Integrative
Plant Biotechnology
ISSN: 3007-2735 (Online), 3007-2727 (Print)

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Research Article

Determination of Acid Value and Saponification Number From *In Vitro* Callus Cultures of Olive (*Olea europaea*) cv. Koroneiki

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ABSTRACT

Olive (*Olea europaea* L.). cv. Koroneiki belongs to the family Oleaceae, which is an evergreen plant of Mediterranean origin. Commonly, this plant is the source of olive oil used for various purposes in human life. To determine the biochemical characteristics of the extracted olive oil from specific callus lines of common olive, we investigated the effects of several hormones on callus induction from field-grown plants. Mature leaves, nodal, and internodal explants were surface-sterilized and inoculated on MS medium. Plant growth regulators in varying amounts were added to MS medium (2,4-D, BAP, IBA, NAA, or TDZ) alone or in combinations, forming a total of 29 treatments for callus induction. The highest (95.45%) callus induction was obtained with 8 + 2 µM BAP + IBA after 35 days of initial culture from leaf explants, followed by 6 µM 2,4-D (75.55%) under the dark culture room conditions. Internode was unable to form callus. Off white, yellowish, fluffy, friable, proliferating calluses were obtained from leaf explants. Such highly proliferating calluses were selected as callus cell lines (CL); CL1 (2, 4-D 4 µM) and CL2 (2, 4-D 6 µM) for oil extraction and subsequent biochemical characteristics of acid value and saponification number. Results demonstrated that CL1 showed the highest acid value (1.25) and saponification number (183). This was followed by CL2, which showed acid values of 1.42 and 1.84, and saponification values of 1.84 and 1.42, respectively. The present study demonstrated an efficient callus induction protocol for subsequent determination of biochemical attributes from the extracted oil of *in vitro* dedifferentiated olive tissues.

Keywords: Acid value, callus Induction, callus lines, olive, saponification number

OPEN ACCESS

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Article History
Received: June 04, 2025
Accepted: June 25, 2025
Published Online: June 29, 2025

Cite this article
Jamil, I., Akram, M., & Aftab, F. (2025). Determination of Acid Value and Saponification Number From In vitro Callus Cultures of Olive (Olea Europea) cv. Koroneiki. Integrative Plant Biotechnology, 3, 153–162.

INTRODUCTION

The olive (*Olea europaea* L.) is a long-lived tree that belongs to the family Oleaceae. The Mediterranean Basin is an ideal place to grow olives, as it provides the favorable conditions necessary for their development. Spain, Italy, and Greece are the primary producers of olive oil, while Spain and Turkey are the primary producers of table olives (Fabbri *et al*., 2008; Gomes *et al*., 2012). Olive oil ranked sixth (Gunstone *et al*., 1994), and a recent study by Abrante-Pascual *et al*. (2024) placed olive oil in fourth place among all vegetable oils worldwide. Due to its culinary qualities and the volatiles that give it a distinct flavour, olive oil has a high market value. Due to the inclusion of many acyl compounds, olive oil has a high nutritional value for a person's daily diet, and its consumption is associated with a lower rate of heart disease (Trevisan *et al*., 1990). Due to various environmental and physiological alterations in the seeds, traditional olive germination is delayed (Graniti *et al*., 2011). The embryo of olive seed contains inhibitory chemicals that cause germination to be postponed for a year or two (Lal *et al*., 2015).

The lengthy germination period of olives limits the utilization of all prior standard methods for *in vivo* germination. Both rootstock production and olive breeding projects benefit from the efficient methods of plant tissue culture, which break the dormancy of olive seeds and shorten the time required for optimal seedling output (Chiancone *et al*., 2008). Special attention is paid to the callus cultivation technique under controlled settings to examine the generation of bioactive compounds from olives. *In vitro* callus induction is a beneficial method for subsequent shoot regeneration and biochemical processes. Callus induction has previously been reported in olives (Shibli *et al*., 2001; Shibli and Al-Juboory, 2002; Capelo *et al*., 2010; Mohammad *et al*., 2019). These reports demonstrated the use of callus cultures for somatic embryogenesis, shoot regeneration, and callus biomass production only in different cultivars of olives. Reports on the determination of biochemical attributes from dedifferentiated *in vitro* tissues of olive callus are scanty. It is, therefore, the present study aimed to induce calluses from the mature tissues of olive cv. Koroneiki for subsequent oil extraction and determination of metabolites from callus cell lines.

MATERIALS AND METHODS

Plant material and culture conditions

One-year-old olive plants of the variety Koroneiki were purchased from the Barani Agriculture Research Institute in Chakwal, Pakistan, and brought to the lab at the Institute of Botany, Punjab University, Lahore. MS (Murashige and Skoog, 1962) medium enriched with several plant growth regulators (PGRs) to induce callus from mature explants of leaves, nodes, or internodes in a Laminar Air Flow Cabinet (Esco Life Sciences Group). The medium was prepared by mixing the specific number of stocks of all nutrients, vitamins, iron EDTA, 3% sucrose and PGRs and pH 5.7 was adjusted solidified with 0.8% agar (Agar Technical, Oxoid, UK) and dispensed 10-15 ml was medium into each Pyrex (Japan) culture vessel (25 × 150 mm). The mouth of culture vessels was sealed with a plastic sheet tied with rubber bands and autoclaved at 121°C and 15 psi pressure. The sterilized medium was then placed in a culture room until use. Leaves, nodes, internodes, and seeds were washed in a 2% detergent suspension for 10 min and surface sterilized with 20% commercial bleach (Robin, Reckitt Benckiser, Pakistan, Ltd.), containing 6% active chlorine, for 15–20 min, followed by 70% ethanol for 30 sec. The explants were then washed twice with sterile distilled water to remove traces of surfactants and inoculated on MS medium supplemented with different PGRs (Table 1). All the surgical items were also sterilized in an autoclave. Glassware was sterilized by keeping it in an incubator for 20 minutes.

Callus induction and maintenance

A variety of auxin and cytokinin concentrations, either separately or in combination, were added to MS agar-solidified media before the leaves (5 mm^2), node (5 mm long), and internode (5 mm long) were inoculated, as indicated in Table 1. After the initial culture period of 35 days, data on callus morphology and induction rate were recorded.

Table 1. Plant growth regulators alone or in combination are used for callus induction in the olive cv. Koroneiki.

Plant growth regulators (µM)					
2,4-D	2,4-D + NAA	2,4-D + BAP + NAA	BAP + IBA	BAP + NAA	TDZ
2	2+2	0.5+2+2	2+2	2+2	1
4	4+2	0.5+5+5	4+2	4+2	2
6	6+2	0.5+8+8	6+2	6+2	3
8	8+2	0.5+10+10	8+2	8+2	4
10	10+2		10+2	10+2	5
Total treatments					29

Whereas, 2,4-D (2,4-Dichlorophenoxyacetic acid), BAP (6-Benzylaminopurine), IBA (Indole-3-butyric acid), NAA (α-Naphthaleneacetic acid), TDZ (Thidiazuron; 1-Phenyl-3-(1,2,3-thiadiazol-5-yl)urea), µM (microMolar).

Callus cell line

Healthy calluses on the corresponding medium that grew quickly were selected as callus cell lines (CL). To later assess the oil contents, calluses of the chosen lines were further cultivated on the appropriate fresh media. To extract the oil, 1g of the callus tissue was placed in a 35 ml centrifuge tube with 10 ml of ethanol and 0.1% butylated hydroxytoluene (BHT). After extensively mixing the samples in a vortex and homogenizing them at the level for 0-120 seconds, 1.5 cc of saturated KOH solution was submerged in a water bath at 60 °C to saponify them. The samples were then vortexed for 15-75 seconds, followed by the addition of distilled water (2 ml), hexane (6 ml), and centrifuged (10 min) at 4 °C. The hexane phase was scraped out. This method was performed three times. Extracts were chilled and collected following water droplet evaporation in a Speed vac evaporator, then kept at 0 °C.

$$Oil\ Contents\ (\%) = \frac{\text{Wt. of the extracted lipids}}{\text{Wt. of the callus tissue}} \times 100$$

Acid value

Fifty milligrams of oil (W) was dissolved in 1.5 ml of ethyl alcohol:diethyl ether solvent in an equal ratio (1:1 v/v) to calculate the acid value. Then, using phenolphthalein as an indicator and titrated against NaOH (0.1N: V). The following formula was used to determine the acid value (Bockisch, 1998).

$$Acid\ Value = \frac{56.1 \times \text{N} \times \text{V}}{\text{w}}$$

The following formula was used to calculate the free fatty acid (FFA) percentage: acid value multiplied by factor 0.503.

$$\text{FFA (\%)} = 0.503 \times \text{acid value}$$

Saponification number

By dissolving 50 mg of oil in 0.5% ethanolic KOH (1 ml) and refluxing it in a water bath for 30 minutes, the saponification number was ascertained. The heated mixture was then titrated with 0.5 N HCl captured in the burette after a few droplets of phenolphthalein were added as an indicator. Applying the following formula, the number for saponification was calculated (Bockisch, 1998).

$$Saponification\ number = \frac{56.1\text{N (V1} - \text{V2)}}{\text{w}}$$

Where, N= HCl Normality, V1= Used HCl volume, V2= Used HCl as blank, W= Oil weight.

Statistical Analysis

The data were analysed by analysis of variance (ANOVA) (Steel and Torrie, 1960), followed by Duncan's multiple range test (Duncan, 1955) for the determination of mean comparison using SPSS v.16.0 at a p≤0.05 probability level. R^2 values were calculated by using the Microsoft Excel function.

RESULTS AND DISCUSSION

***In vitro* seed germination**

After the first 25 days of the initial culture, olive seeds (var. Koroneiki) germinated on a plain MS medium. The germination rate was 30%, and the cotyledons that emerged from the developing seeds had an uneven shape and a yellowish-green hue. Due to insufficient embryo reserve storage and inadequate development of the pro-vascular structures, the germinating seeds failed to progress into the seedling stage (Figure 1A) (Germanà *et al*., 2014). In contrast, Acebedo *et al.* (1997) documented an ideal germination percentage for olive embryos *in vitro*. Similarly, while growing olive seeds in a controlled environment, Kiani *et al*. (2006) achieved an embryo germination rate of 86% before transferring the seedlings to the field.

Callus induction and morphology

Table 2 showed that leaf explants were highly sensitive to PGRs to produce calluses of varying morphology and consistency (Figure 1 A-K). The trend of the effectiveness of explants for callus induction has been demonstrated in Figure 2 A-F. Callus started from the cut margins of leaves after 10 days to 90 days, with varying induction percentages. None of the PGRs showed 100% callogenesis. Amongst the treatments used (Figure 2 A-F), the highest (95.45%) callus induction was obtained at 8 + 2 µM BAP + IBA from leaf explants (Figure 2 E) after 90 days of initial culture (Figure 1 B, C). This was followed by 75.55%, 25.12% callus induction with 4, 6, or 8 µM 2,4-D (Figure 1D, E, F; Figure 2 A-C), respectively, from leaf explants after 30 days of culture. The lowest (2 µM) and highest (10 µM) levels of 2,4-D were unable to induce callus development in either explant type. The nodal explant showed little callus formation at an optimum level of PGRs (Figure 2 A, B, E). Nodal explants could form 22.24% callus on the same medium composition as stated above. The internode remained unaffected (Figure 2). The calluses were off-white to yellowish. Initially, the calluses were compact; however, on subsequent transfer to a fresh induction medium, they changed into a friable consistency. Callus consistency depends upon the type of explants as well as culture conditions (Holme *et al*., 1996). When 0.5 µM 2,4-D is combined with 5 µM BAP or NAA, 60.12% callus induction was obtained from leaf explants. The higher concentrations (8 + 8 µM) of both BA + NAA were less effective, with 30.33% callus formation (Table 2). Node as well as internodal explants did not show any morphological changes in either treatment. The morphology of calluses was greyish green to yellow (Figure 1G). Such calluses were unable to grow further due to necrosis and browning in culture. However, light yellow and friable calluses were also obtained with the remaining treatments.

Table 2. Influence of various PGRs levels on callus induction of olive cv. Koroneiki

PGRs (µM)	Callus induction (%)			Culture's morphology
	Leaf	Node	Internode	
2,4-D				
2	0^{h}	0^{d}	0^{d}	-
4	$22.15±5.55^{ef}$	0^{d}	0^{d}	Off-white, brown callus
6	$75.55±5.21^{b}$	$10.12±2.22^{c}$	0^{d}	Yellowish compact callus
8	$25.22±2.44^{e}$	0d	0^{d}	Light yellow friable callus
10	0^{h}	0^{d}	0^{d}	-
2,4-D + NAA				
2 + 2	0^{h}	0^{d}	0^{d}	-
4 + 2	0^{h}	0^{d}	0^{d}	-
6 + 2	$25.21±2.33^{e}$	0^{d}	0^{d}	Dark greenish-yellow callus
8 + 2	$35.32±4.33^{de}$	$15.23±3.21^{b}$	0^{d}	Compact green-brownish callus
10 +2	0^{h}	0^{d}	0^{d}	-
NAA + BAP				
2 + 2	0^{h}	0^{d}	0^{d}	-
4 + 2	$50.50±4.36^{d}$	0^{d}	0^{d}	Friable dark brown callus
6 + 2	0^{h}	0^{d}	0^{d}	-
8 + 2	0^{h}	0^{d}	0^{d}	-
10 + 2	0^{h}	0^{d}	0^{d}	-
2,4-D + BAP + NAA				
0.5 + 2 + 2	0^{h}	0^{d}	0^{d}	-
0.5 + 5 + 5	$60.12±6.21^{c}$	0^{d}	0^{d}	Compact greyish green callus
0.5 + 8 + 8	$30.33±1.41^{de}$	0^{d}	0^{d}	Yellowish green callus
0.5 + 10 + 10	0^{h}	0^{d}	0^{d}	-
BAP + IBA				
2 + 2	0^{h}	0^{d}	0^{d}	-
4 + 2	0^{h}	0^{d}	0^{d}	-
8 + 2	$95.45±5.55^{a}$	$22.24±4.24^{a}$	0^{d}	Fluffy off-white callus
10 + 2	0^{h}	0^{d}	0^{d}	-
TDZ				
1	0^{h}	0^{d}	0^{d}	-
2	0^{h}	0^{d}	0^{d}	-
3	0^{h}	0^{d}	0^{d}	-
4	$15.14±2.22^{f}$	0^{d}	0^{d}	Granular yellow callus
5	$10.04±1.45^{fg}$	0^{d}	0^{d}	Granular light yellowish grey callus

Mean values (±SE) that are accompanied by distinct lowercase letters indicate statistically significant differences, as determined by Duncan's Multiple Range Test (DMRT) at a significance level of $p \leq 0.05$. Each value represents the average of three replicates, and each experiment was conducted three times. Whereas, 2,4-D (2,4-Dichlorophenoxyacetic acid), BAP (6-Benzylaminopurine), IBA (Indole-3-butyric acid), NAA (α-Naphthaleneacetic acid), TDZ (Thidiazuron; 1-Phenyl-3-(1,2,3-thiadiazol-5-yl) urea), µM (microMolar).

Capelo *et al*. (2010) induced olive callus from leaf explants. Leaf explants are usually the best explant material for *in vitro* callus induction or for subsequent regeneration or somatic embryogenesis (Shibli *et al*., 2001; Shibli and Al-Juboory, 2002; Ahmad *et al*., 2010; Capelo *et al*., 2010; Zhu *et al*., 2018). Shibli *et al*. (2001) reported callus induction from root explants for somatic embryogenesis of olive cv. Nabali. Similarly, somatic embryogenesis has also been reported from olive callus cultures var. sylvestris in 2010 (Capelo *et al*., 2010). In 2002, Shibli and Al-Juboory again reported callus induction for studies to investigate salinity stress and water deficit in dedifferentiated tissues of olive cv. Nabali. Apart from these studies, the highest callus induction (50%) was obtained from stem explants pulsed with silver nanoparticles and further cultured to MS

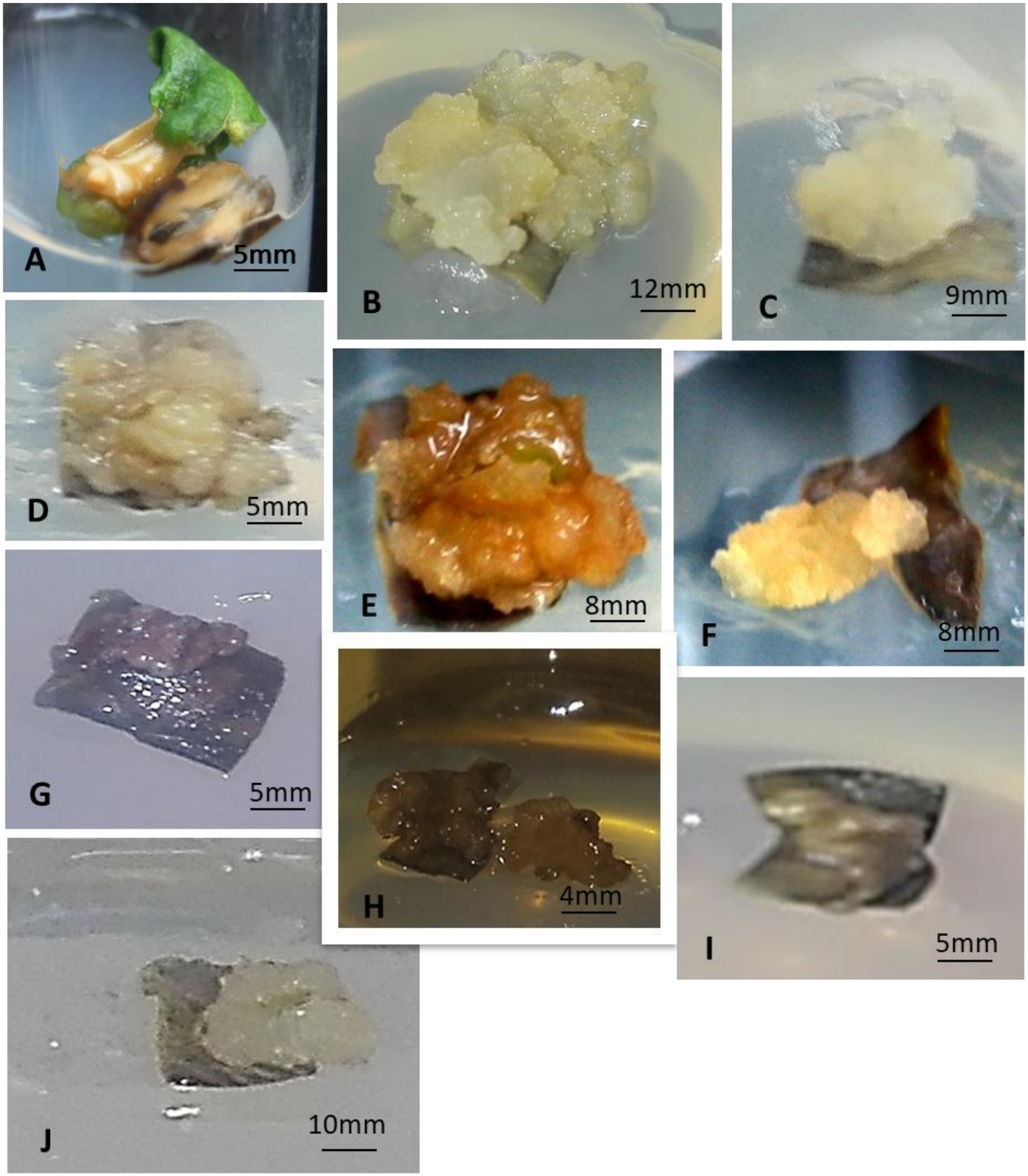

Figure 1. Callus induction from different explants affected by different explants of olive cv. Koroneiki. **A)** *In vitro* seed germination after 25 days of initial culture. **B, C)** 90-day-old calluses at 8μM BAP + 2M IBA. **D)** After 30 days of the initial culture, callus development occurred at 4 μM 2, 4-D. **E)** Following 30 days of initial cultivation, yellow friable callus at 6 μM 2, 4-D. **F)** After 30 days of inoculation, white callus development at 8 μM 2, 4-D. **G)** Greyish green callus formed on MS medium supplemented with NAA + BAP + 2,4-D (5+5+0.5 μM). **H)** After a month of initial cultivation, yellowish brown callus development at 4 + 2 μM NAA + BAP. **I)** A dark greenish-yellow callus formed at 6 + 2 μM 2, 4-D + NAA after 35 days of initial culture. **J)** Granular yellow callus development at 4μM TDZ after 40 days of initial cultivation. 2,4-D (2,4-Dichlorophenoxyacetic acid), BAP (6-Benzylaminopurine), IBA (Indole-3-butyric acid), NAA (α-Naphthaleneacetic acid), TDZ (Thidiazuron; 1-Phenyl-3-(1,2,3-thiadiazol-5-yl) urea), μM (microMolar).

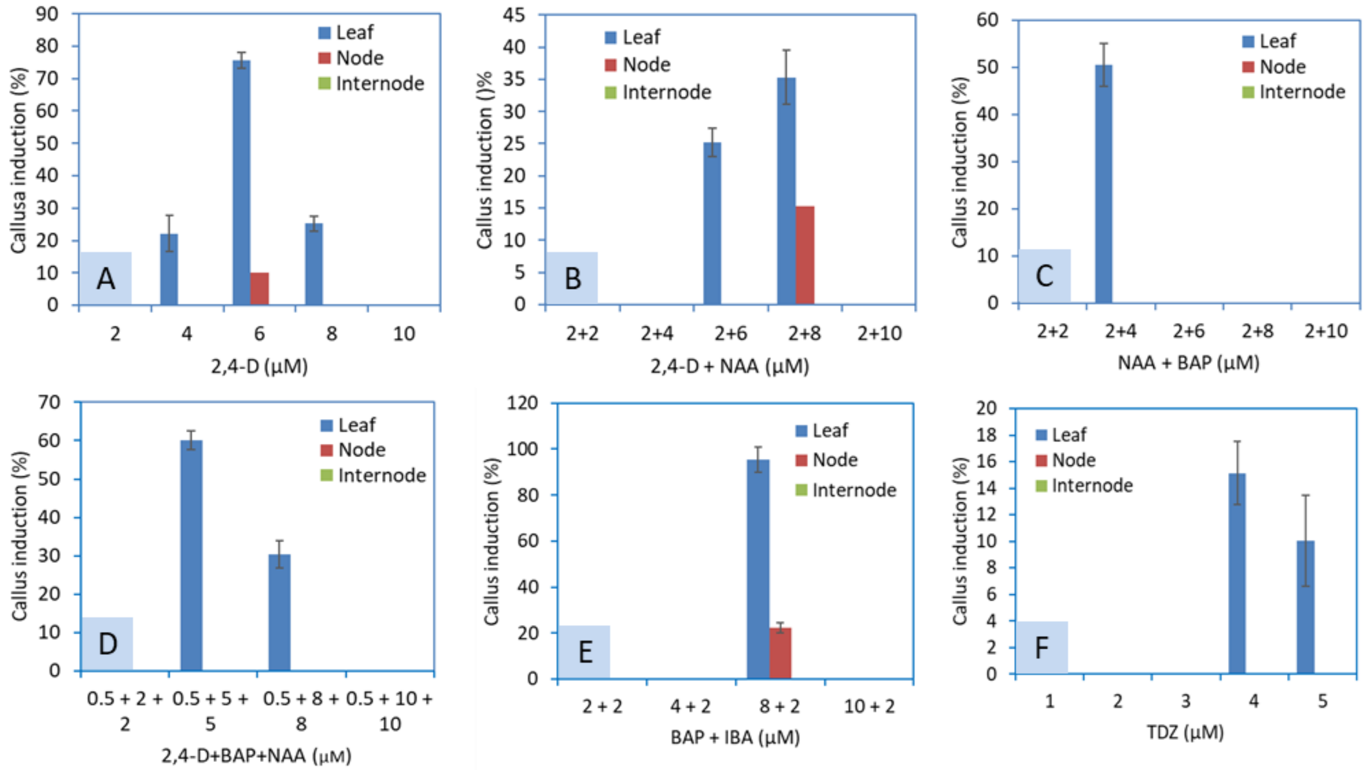

Figure 2. Trend of callus induction by different treatments from different explants of olive v. Koroneiki. Graphs (A-F) show that the leaf explant was most effective for callus induction. Whereas, 2,4-D (2,4-Dichlorophenoxyacetic acid), BAP (6-Benzylaminopurine), IBA (Indole-3-butyric acid), NAA (α-Naphthaleneacetic acid), TDZ (Thidiazuron; 1-Phenyl-3-(1,2,3-thiadiazol-5-yl) urea), µM (microMolar).

media + BAP 2 mg/l, Gibberellic acid 1.5 mg/l + NAA 0.5 mg/l for polyphenols and antioxidant studies. Other than leaf and nodal explants of the present study, petiole explants of mature olives were also the potential source for callus induction and somatic embryogenesis (Capelo *et al.*, 2010). In other species, IBA and BAP have been reported for callus induction from leaf explants with varying morphology (Hossain *et al.*, 2016; Saleem *et al.*, 2024). There was only one treatment (2 + 4 µM) from the combination of BAP + NAA, which formed 50.50% callus from leaf explants after 30 days of initial culture exhibiting yellowish-brown coloration (Figure 1H).

Among the combinations of 2,4-D + NAA, 8 + 2 µM or 6 + 2 µM, only 8 + 2 µM was effective, with 35.32% callus induction from leaf explants (Figure 1I). The former treatment also produced 15.23% callus from nodal explants, whereas the internode was not responsive. Figure 3 shows a comparative analysis of the rate of *in vitro* callus induction. The most effective PGR was 2,4-D, followed by BAP + IBA and 2,4-D + BAP + NAAA, respectively. The rate of callus induction decreased when 2,4-D was used with NAA. Similarly, the effect of BAP also declined when used in combination with NAA.

The R^2 value (0.2765) indicates that the treatments used during the present investigation were not optimal in inducing calluses from olive explants. This may be true in other plant species (Adedeji *et al.*, 2024; Gao *et al.*, 2021). However, Ismaili *et al*. (2011) reported that callus induction in olives is dependent upon various other factors, including chemical treatments.

Among the PGRs in plant tissue culture research, thidiazuron (TDZ) is considered a strong thiourea-based cytokinin by nature, inducing in vitro morphogenesis in plants (Murthy *et al.*, 1998). However, its effect was not promising during the present investigation (Figure 3). TDZ at 4 or 5 µM was only effective with 15.14% and 10.04% callus induction, respectively, with coarse yellowish callus development after 40 days at 4µM TDZ (Figure 1J). Albeit our study, TDZ possessed and reported as a potent regulator induced *in vitro* morphogenesis, callus induction, axillary bud growth, direct or indirect shoot regeneration, somatic embryogenesis, and suspension cell cultures in various crop and woody plants (Murthy *et al.*, 1998; Akram and Aftab, 2008, 2016; Pai and Desai, 2018). Olive is also a woody plant. Unfortunately, TDZ remained modest for optimum callus initiation and morphogenesis during the present investigation (Figure 3).

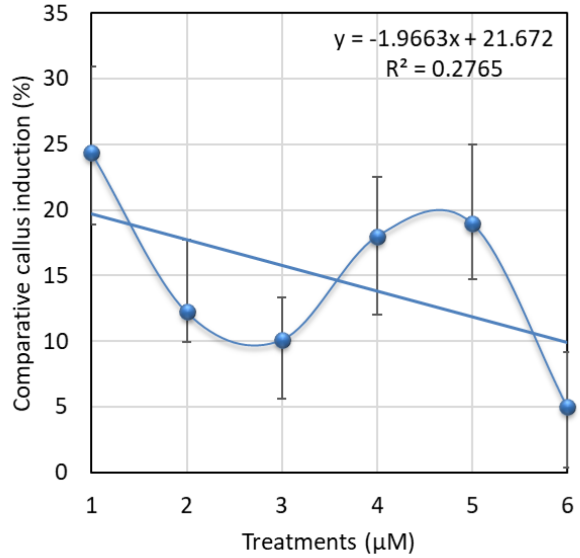

Figure 3. Comparison of the rate of callus induction from leaf explants of olive cv. Koroneiki on different treatments. Linear trend line values demonstrated correlation amongst treatments and the rate of callus induction. 1 = 2,4-D; 2 = 2,4-D + NAA; 3 = NAA + BAP; 4 = 2,4-D + BAP + NAA; 5 = BAP + IBA; 6 = TDZ. Whereas, 2,4-D (2,4-Dichlorophenoxyacetic acid), BAP (6-Benzylaminopurine), IBA (Indole-3-butyric acid), NAA (α-Naphthaleneacetic acid), TDZ (Thidiazuron; 1-Phenyl-3-(1,2,3-thiadiazol-5-yl) urea), µM (microMolar)

Callus cell lines

Callus cultures, which could disintegrate into individual cells for easy determination of metabolites, are regarded as friable and fluffy calluses (Efferth, 2019). In the present investigation, proliferating and friable fluffy calluses were obtained with two concentrations (4, 6 µM) of 2,4-D (Table 3) and set as callus cell lines (CL). CL1 consists of 4 µM 2,4-D, whereas CL2 consists of 6 µM 2,4-D. Efferth (2019) reviewed how culture media and growth regulators significantly influence the nature and texture of calluses. Other physical factors, such as explant type, light, gelling agents (Muzika *et al*., 2024), and temperature, significantly impact the production of various callus textures. Daffalla *et al*. (2019) selected three cell lines based on the color (yellow-brown line) of the calluses. In contrast, friable calluses are often used in biochemical and pharmacological studies establishing cell suspension cultures because of their faster growth and viable biomass production (Keng, 2013).

Table 3. Callus cell lines (CL) of 35-day-old cultures from leaf explants of olive cv. Koroneiki

Culture media	Callus cell lines (CL)	Culture's morphology
2,4-D 4 µM	CL1	Off white, yellowish, fluffy, friable, proliferating calluses
2,4-D 6 µM	CL2	

Whereas, 2,4-D (2,4-Dichlorophenoxyacetic acid), µM (microMolar).

Biochemistry of olive oil (Acid value and saponification number)

To extract oil and perform quantitative biochemical characterization, two varieties of the following selected callus lines (CL) were employed. Callus tissues' fatty acid content can be determined using the acid value, a chemical indicator of a substance's acidity. Whereas the quantity of base needed to saponify a fat sample is measured by the saponification number, which is pertinent to the characteristics of oils and fats. Figure 4 shows that the acid values and callus oil saponification numbers of CLs varied. CL2 produced the highest saponification number (184) and acid value (1.42). According to research findings conducted in 2020 by Hasanuzzaman and his colleagues, these biochemical markers may serve as early indicators of stress. As a result, screening for resistance to abiotic stress can be conducted in vitro. The findings provide new genetic bioengineering and biotechnology, offering insights that enhance this approach. Because it can be modified to meet a variety of requirements, tissue culture is an effective method for conducting metabolic and physiological research, as well as for fostering development.

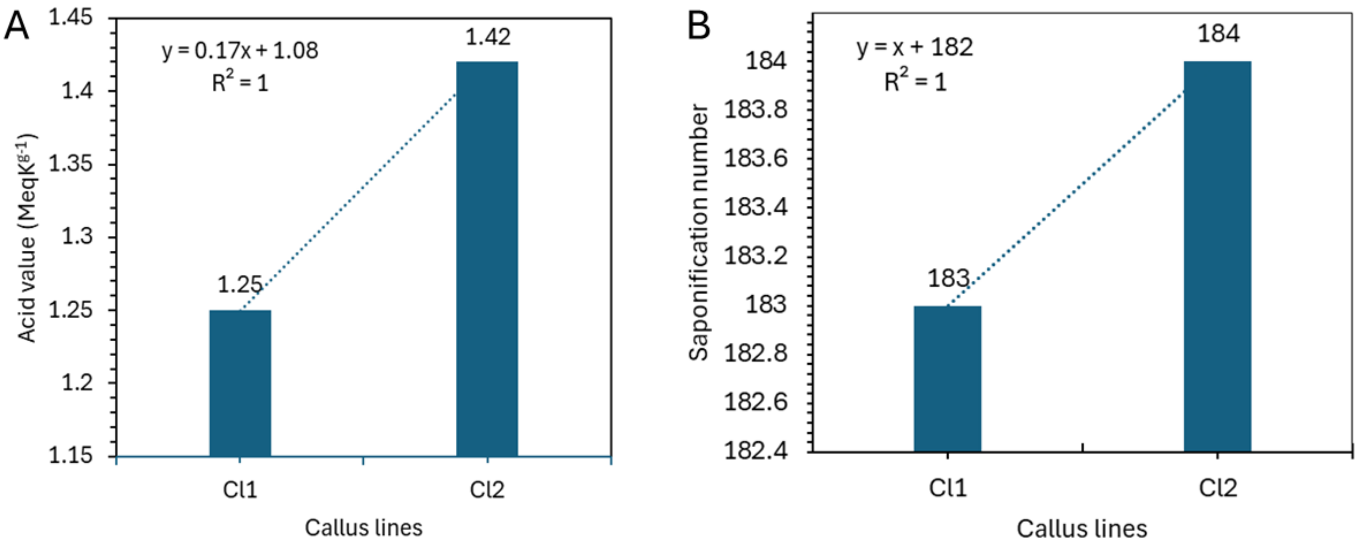

Figure 4. Chemical characteristics of callus oil extracted from olive cv. Koroneiki leaf explants. Whereas Cl1 (Callus line 1), Cl2 (Callus line 2).

The acidity level and saponification of oil from callus are not at all the same as those of olive oil. As per Boskou (2006), the acidity levels of regular fresh olive oil run from 0.2 mg KOH/g to 1.0 mg KOH/g, based on the type of olive tree used and how it was processed. In our study, a higher acid value may be due to immature tissues often having higher lipase activity or insufficient fatty production when grown in a laboratory experiment. Alimentarius (2019) reported that the saponification values of industrial olive oil are usually between 184 and 196 mg KOH/g. This means that there are equal numbers of C18 and C22 fatty acids. The significantly different saponification number in callus-derived oil may lead to different lipid patterns or changes in biochemical pathways *in vitro*. This is because complex lipids or shorter-chain fatty acids have a smaller saponification number than longer-chain fatty acids.

We could not find relevant research in contemporary literature on the determination of acid value and saponification number from the oil of callus tissues of either olive cultivars, including Coroneiki, addressing a gap in the literature regarding the biochemistry of the aforementioned parameters (Baccari *et al*., 2020). The reliable methods for callus induction under controlled biochemical conditions facilitate future research for somatic embryogenesis, metabolite extraction, and stress physiology. Our biochemical parameters, however, have been reported in other plant species (Verma and Juneja, 2014; Bernabé-Antonio *et al*., 2015; Akram and Aftab, 2022).

It might be possible to find out what physiological similarities olive callus cultures have by comparing their biochemical and hormonal reactions to those of other tree species. It was chemicals, demonstrating that combining auxin and cytokinin increased the amount of phenolic compounds in the callus. This, in turn, increased the antioxidant activity of walnut (*Juglans regia*) (Villatoro-Pulido *et al*., 2012). To agree with the findings of Villatoro-Pulido *et al.* (2012), the metabolic process that woody plants go through remains the same throughout their entire life. In 2010, Gambino *et al*. found that 2,4-D and kinetin altered the accumulation of secondary metabolites and the growth of callus in *Vitis vinifera*. This is yet another piece of proof that these two molecules play specific roles during the biological creation process. The method developed for olives may also apply to other green plants that cause problems, provided these similarities are considered. To work correctly, many different applications rely on this one application. These include synthesizing molecules, preserving genetic information, and investigating stress. On the same pitch, the present investigation is the first of its kind to determine such attributes from the callus oil. Our study would serve as a valuable indicator and sample in the field of plant tissue culture and biochemical analyses.

CONCLUSION

It is concluded that olive callus cultures can be induced from mature leaf explants of olive cv. Coroneiki with equal proportion by BAP + IBA or 2,4-D (4, 6 μM) supplemented in MS medium. Such calluses were highly proliferating, with different morphologies selected as callus lines, which were the most successful cultures for extracting the total fixed oil content for subsequent biochemical analysis (acid value and saponification number) of olive oil.

AUTHOR CONTRIBUTIONS

Conceptualization, MA; methodology, MA; software, MA; validation, FA; formal analysis and investigation, IJ; data curation, IJ; writing—original draft preparation, MA; writing—review and editing, MA; supervision, FA. All authors have read and approved the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The present study is part of an M.Phil. thesis of the first author. Plant Developmental and Regenerative Biology Lab of the Institute of Botany, University of the Punjab, Lahore, Pakistan, is acknowledged for providing research facilities and funding for experimental work.

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