and methyl jasmonate (MeJA) was explored in various studies.[5,6,7,17] GABA had been identified as the most effective elicitor for increasing tetrahydrocannabinol (THC) levels compared to SA (Table 1, section a.2). At the same time, SA had been shown to enhance cannabidiol (CBD) levels more significantly than GABA, (Table 1, section a.1). These studies showed that cannabinoid levels, notably THC and CBD, could be increased considerably through elicitation, with effectiveness varying depending on the type of elicitor and treatment conditions. A key distinction in these studies was in the type of plant material used. Treatment with SA, GABA, and MeJA did not enhance the expression of cannabinoid compounds in cell cultures.[17] The mechanism of cannabinoid biosynthesis was considered inactive in undifferentiated *Cannabis* cell cultures, which inhibited production of secondary metabolites.[17] However, when the same treatments were applied to mature plants, cannabinoid production increased significantly.[6] Chemical elicitation appeared more effective when applied to mature *Cannabis* plants than to the undifferentiated cell cultures.

The mechanism of gamma-aminobutyric acid (GABA) as an elicitor had been shown to induce enzyme activity and gene expression associated with cannabinoid biosynthesis in *Cannabis* plants. GABA significantly increased the expression of THCAS, along with other genes such as OLS and prenyltransferase (PT), which were also upregulated in response to this elicitor. OLS, a type III polyketide synthase (PKS), served as the enzyme that produced olivetolic acid (OAC).[5,39] OAC then formed CBGA,[40,41] as showed in Figure 4. However, a decrease in THCA, CBDAS, OLS, and PT levels was observed at higher concentrations of GABA.[5] These results suggested that while GABA could stimulate the expression of key enzyme genes in cannabinoid biosynthesis, excessive inputs of GABA levels triggered feedback inhibition or stress responses that reduced overall cannabinoid production.

The mechanism by which salicylic acid (SA) functioned as an elicitor to enhance cannabinoid biosynthesis was mediated through gene expression and stress induction. Responses were induced by SA that activate genes included in various biosynthetic pathways for plant defense. [17,45] In this context, stress caused by SA was responded to by activating the enzyme geranyl pyrophosphate synthase (GPPS). In *Nepeta cataria* L., GPPS, along with the nepetalactol-related short-chain dehydrogenase enzyme (NEPS), was involved in the nepetalactone biosynthetic pathway, which was associated with a reduction in defense responses. [46] However, in cannabinoid biosynthesis, GPPS catalyzed the formation of geranyl pyrophosphate (GPP), the primary precursor for cannabinoids, as shown in Figure 4. Therefore, increased GPPS activity, as a response to SA-induced stress, was thought to contribute to enhanced cannabinoid biosynthesis in *Cannabis*.

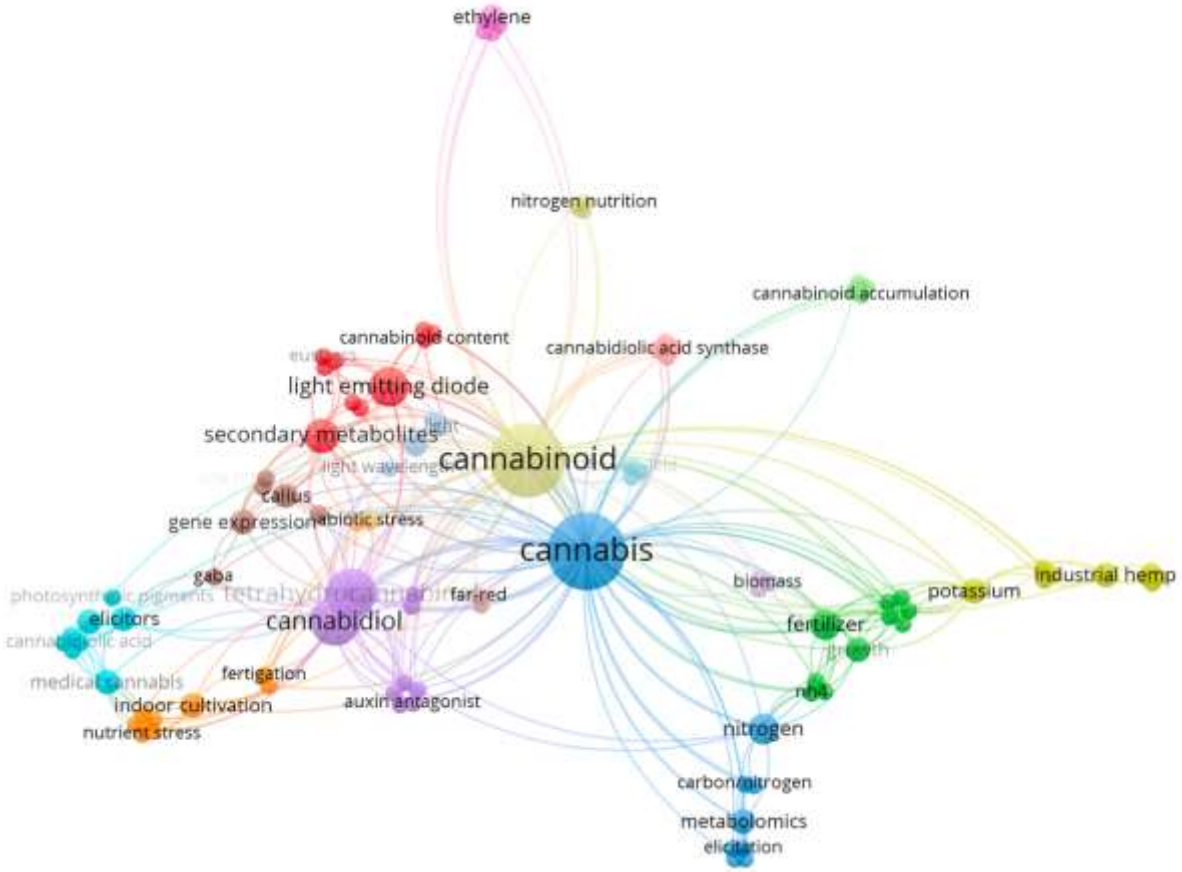

Figure 3: Keyword co-occurrence network

In comparison, treatment with MeJA was found to enhance the regulation of the GPPS gene in plants. This increased GPPS expression had been reported in several species, including *Gossypium* spp., [42] *Catharanthus roseus*, [43] *Litsea cubeba*, [44] and *Tripterygium regelii*, [45] resulting in increased terpenoid content and biomass. MeJA had been shown to enhance the biosynthesis of cannabinoid precursor CBGA in medical *Cannabis*. Therefore, cannabinoid biosynthesis was stimulated by MeJA through the upregulation of the GPPS gene in its biosynthetic pathway.

In addition to chemical elicitation, cannabinoid production was also enhanced by physical elicitation through light-emitting diode (LED) lighting and plant architectural modification, specifically in mature *Cannabis* plants compared to undifferentiated cells. Plant architecture modification improved cannabinoid uniformity by increasing light exposure and reducing shading in lower inflorescences. This reduced variation in cannabinoid concentrations along the plant. [9] Ultraviolet (UV) radiation on cell culture was reported to exhibit no significant effect on cannabinoid production (Table 1, sections c.1 and c.2). [17] Meanwhile, the effects of LED lighting on the upper canopies and top leaves of *in vivo Cannabis* plants had been shown to increase cannabinoid production significantly (Table 1, section c.6). [18,19,20,21,22,23,24] The best results were obtained using red and blue light in the correct proportion. A higher ratio of red light compared to blue light was shown to increase the levels of CBD significantly, cannabidiolic acid (CBDA), THC, and tetrahydrocannabinolic acid (THCA) compared to treatments involving modifications to plant architecture (Table 1, section c.6). LED lighting can induce specific mechanisms and responses in plants, which affected their stress response. Therefore, using LED light with a higher red-to-blue ratio effectively increased cannabinoid production in *Cannabis* plants *in vivo*, rather than the undifferentiated cells.

The 2 cases presented, chemical and physical treatments, showed that differentiated tissues were a more appropriate target, compared to the undifferentiated ones, to increase cannabinoid production. This could be due to the absence of specialized structures, such as trichomes, in the undifferentiated tissues, which were essential for cannabinoid biosynthesis and accumulation. [25] The THCA synthase gene, responsible for THC production, was not expressed in cell cultures and was detected only in tissues containing trichomes, such as leaves and flowers. [17] Furthermore, *Cannabis* suspension cultures were reported not to produce CBDA. [26] Cannabinoids like cannabichromene acid (CBCA), CBGA, and THCA had also been toxic to suspension cell cultures, causing cell death in both suspension and leaf cells. [25,26,27] Cannabinoid synthases were secreted into storage cavities to prevent cellular damage, supporting the hypothesis that trichomes served as protective storage sites for plant cells. [12,27] This explained why undifferentiated cell cultures were less effective for cannabinoid production.

Although undifferentiated cells were generally incapable of producing cannabinoids, an enhancement in cannabinoid production in *Cannabis* callus cultures had been reported under modified microgravity conditions. Microgravity was defined as a condition of reduced gravity used to promote plant growth and development.

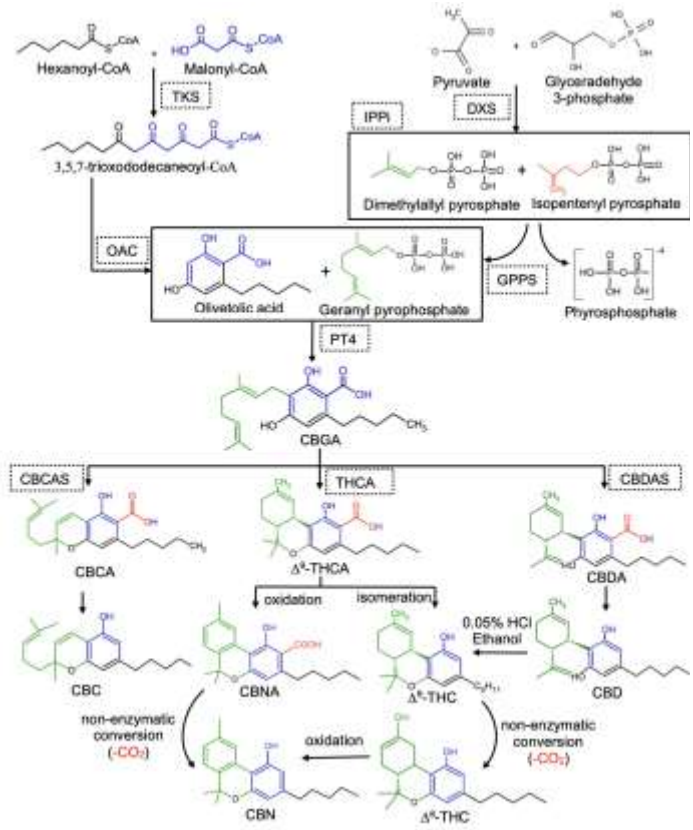

Figure 4: Biosynthesis pathway of cannabinoids in *Cannabis sativa* L. derived from recent reviews.[74,75,76,77] Abbreviations: Δ^9-THC - Δ^9-tetrahydrocannabinol; Δ^8-THC - Δ^8-tetrahydrocannabinol; CBC - cannabichromene; CBCA - cannabichromentic acid; CBCAS - cannabichromentic acid synthase; CBD - cannabidiol; CBDA - cannabidiolic acid; CBDAS - cannabidiolic acid synthase; CBGA - cannabigerolic acid; CBN - cannabinol; CBNA - cannabinol acid; DMAAP - dimethylallyl pyrosphate; DXS - 1-deoxy d-xylulose-5-phosphate; GPPS - geranyl pyrophosphate synthase; IPP - isopentenyl pyrosphate; IPPi - isopentenyl-diphospate; OAC - olivetolic acid; PT4 - methylerythritol phosphate; THCA - tetrahydrocannabinolic acid; THCAS - tetrahydrocannabinolic acid synthase; TKS - tetraketide synthase.

Clinorotation was identified as the most commonly applied technique for simulating microgravity in plant growth studies.[10,28,29] In *Cannabis*, microgravity levels ranging from zero gravity to 1.12×10^{-3} g at the edge of the callus ring were investigated. Increases in CBG and CBD levels and decreases in cannabinol (CBN) and THC levels had been observed in callus subjected to clinorotation (Table 1, section c.10).[10] The enhancement of cannabinoids in the callus was attributed to physiological and molecular modifications induced by microgravity conditions. Increased transcriptions of the olivetolic acid (OA), olivetol synthase (OLS), cannabidiolic acid synthase (CBDAS), and tetrahydrocannabinol acid synthase (THCAS) genes were reported following microgravity treatment.[10] These alterations were validated through high-performance liquid chromatography (HPLC) and molecular assessments. The contribution of microgravity to biomass accumulation in callus cultures had also been documented.[10] Furthermore, several key mechanisms, including changes in cell proliferation, cell wall stiffness, cytoskeleton organization, gene regulation, and energy production efficiency, were influenced by microgravity.[29,30,31,32,33,34,35,36,37,38] In summary, despite the undifferentiated tissues being an ineffective resource under normal gravity, their utility in cannabinoid production was remarkably boosted upon the application of microgravity, which could be attributed to physiological and molecular modulations.

Impact of Nutrient Feeding in Cannabinoid Biosynthesis

Nitrogen (N), phosphorus (P), and potassium (K) were essential macronutrients that indirectly influenced the biosynthesis of cannabinoids. Several studies had shown their impact in enhancing cannabinoid yield.[47,48,49] Optimal combinations of these 3 nutrients in the growth media resulted in a significant increase in the accumulation of cannabidiol (CBD) and tetrahydrocannabinol (THC) compared to controls (Table 1, section 2.1).[50,51] However, another study found that only nitrogen was significantly shown to increase cannabinoid content, while phosphorus and potassium had a negative impact.[52] Although several studies showed the potential role of N and K in cannabinoid production, the correlation remained unclear. However, it was assumed that the appropriate combination of macronutrients influenced cannabinoid biosynthesis indirectly through their effect on plant metabolism.

The concentration of N in the growth media was correlated with cannabinoid yield regardless of cultivar. Song *et al*. (2023) and Dilena *et al*. (2023) reported that an increase in N concentration reduced cannabinoid content (Table 1, section d.3) regardless of the cultivar used, with 'Annapurna' in the former, [47,53,] and 'Clone 97' in the latter.[54] Although 'Annapurna' was characterized as Indica with a THC: CBD ratio of approximately 1:1, and Clone 97 produced female flowers rich in CBD and low in THC (<0.2%), both studies reported a similar pattern, where cannabinoid content decreased as N concentration increased. This effect could be attributed to the role of nitrogen in shifting metabolic priorities, with vegetative growth favored over secondary metabolite production. The synthesis of photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) was generally enhanced by increased N, which supported photosynthesis and vegetative growth.[47,55] However, this shift in metabolic priority could occur at the expense of secondary metabolite production, such as cannabinoids. Based on the results from Song *et al*. (2023) and Dilena *et al*. (2023), a lower N concentration in the growth media was recommended to enhance cannabinoid accumulation.

Similar to nitrogen, studies had also shown that soil potassium (K) content impacted cannabinoid yield, although conflicting results were reported. De Prato *et al* (2022) applied K through irrigation using K_2SO_4 solutions to potted ECO-GH15 plants grown in cocopeat and perlite. This resulted in increased levels of Δ^8-tetrahydrocannabinol (Δ^8-THC), Δ^9-tetrahydrocannabinol (Δ^9-THC), THCA, CBGA, CBD, and cannabidivarin (CBDV), in cultivar ECO-GH15 (Table 1, section d.2).[56] Saloner and Bernstein (2022) supplied K^+ through fertigation at increasing concentrations to 'Royal Medic' and 'Desert Queen' cultivars grown in perlite, and observed a decrease in cannabinoid content (Table 1, section d.2).[49] Different results could be attributed to some experimental variations, but it was also possible that different *Cannabis* genotypes responded differently to potassium supply.

Despite the showed effects of the macronutrients on cannabinoid biosynthesis, the existing studies had not yet established the regulation mechanisms at the molecular level. Therefore, further exploration of how these macronutrients influenced the expression of key genes in cannabinoid biosynthesis was becoming an intriguing area for future studies. For instance, studying how N and K influenced the expression of key genes in cannabinoid biosynthesis, such as THCAS, CBDAS, OLS, and GPPS.

Plant growth regulators (PGRs) and hairy root

Adding PGRs to the growth medium had been reported to trigger stress responses in *Cannabis* plants. A study was reported using auxins such as NAA, IBA, and IAA to induce hairy root cultures in *Cannabis* and naturally low content of cannabinoids in root (Table 1, section h.1).[57] This approach had shown limited effectiveness for cannabinoid production due to the lack of glandular trichomes in root tissues.[58] Particular PGRs were known to enhance cannabinoid production, such as ethephon,[59,60] α-(2-oxo-2-phenylethyl)-1H-indole-3-acetic acid (PEO-IAA),[61] and diniconazole (DIN),[62] (Table 1, section e.2, e.3, e.1). Ethylene was released by ethephon, causing stress and activating the biosynthesis pathways of secondary metabolites, including cannabinoids, as part of the plant's defence mechanism.[63,64] The stress induced by ethephon could affect the activity of key enzymes such as THCA synthase and CBDA synthase, which converted CBGA into tetrahydrocannabinol (THC) and cannabidiol (CBD), respectively.

Table 2: Keyword co-occurrence network analysis highlights based on keywords

Clusters	Keywords	Thematic interpretation
Cluster 1 (red colors/10 items)	Cannabinoid content, cell viability, dynamic lighting, eustress, harvest index, light emitting diode, light exposure, light spectrum, secondary metabolites, ultraviolet (UV)	This cluster highlights that dynamic lighting with LEDs and varying light spectra can influence cell viability, leading to growth under eustress conditions, cannabinoid and secondary metabolites production, which ultimately impact the harvest index in *Cannabis* cultivation
Cluster 2 (green colors/10 items)	Cultivation, deficiency, development, fertilization, fertilizer, growth, NH_4, nitrate, NO_3, nutrition	This cluster highlights the important relationship between nutrient management in fertilization, specifically through the application of NH_4 and NO_3, and the impact of nutrient deficiency on growth, development, and secondary metabolite production in *Cannabis* cultivation.
Cluster 3 (dark blue colors/9 items)	*Cannabis*, carbon/nitrogen, elicitation, gene, glutamyl-tyramine, metabolomics, nitrogen, secondary metabolism, thca synthase gene	This cluster highlights the production of secondary metabolites, focusing on carbon/nitrogen nutrient ratios, elicitation processes, and specific genes involved in biosynthesis, including the THCA synthase gene and glutamyl-tyramine in the plant *Cannabis*.
Cluster 4 (yellow colors/9 items)	Crop nutrition, dwarfism, gibberellin acid, industrial hemp, physiology, plant factory, potassium, slow-release fertiliser, triazole	This cluster explores the relationship between crop nutrition and plant physiology. It uses compounds such as gibberellin acid and triazole to induce dwarfism in industrial hemp and considers while also considering the use of slow-release fertilizers in *Cannabis* cultivation
Cluster 5 (purple colors/9 items)	Auxin antagonist, cannabidiol, CBC, defoliation, *in vitro*, PEO-IAA, pruning, shoot propagation, tetrahydrocannabinol	This cluster connects the effects of auxin antagonists such as PEO-IAA, defoliation, pruning, and *in vitro* shoot propagation techniques on the production of cannabinoid compounds, including cannabidiol (CBD), cannabichromene (CBC), and tetrahydrocannabinol (THC).
Cluster 6 (light blue colors/ 8 items)	Cannabidiolic acid, elicitors, jasmonic acid, medical *Cannabis*, photosynthetic pigments, salicylic acid, tetrahydrocannabinol acid, γ-aminobutyric acid	This cluster explores the role of elicitors such as jasmonic acid, salicylic acid, and γ-aminobutyric acid in the production of cannabidiolic acid and tetrahydrocannabinol acid, as well as their relationship with photosynthetic pigments in *Cannabis* plants for medical purposes.
Cluster 7 (orange colors/8 items)	Environmental impact, fertigation, indoor cultivation, mineral fertilization, nutrient stress, nutrient use efficiency, organic fertilization, soilless-cultivation	This cluster examines interconnected concepts such as environmental impact, fertigation, indoor cultivation, mineral fertilization, nutrient stress, nutrient use efficiency, organic fertilization, and soilless cultivation to influence cannabinoid production in *Cannabis* cultivation.
Cluster 8 (brown colors/6 items)	Callus, DNA methylation, GABA, gene expression, hairy root, transcription	This cluster examines callus development into hairy root cultures, and the use of DNA methylation methods, GABA, gene expression, and transcription in plant development and cannabinoid production in *Cannabis* plants.
Cluster 9 (pink colors/5 items)	2-chlorethyl phosphonic acid, ethephon, ethylene, feminization, sexual expression	This cluster examines the effects of 2-chloroethyl phosphonic acid (ethephon) and ethylene on feminization and sexual expression in plants, highlighting their roles in manipulating the reproductive traits of *Cannabis* plants.
Cluster 10 (coral colors/4 items)	Cannabidiolic acid synthase, glandular trichomes, osmolytes, tetrahydrocannabinolic acid synthase	This cluster links cannabinoid biosynthesis in glandular trichomes with the roles of cannabidiolic acid synthase and tetrahydrocannabinolic acid synthase in producing CBD and THC, as well as osmolytes in this process in *Cannabis* plants.

Cluster 11 (light green/4 items)	Cannabinoid accumulation, cellular stress, LED light composition, reactive oxygen species	This cluster refers to cannabinoid accumulation influenced by LED light composition. These can affect cellular stress and reactive oxygen species, which in turn impact the growth and development of *Cannabis* plants.
Cluster 12 (light steel blue/4 items)	HPS, light, light wavelength, spectrum	The effects of HPS (high-pressure sodium) and LED lights with varying light wavelengths on the growth, development, and cannabinoid production in plants are examined in this cluster.
Cluster 13 (light yellow/4 items)	Cannabinoid, nitrogen nutrition, phenotype, time to flowering	This cluster examines the relationship between nitrogen nutrition and plant phenotype to cannabinoid production and time to flowering in *Cannabis* plants.
Cluster 14 (light purple/3 items)	Biomass, metabolism, nitrogen/metabolism	This cluster examines the interaction between nitrogen utilization in biomass production and its impact on cannabinoid production in *Cannabis* plants.
Cluster 15 (sky blue /3 items)	Flower yield, microbial growth medium, plant growth-promoting rhizobacteria	This cluster highlights the effects of plant growth-promoting rhizobacteria on microbial growth media and their impact on flower yield in cannabinoid production.
Cluster 16 (light orange /2 items)	Abiotic stress, biotic stress	This cluster examines the effects of induced abiotic and biotic stress on plant cannabinoid production.
Cluster 17 (light brown /2 items)	Far-red, UVB	This cluster examines the effects of far-red and UVB light on plant growth and development and as well as their impact on cannabinoid production.

Abbreviations: CBC - cannabichromene; CBD - cannabidiol; HPS - high-pressure sodium; LED - Light emitting diode; N - nitrogen; NH_4 - ammonium; NO_3 - nitrate; THC - tetrahydrocannabinol; UV - ultraviolet.

This could lead to increased levels of THC, CBD, and cannabigerol (CBG).[59,60] The appropriate concentration of ethephon was considered crucial for maximizing the stress response that drove cannabinoid biosynthesis. In this context, a concentration of 1 µM was effective (see Table 1, section e.2). Therefore, ethephon could enhance cannabinoid production by managing plant stress.

Plant Growth Promoting Rhizobacteria

Plant Growth Promoting Rhizobacteria (PGPR) used in nutrient-rich King's B medium had been shown to affect cannabinoid production. Bacteria such as *Bacillus* sp., *Mucilaginibacter* sp., and *Pseudomonas* sp. applied to this medium were found to increase the accumulation of cannabidiol (CBD) and tetrahydrocannabinol (THC) (Table 1, section f.1, f.2, f.3).[65] The consistency of these results was reinforced by a study that also reported the positive role of PGPR in cannabinoid production.[66] Therefore, further exploration of potential microbes that could enhance the efficiency of *Cannabis* production was of great importance.

The mechanism behind the increased cannabinoid biosynthesis was linked to nutrient uptake enhanced by PGPR, which supported the biosynthesis of secondary metabolites.[65] Through mechanisms such as nitrogen fixation, phosphate solubilization, and indole-3-acetate acid (IAA) production, the availability of essential nutrients required for secondary metabolite biosynthesis was increased.[67,68] Besides supplying raw materials, the interaction between PGPR and plants had modulated metabolic pathways involved in cannabinoid synthesis and plant defense mechanisms against environmental stress.[65] This suggested that the increased cannabinoid content in PGPR-inoculated plants was likely due to a combination of improved nutrient availability and the activation of related biosynthetic pathways.

Precursor Feeding

Precursor feeding in *Cannabis* had only been reported in the context of CBGA supplementation in synthetic trichomes, with no studies yet exploring upstream precursors beyond CBGA. Most existing studies focused on genetic engineering [69,70] and metabolic pathway optimization in microorganisms to enhance production of precursors, such as olivetolic acid (OAC), a precursor to CBGA.[71] While such studies focused on microbial production, this review emphasized precursor feeding as a non-genetic strategy for enhancing cannabinoid biosynthesis. The concept of precursor feeding had been successfully applied in azadirachtin production in *Azadirachta indica*,[72] suggesting that a similar approach could be developed to increase cannabinoid levels in *Cannabis*. Therefore, this strategy still required further exploration to determine its effectiveness in enhancing cannabinoid biosynthesis.

The addition of CBGA precursor had been reported to increase cannabinoid content in synthetic trichomes (Table 1, section g.1). CBGA was converted into tetrahydrocannabinolic acid (THCA) or cannabidiolic acid (CBDA), which served as precursors for THC and CBD, respectively (refer to Figure 4). This conversion process was catalyzed by the enzymes tetrahydrocannabinol acid synthase (THCAS) and cannabidiolic acid synthase (CBDAS), which were active both within trichome cells and in the trichome exudates (the fluid secreted by trichomes), allowing the conversion to occur outside the cells.[11] Therefore, adding CBGA as a precursor enhanced production of tetrahydrocannabinol (THC) and cannabidiol (CBD) in synthetic trichomes through intracellular and extracellular conversion.

In addition to CBGA, the unique properties of trichome exudates supported enzymatic activity in cannabinoid biosynthesis, even outside plant cells. These exudates contained amphipathic components that enabled the conversion reactions by THCAS and CBDAS within the trichomes' hydrophobic environment.[11] This showed that cannabinoid biosynthesis occurred inside the cells and in trichome exudates outside the secretory cells, opening new opportunities for increased cannabinoid production.

Conclusion

In conclusion, the age of the plant tissue plays a significant role in production of cannabinoids. Mature plants with more trichomes, the primary sites for cannabinoid biosynthesis, tend to produce higher amounts of cannabinoids. However, undifferentiated tissues lacking trichomes are not preferable for producing cannabinoids. Microgravity has been reported to activate genes responsible for cannabinoid biosynthesis in undifferentiated tissues. Therefore, callus cultures can be further explored as a resourceful material. The microgravity's mode of action in regulating cannabinoid gene expression remains a large area for study. Due to the limited non-genetic-engineering reports on *Cannabis* available at present, an extensive study is necessary to

determine the optimal conditions that facilitate cannabinoid biosynthesis across different stages of plant tissue development. Using mature, fully differentiated tissues and applying microgravity in callus culture seems to be the most practical and shortest non-genetic approach to advance cannabinoid production.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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