Original Research Article

Shoot Culture of Ocimumsp and ItsPhytochemical profile

Abstract.

The success of the in vitro culture technique is influenced by many factors, including the type of explants, basic medium and exogenous plant growth regulators (PGR). These factors greatly influence the speed and effectiveness of plant regeneration as well as the quantity and quality of the phytochemical compounds produced. Therefore, this study aims to determine the effect of the PGR combination on the growth response of in vitro nodal explants as well as the phytochemical profiles of Ocimum sp. Nodal explants obtained from 2 weeks old in vitro seedlings were cultured on MS medium with the addition of ZPT, namely BAP and Kinetin (0.2 and 5 mg/l) combined with NAA (0.1 and 0.2 mg/l) and synthetic cytokinins namely Thidiazuron (TDZ) (1, 3, and 5 mg/l). The growth response of the explants and the potential for regeneration were observed for 8 weeks of culture. Effect of adding activated charcoal (AC) to root media on growth of plantlets aged 2 weeks. Phytochemical profile of in vitro shoot was analyzed using GCMS and LCMS to be compared with its profile in in vitro callus tissue. The results showed that MS medium with the addition of a combination of cytokinin and auxin was able to induce shoot regeneration in nodal explants ofOcimum sp. in vitro. The Kinetin/NAA combination produced better shoot height growth, while the BAP/NAA combination produced a higher leaves number. Thidiazuron at all concentrations was able to induce shoots that were more likely to form rosettes. The addition of AC to the rooting medium did not have a positive effect on the response of shoot and plantlet growth. Chromatographic screening showed different profiles of secondary compounds in the callus and shoottissues of Ocimum sp. in vitro. Callus composed of actively dividing cells do not produce some of the secondary compounds produced in vitro shoots. This shows the difference in the potential of cells or tissues in synthesizing secondary metabolites.

1. Introduction

Basil is a medicinal plant that is a member of the genus Ocimum of the family Lamiaceae, which has more than 150 species (Dzoyem et al., 2017). Basil plants or Ocimum species are aromatic or ornamental plants which contain many essential oils and various secondary metabolites such as anthocyanins, flavonoids, and rosmarinic acid.For pharmaceutical purposes, the utilization of Lamiaceae species has major constraints, namely variations at the individual level caused by heterogeneous genetics and biochemistry since hybridization and polyploidy between species occur frequently in this genus (Harley & Heywood, 1992). In addition, propagation by seed has problems due to poor viability and low germination frequency. In vitro culture technique is an alternative method to be able to produce offspring that are true-to-type and could improve secondary metabolite products. In vitro systems have been widely implemented in the medicinal plants micropropagation. The production of secondary metabolites which naturally depend on the growth phase of plants and only in small amounts makes tissue culture techniques an alternative solution to these problems.

In vitro micropropagation is an effective and relatively fast way of propagation of species that require high progeny uniformity. Plant cell, tissue and organ culture technology has been proven to be an alternative for all plant systems in producing natural products. Therefore, large-scale propagation of aromatic and medicinal plants using in vitro techniques is gaining increasing interest(Grigoriadou et al., 2019; Máthé et al., 2015).The success of the in vitro culture technique is influenced by many factors, such as the type of explants, basic medium and exogenous growth regulators (PGR). These factors greatly influence the speed and effectiveness of plant regeneration and the phytochemical compounds produced both quantitatively and qualitatively.In vitro studies in the genus Ocimumhave been reported using a variety of explants, such as node segment explants(Ahuja et al., 1982; Begum et al., 2000; Shahzad & Siddiqui, 2000), leaves (Phippen & Simon, 2000), young flowers (Singh & Sehgal, 1999) and axillary buds(Begum et al., 2000, 2002).In vitro system can produce different profiles of secondary metabolites when compared to the original plant(Al-Qudah et al., 2011; Miguel, 2018). Therefore, the goal of this study is to determine the combination of growth regulators effect on the shoot induction of Ocimum sp. nodal explants in vitro and itsphytochemical profiles.

2. Materials and Methods

2.1. Seedgermination and explants preparation

Basil seeds (Ocimum sp.) were washed with running tap water for 1 hour and sterilized with 10% commercial bleach solution for 15 min. Subsequently, seeds were rinsed with sterile distilled water for 5 minutes three times. The sterilized seeds were germinated in water medium solidified with agar without the addition of either macro and micronutrients or PGR. Two weeks old seedling derived from seed germination were ready to be used as a source of explants.

2.2. Shoot induction media

Murashige and Skoog (MS) basal salt medium were used supplemented with 3% sucrose and solidified with 1.1 % (w/v) agar. ThepH was adjusted to 5.8 by adding NaOH or HCl 0.1 N prior to sterilization using autoclave at 121 °C, 1.5 atm for 15 minutes. A combination of cytokinins (BAP/ kinetin 2 and 5 mg/l) and auxin NAA (0, 0.1 and 0.2 mg/l) as well as a single thidiazuron in several concentrations (1, 3, and 5 mg/l) was added to observe their effect on regeneration and multiplication of shoot.

2.3 Shoot regeneration and multiplication

The first nodes of seedling were used as explants and placed vertically on shoot induction medium. Six bottles were repeated for each type of explant and each culture was filled with two explants. All cultures were kept in incubation room under continuous illumination from fluorescent white light (light intensity about 600 lux) at 24±2°C,with relative humidity of 60-65%. The leaves number were counted, and shoot height were measured every week for four weeks.

Comment [h1]: Give the total number of explants cultured by type of explant and by medium

2.3. In vitro rooting

Single shoots were separated from the shootsclupms and then transferred to the rooting medium (MS medium without hormones) with or without the addition of 0.1% (w/v) AC (activated charcoal). Shoot and root development was observed two weeks after subculture.

2.4. *Phytochemical analysis using GCMS and LCMS*

Phytochemical analysis using GC-MS and LC-MS based on (Hassan et al., 2022) with modification. Phytochemical screening and identification of phytochemical compounds were carried out on in vitro shoots and callus of Ocimum. The screening stage includes sample extraction using 95% methanol with a ratio of 1:5, followed by dilution of the extract with 95% methanol solvent to a concentration below 100 ppm. After being homogenized with a vortex and centrifuged at 8000 rpm for 10 minutes the resulting supernatant was used for the protein precipitation and purification stages with Solid Phase Extraction (SPE). The purified solution was then filtered with a 0.45 µm cellulose acetate filter membrane and the solution was ready to be analyzed by LC-MS. The Shimadzu LCMS – 8040 LCMS was used for this study. The filtration was carried out using polytetrafluoroethylene (PTFE) membrane filter (0.45 μm size). One microliter (1 μL) of the filtrate was introduced (injected) into the liquid chromatographical system. This was separated on Shimadzu Shim Pack FC-ODS (2 mm x 150 mm, 3 µm) column. Running was undertaken at a flow rate of 0.5 mL/min. The column temperature was fixed at 35°C with isocratic mobile phase mode. Scan range from *0,6 sec/scan (mz: 10-1000)* was used to acquire mass spectra.

Comment [h2]: Give the total number of shoot samples and calluses used for each type of analysis.
Specify the culture medium(s) from which the calluses and shoots originated

The GC system (Shimadzu GCMS QP 2010 SE) with ZB – AAA (10 mL x 0,25 mmLD (Phenomenex Inc) capillary column was used for this study. Helium (99.99%) gas was used to maintain flow rate of 0.6 mL/min at a constant column. GC-MS spectral lines was detected using the ionization energy method, in a 0.2 second scan time with a ranging fragment from 40 to 600 m/*z*, with one microliter (1 μL) injection quantity (split ratio 10:1). The temperature was maintained at 250°C. The column oven temperature was at 50°C running for 3 minutes and 10°C temperature increase per minutes up to 280°C, with a final temperature of about 300°C for 10 minutes. The compound profiles icluding peak number, RT (retention time) per minute, curve area, composition and the structure and analysis were compared with the standard library of phenolic compounds.

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3. Results and Discussion

3.1. *Shoot induction*

The node explants showed a positive response in shoot induction on all media with various combinations of cytokinin:auxin. In general, shoot induction begins with the emergence of lateral shoots in the leaf axils 3-7 days after (Figure 1A). Furthermore, adventitious shoots emerge from the node tissue in varying numbers (Figure 1B). After four weeks of culture, every combination of cytokinin:auxin showed different effect on shoot growth (Figure 2). In general, the kinetin/NAA combination produced higher shoots (0.59 – 2.41 cm) than those produced by the BAP/NAA combination (0.2 – 0.9 cm). On the other hand, the kinetin/NAA combination produced fewer leaves (0.59 - 1.38) than those produced by the BAP/NAA combination (5.88 - 30.0). The addition of a single cytokinin either kinetin or BAP had a less favorable growth effect than the addition of a combination of cytokinin/auxin. According to (Yang et al., 2017) auxin and cytokinins are needed synergistically to control shoot growth. The highest leaf number was produced in the medium with the addition of 2 or 5 mg/l BAP combined with 0.1 and 0.2 mg/l NAA.

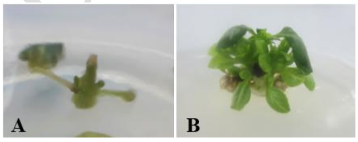

Figure 1. Development of nodal explants and shoot regeneration. A. lateral shoots in the leaf axils, B. Multiple shoots derived from nodal tissues.

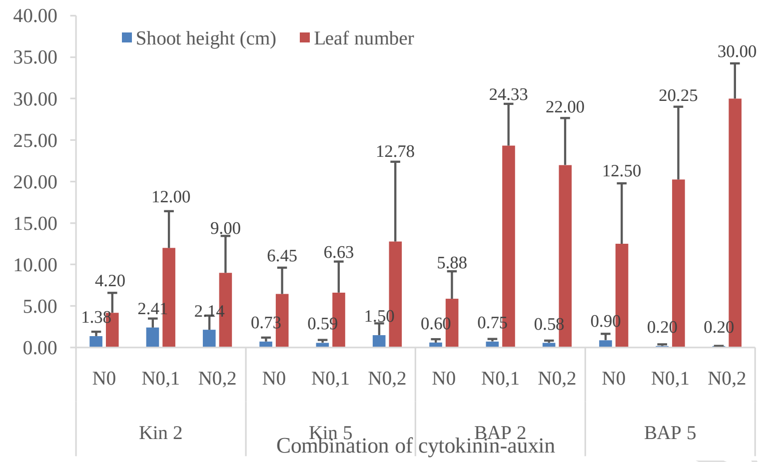

Figure 2. Shoot regeneration derived from nodal explants of *Ocimum* sp. four weeks after culture. ~~Notes:~~ Kin: Kinetin, N: NAA

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The concentration of thidiazuron tested was able to produce leaves but the number was less than the use of BAP or kinetin. After four weeks of culture the medium supplemented with 3 and 5 mg/l thidiazuron produced the number of leaves 14.7and 14.2, respectively (Figure 3). Shoots derived from Thidiazuron treatment were generally clustered to form a rosette (Figure 3A). Shoot height on medium with the addition of thidiazuron could not be measured properly when compared to shoots produced on media with the addition of a combination of cytokinins and auxin (Figure 3B). Thidiazuron also showed poor response in shoot formation of *O. sanctum* when compared with zeatin and BA(Manjudevi et al., 2022). However, thidiazuron could induce rapid micropropagation of *O. basilicum* using shoot tip explants (Siddique & Anis, 2007) and secondary shoot growth with various types of explants except root (Enkhbileg et al., 2019). An efficient method for micropropagation of basil on MS medium supplemented with thidiazuronwas established using epicotyl, hypocotyl and shoot tip explants cultured (Ekmekci & Aasim, 2014).

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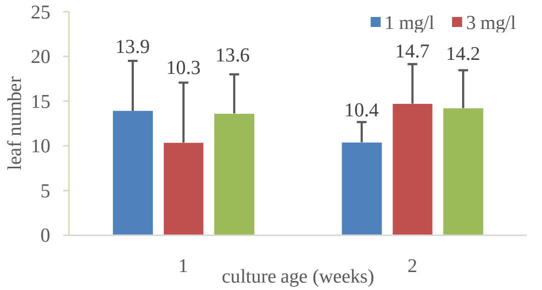

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Figure 3. The leaf number of Ocimum sp. in vitro in media containing thidiazuron

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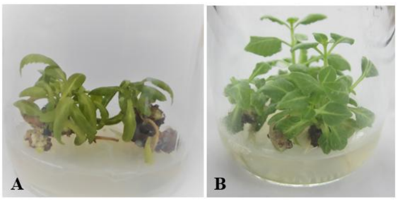

Figure 4. Shoot growth of Ocimum sp. in vitro. A. MS medium + single TDZ, B. MS medium + combined cytokinin-auxin

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3.2. Root Induction

Four weeks after culture to the shoot induction medium the regenerated shoot was able to form roots without subculture to rooting medium. The quality and quantity of roots formed on shooting medium were varied. In general,medium supplemented with BAP and NAA showed poor ~~respons~~ responsein root formation (Figure 5). Meanwhilethe combination of Kinetin and NAA appears to be most suitable for producing large quantities of roots. This is contrary to the effect of the combination of these growth regulators on shoot height (Figure 2). The addition of kinetin alone produces more roots than the addition of BAP alone.Kinetin is a synthetic cytokinin which generally has a stronger effect than its natural compounds.

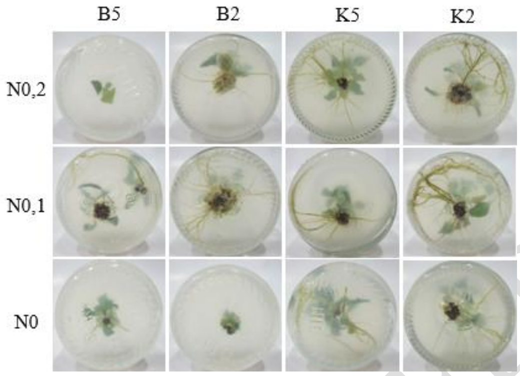

Figure 5. Rooting in shoot induction medium with various combinations of auxin-cytokinin (mg/l) (N = NAA, B = BAP, K= Kinetin)

In this study the addition of AC to the medium tends to reduce the growth of Ocimum sp. in vitro. Shoot and leafnumber of in the medium without AC was always higher than in the medium with the AC (Figure 6). While the addition of AC slightly increased shoot height. In Ocimum sanctum micropropagation, the percentage of roots, roots number, and root length were positively affected by the addition of AC when compared to control media without AC(Shukla et al., 2021). According to (Winarto, 2011) the addition of AC to the medium can produce root morphology with a larger diameter. This suggests that AC appears to affect nutrient absorption in the culture medium. However, in this study ACdid not show a significant effect on the root's growth (Figure 7).

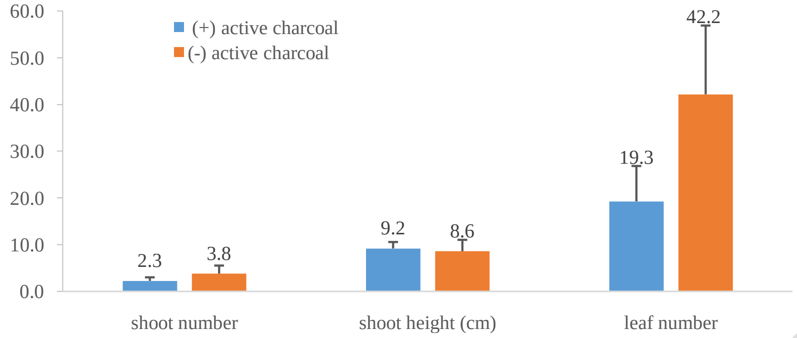

Figure 6. Effect of AC (activated charcoal) in hormone free MS medium on shoot growth of Ocimum sp.

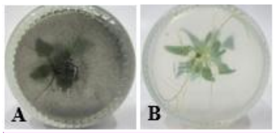

Figure 7. Effect of AC (activated charcoal) on root induction. A. medium with the addition of AC, B. medium without the addition of AC.

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Activated charcoal (AC) is a porous carbonized substance with a large inner surface area (Dong et al., 2020) therefore they can adsorbe many substances (Thomas, 2008). Although AC can adsorp unwanted substances/inhibiotors(Buckseth et al., 2018) the addition of AC into tissue culture media is often associated with its role in increasing cell growth and development. Analysis of the main metabolic pathways in wheat seedling revealed that AC stimulates the expression of nine genes in the phenylpropanoid biosynthetic pathway that promote cell differentiation and seedling growth (Dong et al., 2020). However, its addition to culture media can enhance or inhibit growth in vitro, depending on the species and tissue used (Pan & Van Staden, 1998). Even the addition of AC to the culture media has an unfavorable effect, namely a drastic reduction in the concentration of PGR and other organicsupplements. Activated charcoal in low and moderate concentrations were impelling shoot formation *of Oroxylum indicum* the shoots, while root induction did not occurred in high concentrations of AC (Bansal & Gokhale, 2012). It is possible that the addition of high concentrations of AC may induce nutrient deficiencies in culture media. Such a deficiency will affect explant growth (Pan & Van Staden, 1998). The effects of AC can be attributed to the formation of a dark environment which can promote rooting (Sharma et al., 2012).

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3.3. Phytochemical profiles of Ocimum sp. *in vitro*

The results of phytochemical analysis of Ocimumsp. using GC-MS methods separated 78 volatile polar compounds with retention times ranging from 1.176 to 36.591 min Figure 8A-B). Meanwhile, analysis using LCMS method detected 97 compounds with retention times ranging from 1.475 to 58.098 min (Figure 8C-D). The first peak wasβ-ocimene with molecular weight of 136.2380 (Figure 9a), while the last peak was ocimumoside B with molecular weight of 893.2060 (Figure 9b). The compound with the smallest curve area of 73.9199 was germacrene B (retention time 5.483 min) (Figure 9c) while the compound with the largest curve area of 1554.2209 was gallic acid (retention time 3.042 min) (Figure 9d).

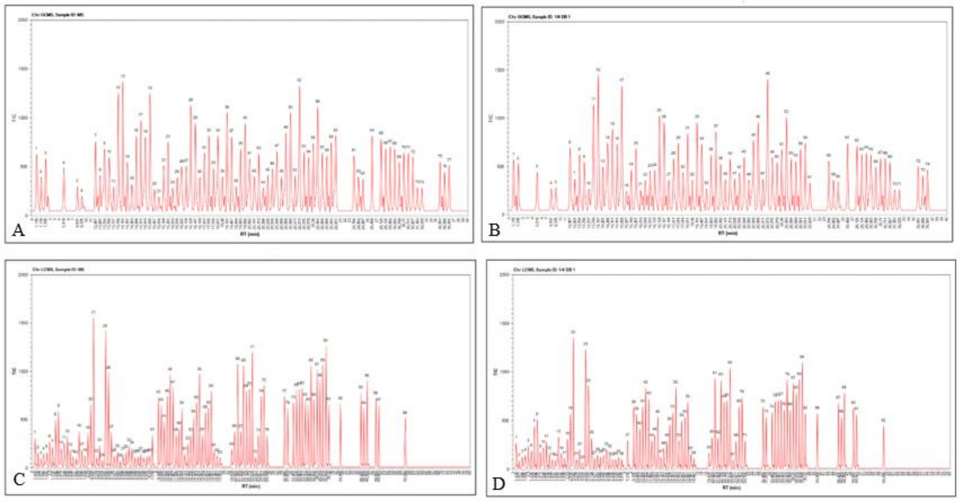
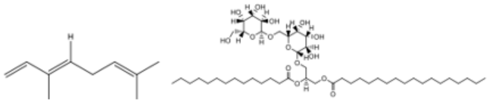


Figure 8. Chromatography profile of Ocimum sp. in vitro. A-B. GCMS analysis, B-C. LCMS analysis, A,C Shoot tissues, B,D callus tissues



(a) β-ocimene ($C_{10}H_{16}$)
BM: 136.2380

(b) ocimumoside B ($C_{47}H_{88}O_{15}$)
BM: 893.2060

(c) Germacrene B($C_{15}H_{24}$)
BM: 204.3570

(d) Galic acid ($C_7H_6O_5$)
BM: 170.1200

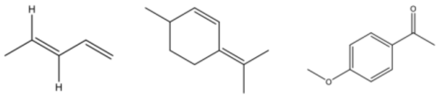
Figure 9. Phytochemical types in in vitro Ocimum sp. (a). the first retention time, (b). the last retention time, (c) the smallest curve area, (d) the largest curve area

The results of analysis using the GC-MS and LC-MS methods showed differences in the phytochemical profiles of Ocimum shoots and callus in vitro. There were 78 volatile non-polar compounds detected by GC-MS (Table 1). Seventy-three of these compounds were produced by both shoot and callus in vitro. However, there are four compounds produced by shoot but not detected in callus, namely 1,3-pentadiene (C_5H_8), p-menthadiene ($C_{10}H_{16}$), 4'-methoxyacetophenone ($C_9H_{10}O_2$), and α-calcorene ($C_{15}H_{20}$). In contrast, the longifolene compound ($C_{15}H_{24}$) only appeared in callus but not in shoot (Figure 10).

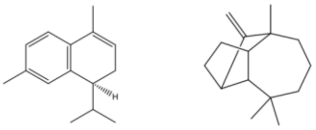
Table1. Volatile non-polar compounds in shoot and callus in vitro of Ocimum

No	Compounds	Shoot	Callus	No	Compounds	Shoot	Callus
1	methyl formate	+	+	40	citronellylformate	+	+
2	**1,3-pentadiene****	**+**	**-**	41	geranyl acetate	+	+
3	methyl butyrate	+	+	42	lavandulyl acetate	+	+
4	1-octen-3-ol	+	+	43	citronellyl acetate	+	+
5	2-phenylethyl alcohol	+	+	44	**α-calcorene****	**+**	**-**
6	p-cymene	+	+	45	(E,E)-α-farnesene	+	+

7	β-phellandrene	+	+	46	α-bourbonene	+	+
8	α-terpinolene	+	+	47	α-cadinene	+	+
9	α-pinene	+	+	48	α-cubebene	+	+
10	β-pinene	+	+	49	α-humulene	+	+
11	β-myrcene	+	+	50	α-muurolene	+	+
12	limonene	+	+	51	γ-muurolene	+	+
13	β-ocimene	+	+	52	α-ylangene	+	+
14	α-terpinene	+	+	53	germacrene A	+	+
15	**p-menthadiene****	**+**	**-**	54	β-bisabolene	+	+
16	γ-terpinene	+	+	55	germacrene B	+	+
17	3-hexenyl acetate	+	+	56	germacrene D	+	+
18	(2R)-nonan-2-ol	+	+	57	β-caryophyllene	+	+
19	thymol	+	+	58	β-chamigrene	+	+
20	4'-ethylacetophenone	+	+	59	β-elemene	+	+
21	**4'-methoxyacetophenone****	**+**	**-**	60	bicyclo-germacrene	+	+
22	dehydro-1,8-cineole	+	+	61	**longifolene***	**-**	**+**
23	geranial	+	+	62	allo-aromadendrene	+	+
24	sabinene	+	+	63	β-selinene	+	+
25	1,4-cineole	+	+	64	γ-cadinene	+	+
26	cis-rose oxide	+	+	65	geranyl propionate	+	+
27	linalool	+	+	66	elemol	+	+
28	citronellal	+	+	67	farnesol	+	+
29	geraniol	+	+	68	α-copaene	+	+
30	isoborneol	+	+	69	spathulenol	+	+
31	citronellol	+	+	70	10-epi-γ-eudesmol	+	+
32	isomenthol	+	+	71	β-eudesmol	+	+
33	neoisomenthol	+	+	72	11-selina-4-α-ol	+	+
34	eugenol	+	+	73	nerolidol	+	+
35	safrole	+	+	74	T-cadinol	+	+
36	1-octen-3-yl acetate	+	+	75	selina-4,11-diene	+	+
37	methyleugenol	+	+	76	geranyl butyrate	+	+
38	coniferylalcohol	+	+	77	geranyl isobutyrate	+	+
39	geranyl formate	+	+	78	citronellyl butyrate	+	+



(a) 1,3-pentadiene (b) p-menthadiene (c) 4'-methoxyacetophenone



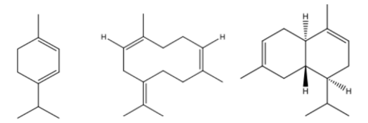
(d) α-calcorene (e) longifolene

Figure 10. Specific volatile non-polar compounds detected in shoot and callus in vitro of Ocimum sp. (a-d). the compounds only detected in shoot, (e) the compound olny detected in callus

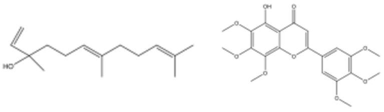
The type of tissues affected the composition of the secondary compounds synthesized in Ocimum sp. cultured in medium MS + 0.25 mg/l 2,4-D + 1 mg/l BAP. Non-volatile compounds in Ocimum shoot tissue in vitro detected by HPLC were 97 compounds. However, only 92 compounds were detected in callus tissue (Table 2). Five compounds, namely α-terpinene ($C_{10}H_{16}$), germacrene B ($C_{15}H_{24}$), ocimarin ($C_{12}H_{12}O_4$), nerolidol ($C_{15}H_{26}O$) and gardenin A ($C_{21}H_{22}O_9$) were not detected in callus culture (Figure 11).

Table 2. Nonvolatile compounds in shoot and callus in vitro of Ocimum

No	The compounds	Shoot	Callus	No	The compounds	Shoot	Callus
1	β-ocimene	+	+	50	thymoquinol 2-O-B-glucopyranoside	+	+
2	**α-terpinene***	**+**	**-**	51	cirsillineol	+	+
3	α-pinene	+	+	52	eupatorin	+	+
4	limonene	+	+	53	nevadensin	+	+
5	α-terpinolene	+	+	54	gardenin B	+	+
6	β-myrcene	+	+	55	5,6,4'-trihyfroxy-7,3'- dimethoxyflavone	+	+
7	β-phellandrene	+	+	56	chlorogenic acid	+	+
8	cinnamic acid	+	+	57	gardenin D	+	+
9	estragole	+	+	58	gardenin E	+	+
10	thymol	+	+	59	labiatenic acid	+	+
11	1,8-cineole	+	+	60	thymonin	+	+
12	geraniol	+	+	61	squalene	+	+
13	borneol	+	+	62	gardenin C	+	+
14	linalool	+	+	63	**gardenin A***	**+**	**-**
15	p-coumaryl alcohol	+	+	64	α-amyrin	+	+
16	citronellal	+	+	65	apigenin-7-O-glucoside	+	+
17	4-methoxycinnamaldehyde	+	+	66	cosmoslin	+	+
18	p-coumaric acid	+	+	67	kampferol-3-O-rhamnoside	+	+
19	safrole	+	+	68	acacetin-7-glucoside	+	+
20	eugenol	+	+	69	acacetin-7-galactoside	+	+
21	gallic acid	+	+	70	quercetin-3-O-rhamnoside	+	+
22	coniferaldehyde	+	+	71	luteolin-7-glucoside	+	+
23	methyleugenol	+	+	72	betullinic acid	+	+
24	coniferylalcohol	+	+	73	hirsutrin	+	+
25	caffeic acid	+	+	74	quercetin-3-glucoside	+	+
26	ferulic acid	+	+	75	chicoric acid	+	+
27	menthyl acetate	+	+	76	kampferol-3-(2"-acetylrhamnoside)	+	+
28	germacrene A	+	+	77	luteolin-7-O-(6"-malonylglucoside)	+	+
29	α-cubebene	+	+	78	quercetin-3-O-malonylglucoside	+	+
30	**germacrene B***	**+**	**-**	79	apigenin-7-(6"-p-coumarylglucoside)	+	+
31	α-copaene	+	+	80	luteolin-7-aplosyl (1-2) glucoside	+	+
32	β-caryophyllene	+	+	81	naringin	+	+
33	β-chamigrene	+	+	82	apigenin-7-[rhamnosyl (1-2) galacturoside]	+	+
34	α-farnesene	+	+	83	acacetin-7-rutinoside	+	+
35	β-selinene	+	+	84	kaempferol-7-rhamnoside-4'-glucoside	+	+
36	germacrene D	+	+	85	luteolin-3'-methyl ether-7-apiosyl (1-2) glucoside	+	+
37	α-zingiberene	+	+	86	apigenin-7-glucoside-4'-trans-caffeate	+	+
38	**β-cadinene***	**+**	**-**	87	kampferol-3-(5"-feruloylaloside)	+	+
39	ocimarin	+	+	88	luteolin-7-O-rutinoside	+	+
40	**nerolidol**	**+**	**-**	89	kampferol-3-(6"-caffeoyglucoside)	+	+
41	sinapic acid	+	+	90	quercetin-3-(3"-p-coumarylglucoside)	+	+
42	apigenin	+	+	91	tulsinol E	+	+
43	acacetin	+	+	92	apigenin-7-rhamnoside-4'-rutinoside	+	+
44	genkwanin	+	+	93	kaempferol-3-glucoside-2"-rhamnoside-7-rhamnoside	+	+
45	luteolin	+	+	94	apigenin-7-rutinoside-4'-trans-caffeate	+	+
46	kaempferol	+	+	95	quercetin-3,7,4'-triglucoside	+	+
47	quercetin	+	+	96	ocimumoside A	+	+
48	ladanein	+	+	97	ocimumoside B	+	+
49	salvigenin	+	+				



(a) α-terpinene (b) germacrene B (c) β-cadinene



(d) nerolidol (e) gardenin A

Figure 11. Specific non-volatile compounds only detected in shoot in vitro of Ocimum sp.

In vitro culture techniques are often used as a strategy to produce secondary metabolites. According to (Marchev et al., 2014) in vitro culture can change the pathway of secondary metabolite synthesis by expanding the production of phytochemicals in plants. Metabolite production which may represent a similar metabolite profile to native plants have been reported in types of ogan culture such as shoots or roots (Karuppusamy, 2009). Callus culture also has the potential to produce secondary metabolites that have therapeutic significance(Fazili et al., 2022). Even callus culture has been reported to be more reliable than collecting plant material from the wild for extracting therapeutic metabolites (Chandran et al., 2020). On the other hand, PGR are one of the most important factors influencing cell growth, differentiation, and formation of metabolites(Jain et al., 2012). The composition of in vitro culture and tissue media also determines the accumulation of secondary compounds. In this study, the different phytochemical profiles in shoot and callus tissue were thought to be caused by the PGR involved. Therefore, knowledge related to the use of PGR and the types of secondary metabolites produced is very important.

4. Conclusion

This study showed that in vitro Ocimum shoots were successfully induced by the addition of a combination of NAA and BAP. The adding of activated charcoal had no significant effect on shoot growth or root formation. Regenerated shoots from nodal explants from in vitro callus tissue had a different phytochemical profile. This proves that PGRin addition to affect cell growth and development it also the synthesis or production of secondary compounds.

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