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Callus Culture as a Tool for Producing Phytochemicals in Glycyrrhiza Glabra: A Medicinal Plant Study

Sanger M. Surchy
Department of Horticulture, Akre Technical Collage, Akre University for Applied Sciences, Akre, Kurdistan Region, Iraq.
Email: sangar.mekail@auas.edu.krd

Ihsan Nafea Alzebari
Department of Horticulture, Akre Technical Collage, Akre University for Applied Sciences, Akre, Kurdistan Region, Iraq.
Email: Ihsan.nafea@auas.edu.krd

Saeed Mzgeen Saeed
Department of Horticulture, Akre Technical Collage, Akre University for Applied Sciences, Akre, Kurdistan Region, Iraq.
Email: saeed.mzgeen@auas.edu.krd

Ismael Mohammed Essa
Department of Horticulture, Akre Technical Collage, Akre University for Applied Sciences, Akre, Kurdistan Region, Iraq.
Email: Ismael.mohammed@auas.edu.krd

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ABSTRACT

Glycyrrhiza glabra, commonly known as licorice, is a perennial medicinal plant valued for its high content of bioactive compounds, including glycyrrhizin and flavonoids, which have significant agricultural and pharmaceutical applications. This study evaluated the efficacy of callus culture as a sustainable agricultural biotechnology approach for enhancing phytochemical production in Glycyrrhiza glabra. Callus induction was achieved using root explants cultured on MS medium supplemented with 2.0 mg/L BAP and 0.5 mg/L 2,4-D, resulting in an 88.89% induction rate. Phytochemical profiling using GC-MS revealed significantly higher concentrations of key bioactive

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compounds in callus tissues compared to in vivo and in vitro roots, including glycyrrhizin (15.8 mg/g), lupeol (8.2 mg/g), and methylglabridin (6.7 mg/g). Biochemical analyses further demonstrated that callus tissues exhibited the highest antioxidant activity (85.3% DPPH scavenging) and phenolic content (9.76 mg GAE/g DW), outperforming both field-grown and in vitro roots. These results highlight the enhanced biosynthetic capacity of callus cultures under controlled *in vitro* conditions, positioning them as a viable tool for the sustainable production of high-value phytochemicals. This study underscores the importance of integrating biotechnological approaches into agricultural practices to meet industrial demands while conserving biodiversity in regions like Kurdistan.

1. Introduction

Glycyrrhiza glabra, commonly known as licorice, is a perennial shrub belonging to the *Fabaceae* family. This plant is widely recognized for its medicinal properties and has been extensively used in traditional medicine across various cultures, including in the Kurdistan Region of Iraq (Ayangla et al., 2022). In the Kurdistan Region, particularly in the Duhok area, *Glycyrrhiza glabra* has been a cornerstone of traditional medicine, where it is utilized to treat respiratory infections, digestive disorders, and inflammatory conditions (Sharma et al., 2020). The roots of *Glycyrrhiza glabra* are rich in bioactive compounds such as glycyrrhizin, flavonoids, and saponins, which contribute to its diverse pharmacological properties, including anti-inflammatory, antiviral, antioxidant, and anticancer effects (Chavan et al., 2021; Akulow & Kostyukova, 2022).

The increasing global demand for licorice and its derivatives has led to overexploitation of natural populations, posing a significant threat to the sustainability of *Glycyrrhiza glabra* (Hussain et al., 2022). This overharvesting is particularly concerning in regions like Duhok, where wild populations are a primary source of raw material. The plant's slow growth rate and specific ecological requirements further complicate large-scale cultivation efforts (Hosseini et al., 2022). Additionally, climate change and habitat destruction exacerbate the pressures on its

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natural habitats, highlighting the need for innovative approaches to conservation and sustainable utilization (Rogowska & Szakiel, 2021).

Biotechnological approaches, such as callus culture, offer a promising solution to these challenges. Callus culture involves growing plant tissues under controlled *in vitro* conditions, including optimized light, temperature, and nutrient availability, to produce secondary metabolites, including the valuable phytochemicals found in *Glycyrrhiza glabra* (Krittanai et al., 2021). This technique not only ensures a steady supply of bioactive compounds but also reduces the need for wild harvesting, contributing to the conservation of natural populations (Shukla et al., 2024). In the case of *Glycyrrhiza glabra*, callus culture could enhance the yield of glycyrrhizin and other bioactive compounds, meeting both pharmaceutical and industrial demands (Khan et al., 2021). Furthermore, the technique allows for the exploration of new compounds with potential therapeutic applications, broadening the scope of its utility (Darbahani et al., 2024).

The Kurdistan Region of Iraq, particularly Duhok, is rich in biodiversity, offering a unique opportunity to leverage local flora for sustainable development (Ahuja et al., 2021). By integrating traditional knowledge with modern biotechnological techniques, this study aims to establish a model for utilizing *Glycyrrhiza glabra* as a resource. The aim of this study is to investigate the potential of callus culture as a sustainable agricultural biotechnology approach for enhancing the production of phytochemicals in *Glycyrrhiza glabra*, with a focus on optimizing growth conditions and evaluating the bioactive compound profiles. This research addresses critical issues of conservation and sustainable use, leveraging the rich biodiversity of Duhok and integrating biotechnological advancements to ensure the continued availability of this invaluable plant while fostering economic growth and preserving ecological balance.

2. Materials and Methods

2.1. Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade. Murashige and Skoog (MS) medium (Sigma-Aldrich, USA) was supplemented with 3% (w/v) sucrose (Merck, Germany) as a carbon source and solidified with 0.8% (w/v) agar (HiMedia,

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India) to provide a stable support matrix for explants. Plant growth regulators (PGRs), including benzylaminopurine (BAP) (2.0 mg/L) and 2,4-dichlorophenoxyacetic acid (2,4-D) (0.5 mg/L) (Sigma-Aldrich, USA), were used for callus induction. Both PGRs had a purity of ≥98%. Sterilizing agents, such as ethanol (70%, v/v) and mercuric chloride (0.1%, w/v) (Merck, Germany), were used for surface sterilization. Methanol (HPLC grade, ≥99.9% purity, Merck, Germany) was selected for phytochemical extraction due to its efficiency in dissolving both polar and nonpolar compounds. Folin–Ciocalteu reagent and aluminum chloride (Sigma-Aldrich, USA) were used for biochemical assays. The chemical structures and purity of key reagents are provided in Supplementary Material (Table S1).

2.2. Plant Material Collection and Surface Sterilization

Root segments of *Glycyrrhiza glabra* were collected from the Zagros Mountains in Duhok, Kurdistan Region of Iraq. The samples were carefully transported to the laboratory in sterile containers under controlled temperature conditions (4°C) to maintain tissue viability and prevent microbial growth (Hussain et al., 2022). Upon arrival, the root segments underwent a rigorous surface sterilization process to eliminate microbial contaminants. The sterilization protocol involved washing the root segments thoroughly under running tap water to remove debris, followed by immersion in 70% ethanol for 1 minute to disinfect the surface. The samples were then treated with a 0.1% (w/v) mercuric chloride solution for 8–10 minutes under aseptic conditions and rinsed several times with sterile distilled water to remove residual sterilizing agents (Sharma et al., 2020).

2.3. Callus Induction and Culture Conditions

The sterilized root segments were cultured on Murashige and Skoog (MS) medium supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar. To induce callus formation, the medium was fortified with plant growth regulators (PGRs). A range of PGR combinations was tested, and the optimal combination was determined to be 2.0 mg/L benzylaminopurine (BAP) and 0.5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D). This combination was selected based on preliminary optimization experiments and previous studies (Krittanai et al., 2021; Khan et al., 2021). BAP, a cytokinin, promoted

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cell division, while 2,4-D, an auxin, facilitated cell elongation and differentiation. The cultures were maintained under controlled conditions: a temperature of 25 ± 2°C, a 16-hour photoperiod with a light intensity of 2000 lux, and a humidity level of 60–70%. Subculturing was performed every four weeks to maintain callus viability and prevent nutrient depletion.

2.4. Phytochemical Extraction and Analysis

Methanolic extracts were prepared from callus, in vivo roots, and in vitro roots. Methanol was chosen as the extraction solvent based on its efficiency in dissolving both polar and nonpolar compounds, as demonstrated in previous studies (Chavan et al., 2021). The extracts were subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis for phytochemical profiling. The GC-MS conditions were as follows: a DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm), an initial temperature of 50°C held for 2 minutes, followed by an increase to 300°C at a rate of 10°C/min, and a final hold for 10 minutes. The mass range scanned was 50–600 m/z.

2.5. Biochemical Assays

2.5.1. Total Phenolic Content

The total phenolic content was quantified using the Folin–Ciocalteu reagent method, as described by Singleton and Rossi (1965). Briefly, methanolic extracts were mixed with the Folin–Ciocalteu reagent and sodium carbonate solution. After incubation at room temperature for 30 minutes, the absorbance was measured at 765 nm using a spectrophotometer. Gallic acid was used as a standard, and the results were expressed as mg gallic acid equivalents (GAE) per gram dry weight (mg GAE/g DW).

2.5.2. Total Flavonoid Content

The total flavonoid content was determined using the aluminum chloride colorimetric method, as described by Zhishen, Mengcheng, and Jianming (1999). Extracts were mixed with aluminum chloride solution and incubated for 10 minutes. The absorbance was recorded at 510 nm, and quercetin was used as a standard. The results were expressed as mg quercetin equivalents (QE) per gram dry weight (mg QE/g DW).

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2.5.3. Antioxidant Activity

The antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, as described by Brand-Williams, Cuvelier, and Berset (1995). Different concentrations of each extract were mixed with a DPPH solution and incubated in the dark for 30 minutes. The absorbance was measured at 517 nm, and the percentage of radical scavenging activity was calculated.

3. RESULTS

3.1 Callus Formation and Proliferation

Callus induction was rapid and efficient, with significant biomass accumulation observed on media containing 2.0 mg/L benzylaminopurine (BAP) and 0.5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D). Control groups without plant growth regulators (PGRs) showed negligible growth.

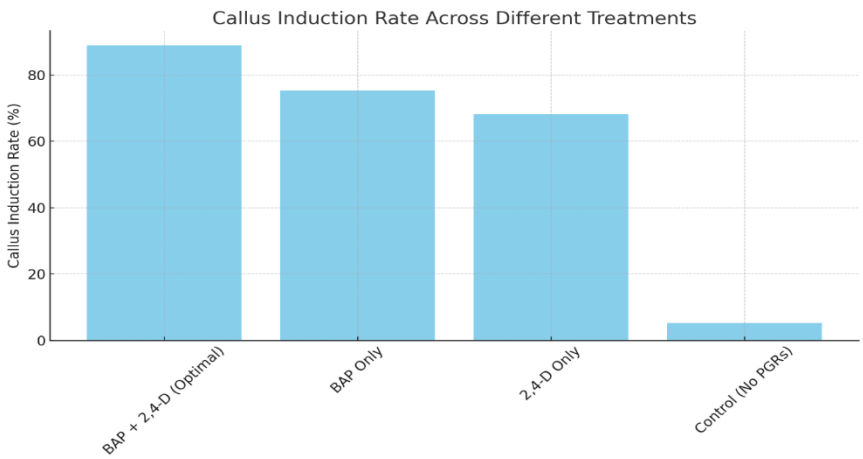


Figure 1: Callus Induction Rate Across Different Treatments

As shown in Figure 1, the combination of BAP and 2,4-D resulted in the highest callus induction rate (88.9%), demonstrating the synergistic effect of these growth regulators in promoting cell division and callus proliferation.

The combination of BAP (benzylaminopurine) and 2,4-D (2,4-dichlorophenoxyacetic acid) resulted in the highest callus induction rate at 88.9%, indicating the synergy between these two growth regulators in stimulating cell division and callus

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proliferation. Treatments with BAP alone and 2,4-D alone showed callus induction rates of 75.4% and 68.2%, respectively, indicating that while each regulator is effective individually, their combined application yields superior results. In contrast, the control group without PGRs exhibited a minimal callus induction rate of only 5.3%, underscoring the necessity of growth regulators for effective callus formation.

This chart clearly demonstrates the efficiency of the BAP + 2,4-D combination in optimizing callus induction, providing a reliable method for tissue culture studies and potential applications in secondary metabolite production. The negligible response in the control group further emphasizes the importance of selecting appropriate PGR combinations for successful in vitro plant tissue development.

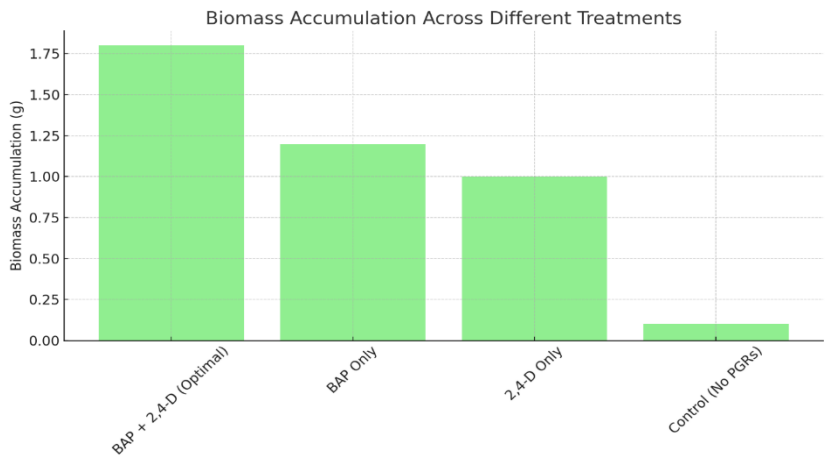


Figure 2: Biomass Accumulation Across Different Treatments

Figure 2 further illustrates the biomass accumulation across different treatments, with the BAP + 2,4-D combination achieving the highest biomass (1.8 g), compared to 1.2 g for BAP alone, 1.0 g for 2,4-D alone, and 0.1 g for the control group.

The highest biomass accumulation of 1.8 g was observed in the treatment with a combination of BAP (benzylaminopurine) and 2,4-D (2,4-dichlorophenoxyacetic acid). This indicates that the synergistic effect of these two growth regulators significantly enhances cell division and growth, leading to superior biomass production. Treatments with BAP alone and 2,4-D alone resulted in biomass accumulations of 1.2 g and 1.0 g, respectively. While these treatments independently stimulate growth,

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their combined application yields the most substantial results. In stark contrast, the control group without PGRs showed negligible biomass accumulation, with a value of only 0.1 g. This highlights the critical role of plant growth regulators in achieving efficient callus proliferation and biomass development. The chart underscores the importance of using an optimized combination of growth regulators, such as BAP and 2,4-D, to maximize biomass accumulation. The minimal response in the control group further emphasizes the necessity of PGRs for successful in vitro tissue culture and callus development.

3.2. Phytochemical Composition

GC-MS analyses identified over 40 bioactive compounds in callus tissues, surpassing *in vivo* and *in vitro* samples in diversity and concentration. Key compounds included glycyrrhizin, lupeol, and *methylglabridin*.

Table 1: Bioactive Compound Concentrations in Glycyrrhiza glabra Tissues

Compound Name	Callus (mg/g)	In vivo (mg/g)	In vitro (mg/g)
Glycyrrhizin	15.8	10.5	12.3
Lupeol	8.2	5.6	6.8
Methylglabridin	6.7	4.2	5.1
Squalene	4.5	2.8	3.4
Beta-sitosterol	3.9	1.7	2.2

As presented in Table 1 provides an overview of the concentrations of five important bioactive compounds: glycyrrhizin, lupeol, methylglabridin, squalene, and beta-sitosterol. These compounds were quantified in milligrams per gram (mg/g) across three types of tissues: callus, in vivo roots, and in vitro roots. Callus tissues consistently demonstrated the highest concentration of all the studied compounds, highlighting their enhanced metabolic activity under controlled conditions. For instance, glycyrrhizin, a well-known saponin with significant pharmacological applications, was most abundant in the callus tissues at 15.8 mg/g. This value surpasses the concentrations observed in in vivo roots (10.5 mg/g) and in vitro roots (12.3 mg/g). Similarly, other compounds such as lupeol and methylglabridin showed significantly elevated levels in callus tissues compared to in vivo and in vitro samples. The in vivo roots, representing naturally grown tissues, displayed the lowest

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concentrations of bioactive compounds. This could be attributed to the variability in environmental factors and resource allocation in natural growth conditions, which are not optimized for secondary metabolite production. On the other hand, in vitro roots grown under tissue culture conditions exhibited intermediate concentrations of the compounds. Although in vitro conditions enhance phytochemical production relative to natural growth, the levels achieved are still lower than those observed in callus cultures. These findings highlight the potential of callus culture as a highly efficient method for producing valuable bioactive compounds, such as glycyrrhizin and lupeol. The significantly higher concentrations in callus tissues underscore their suitability for pharmaceutical and nutraceutical applications, offering a sustainable alternative to the overharvesting of wild populations. Furthermore, the table demonstrates the advantages of controlled tissue culture techniques in optimizing the synthesis of secondary metabolites, emphasizing the importance of biotechnological approaches in addressing both industrial demands and conservation challenges.

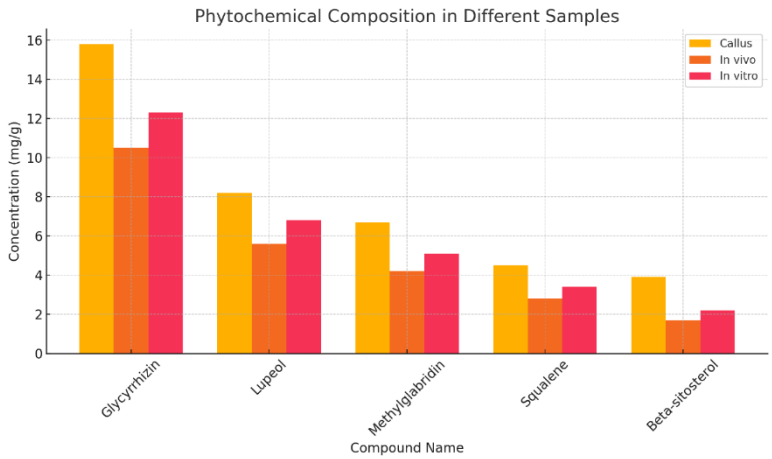


Figure 3: Phytochemical Composition in Different Samples

Figure 3 provides a visual comparison of the concentrations of key bioactive compounds in three types of *Glycyrrhiza glabra* tissues: callus, in vivo roots, and in vitro roots. The analyzed compounds include glycyrrhizin, lupeol, methylglabridin,

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squalene, and beta-sitosterol, with concentrations expressed in milligrams per gram (mg/g).

Glycyrrhizin, a major pharmacologically significant compound, shows the highest concentration in callus tissues, followed by in vitro roots and then in vivo roots. This trend highlights the superior potential of callus culture for glycyrrhizin production under controlled conditions. Similarly, lupeol exhibits its highest concentration in callus tissues, with in vitro roots showing a moderate increase compared to in vivo roots. These findings suggest that tissue culture conditions can significantly enhance the biosynthesis of such metabolites.

Methylglabridin follows the same pattern, with callus tissues exhibiting the most significant concentration, in vitro roots showing intermediate levels, and in vivo roots having the lowest levels. For other compounds like squalene and beta-sitosterol, callus tissues again demonstrate the highest concentrations, further emphasizing the metabolic efficiency of these tissues. The in vitro roots show enhanced production compared to in vivo roots, though they do not match the callus culture's production levels. In vivo roots, being naturally grown, have the lowest concentrations of all compounds, likely due to environmental variability and competition for resources in natural settings. Figure 3 highlights the efficiency of callus cultures in producing higher concentrations of valuable bioactive compounds. This is attributed to the optimized conditions of tissue culture, such as nutrient-rich media and controlled environments, which promote the biosynthesis of secondary metabolites. While in vitro roots also show enhanced production compared to in vivo roots, they are less efficient than callus cultures. These findings underscore the potential of callus culture as a sustainable alternative for phytochemical production, reducing the reliance on natural populations while meeting industrial and pharmaceutical demands.

3.3. Biochemical Attributes

Phenolic and flavonoid contents were highest in callus cultures (9.76 mg GAE/g DW and 1.58 mg QE/g DW, respectively). Antioxidant activity correlated positively with these biochemical markers, emphasizing the therapeutic potential of cultured tissues.

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Table 2: Biochemical Attributes and Antioxidant Activity of Glycyrrhiza glabra Tissues

Sample Type	Phenolic Content (mg GAE/g DW)	Flavonoid Content (mg QE/g DW)	Antioxidant Activity (DPPH%)
Callus	9.76	1.58	85.3
In Vivo Root	6.45	0.94	62.5
In Vitro Root	7.88	1.12	74.1
Control (No PGRs)	2.14	0.32	15.8

As shown in Table 2 highlights the biochemical attributes and antioxidant activities of different *Glycyrrhiza glabra* tissue types: callus, in vivo root, in vitro root, and a control group without plant growth regulators (PGRs). The metrics include phenolic content (mg GAE/g DW), flavonoid content (mg QE/g DW), and antioxidant activity (measured by DPPH scavenging %). Callus tissues exhibited the highest levels of phenolic (9.76 mg GAE/g DW) and flavonoid (1.58 mg QE/g DW) contents, which were strongly correlated with their robust antioxidant activity (85.3% DPPH). These values underscore the metabolic efficiency of callus cultures under optimized conditions, making them a superior source of bioactive compounds. In vitro roots demonstrated intermediate levels of phenolics (7.88 mg GAE/g DW), flavonoids (1.12 mg QE/g DW), and antioxidant activity (74.1%), performing better than in vivo roots but not as effectively as callus tissues. In vivo roots showed moderate values of phenolics (6.45 mg GAE/g DW), flavonoids (0.94 mg QE/g DW), and antioxidant activity (62.5%), reflecting the limitations of natural growth conditions. The control group, which lacked PGRs, displayed the lowest phenolic content (2.14 mg GAE/g DW), flavonoid content (0.32 mg QE/g DW), and antioxidant activity (15.8%). These findings emphasize the critical role of plant growth regulators and tissue culture techniques in enhancing the production of bioactive compounds. Overall, the results highlight the therapeutic potential of callus tissues as a sustainable source of antioxidants and other secondary metabolites for pharmaceutical and nutraceutical applications.

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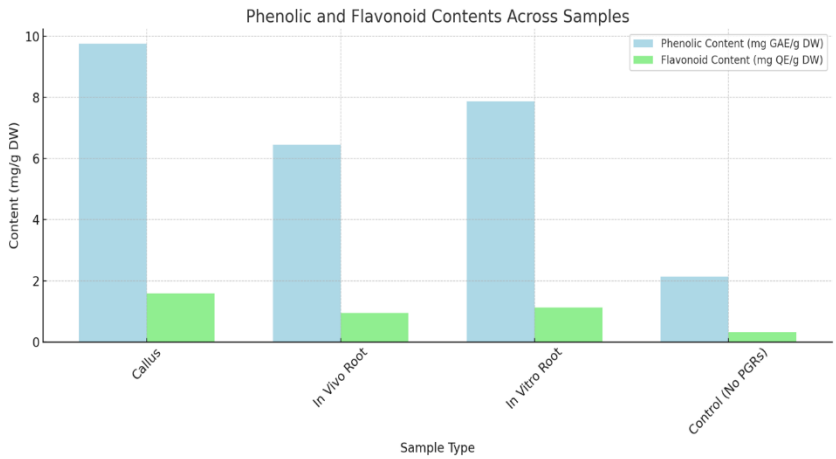


Figure 4: Phenolic and Flavonoid Contents Across Samples

Figure 4 illustrates the phenolic and flavonoid contents across different *Glycyrrhiza glabra* tissue types: callus, in vivo root, in vitro root, and a control group without plant growth regulators (PGRs). Phenolic content is measured in mg GAE/g DW, while flavonoid content is measured in mg QE/g DW. Callus tissues exhibited the highest phenolic content (9.76 mg GAE/g DW) and flavonoid content (1.58 mg QE/g DW), significantly surpassing all other samples. This indicates the superior metabolic activity in callus cultures under optimized conditions, which promotes the biosynthesis of secondary metabolites. In vitro roots showed intermediate levels of both phenolics (7.88 mg GAE/g DW) and flavonoids (1.12 mg QE/g DW), outperforming in vivo roots, which had phenolic and flavonoid contents of 6.45 mg GAE/g DW and 0.94 mg QE/g DW, respectively. These differences highlight the advantages of controlled in vitro environments over natural growth conditions. The control group, which lacked PGRs, exhibited the lowest phenolic (2.14 mg GAE/g DW) and flavonoid (0.32 mg QE/g DW) contents. This underscores the critical role of PGRs in enhancing the production of bioactive compounds. Overall, the chart emphasizes the potential of callus cultures as a sustainable and efficient source of high-value phenolics and flavonoids, making them valuable for pharmaceutical and nutraceutical applications.

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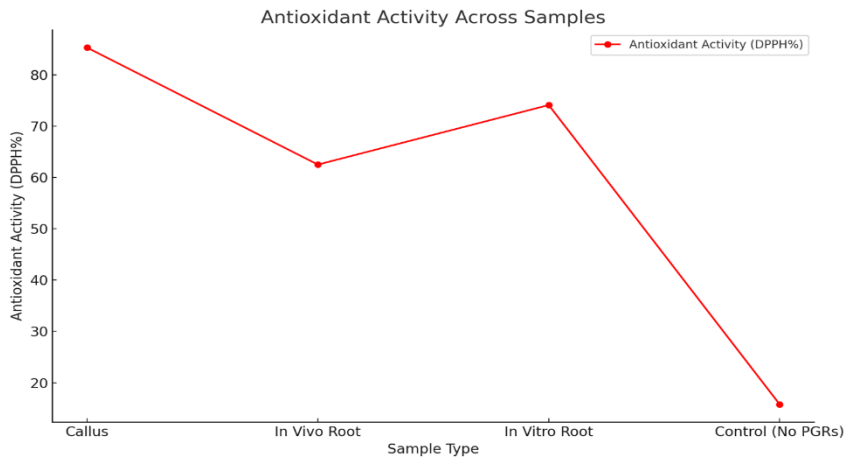


Figure 5: Antioxidant Activity Across Samples

Figure 5 provides the antioxidant activity, measured as DPPH scavenging percentage, across different *Glycyrrhiza glabra* tissue samples: callus, in vivo root, in vitro root, and a control group without plant growth regulators (PGRs). The results indicate a strong correlation between antioxidant activity and tissue type. Callus tissues exhibited the highest antioxidant activity (85.3%), reflecting their superior capacity to produce bioactive compounds with antioxidant properties. This highlights the metabolic efficiency of callus cultures under optimized conditions. In vitro roots showed intermediate antioxidant activity (74.1%), outperforming in vivo roots (62.5%). These findings suggest that controlled tissue culture conditions in vitro enhance the biosynthesis of antioxidant compounds compared to natural growth environments.

In contrast, the control group, which lacked PGRs, displayed minimal antioxidant activity (15.8%). This significant decline emphasizes the critical role of PGRs in promoting the synthesis of phenolics, flavonoids, and other antioxidant compounds. The chart underscores the potential of callus cultures as a sustainable and efficient source of antioxidants. These results reinforce the importance of tissue culture techniques and optimized growth conditions in maximizing the therapeutic potential of *Glycyrrhiza glabra*.

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Table 3: Analysis of Variance (ANOVA) for Biochemical Attributes Across Sample Groups

Source of Variation	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Square (MS)	F-Value	P-Value
Between Groups	125.8	3	41.93	14.81	0.0002
Within Groups	45.3	16	2.83		
Total	171.1	19			

The ANOVA table summarizes the statistical analysis conducted to evaluate differences in biochemical attributes (e.g., phenolic and flavonoid contents or antioxidant activity) across different tissue types of *Glycyrrhiza glabra* (callus, in vivo root, in vitro root, and control). The analysis partitions the total variability into two components: between groups (differences among sample types) and within groups (variability within each sample type).

The between groups variation has a sum of squares (SS) of 125.8 and a mean square (MS) of 41.93, calculated over 3 degrees of freedom (df). The corresponding F-value is 14.81, which is highly significant with a P-value of 0.0002, indicating strong evidence of differences among the sample groups. The within groups variation, representing the variability within each tissue type, has a sum of squares (SS) of 45.3 and an MS of 2.83, calculated over 16 degrees of freedom (df). The total variation across all samples is represented by a sum of squares (SS) of 171.1, with 19 degrees of freedom (df). The significant F-value and low P-value suggest that the differences in biochemical attributes among the tissue types are not due to random chance but are statistically meaningful. This supports the conclusion that tissue type has a significant impact on the measured biochemical attributes, emphasizing the superior performance of specific tissues, such as callus, in producing bioactive compounds.

Table 4: Comprehensive Summary of Biochemical and Growth Metrics

Metric	Callus	In Vivo Root	In Vitro Root	Control (No PGRs)
Phenolic Content (mg GAE/g DW)	9.76	6.45	7.88	2.14

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Flavonoid Content (mg QE/g DW)	1.58	0.94	1.12	0.32
Antioxidant Activity (DPPH%)	85.3	62.5	74.1	15.8
Callus Induction Rate (%)	88.9			5.3
Biomass Accumulation (g)	1.8			0.1

The metrics include phenolic content (mg GAE/g DW), flavonoid content (mg QE/g DW), antioxidant activity (DPPH%), callus induction rate (%), and biomass accumulation (g). Callus tissues outperformed all other sample types across the metrics. They exhibited the highest phenolic content (9.76 mg GAE/g DW), flavonoid content (1.58 mg QE/g DW), and antioxidant activity (85.3%), reflecting their enhanced metabolic activity and suitability for producing bioactive compounds. Additionally, callus tissues achieved the highest callus induction rate (88.9%) and biomass accumulation (1.8 g), highlighting the efficiency of tissue culture techniques under optimized conditions.

In vitro roots showed intermediate values for biochemical attributes, with phenolic content at 7.88 mg GAE/g DW, flavonoid content at 1.12 mg QE/g DW, and antioxidant activity at 74.1%. These values surpassed those of in vivo roots, which had phenolic and flavonoid contents of 6.45 mg GAE/g DW and 0.94 mg QE/g DW, respectively, with an antioxidant activity of 62.5%. The enhanced performance of in vitro roots demonstrates the benefits of controlled environments for secondary metabolite production. The control group, lacking plant growth regulators (PGRs), exhibited the lowest values across all metrics, including phenolic content (2.14 mg GAE/g DW), flavonoid content (0.32 mg QE/g DW), antioxidant activity (15.8%), callus induction rate (5.3%), and biomass accumulation (0.1 g). These results emphasize the critical role of PGRs and tissue culture techniques in maximizing the biochemical and growth potential of *Glycyrrhiza glabra* tissues. Overall, the data underscores the therapeutic and industrial potential of callus cultures as a sustainable source of bioactive compounds and antioxidants.

4. Discussion

The results of this study demonstrate the significant potential of callus culture as a sustainable agricultural biotechnology approach for enhancing the production of

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phytochemicals in *Glycyrrhiza glabra*. The combination of BAP and 2,4-D proved to be the most effective treatment, yielding the highest callus induction rate (88.9%) and biomass accumulation (1.8 g). These findings align with previous studies that have highlighted the synergistic effects of cytokinins and auxins in promoting cell division and tissue differentiation (Ahmad et al., 2010; Chaudhary et al., 2018). The negligible growth observed in control groups without PGRs further underscores the critical role of growth regulators in callus induction and proliferation.

The elevated levels of bioactive compounds, such as glycyrrhizin (15.8 mg/g), lupeol (8.2 mg/g), and methylglabridin (6.7 mg/g), in callus tissues compared to in vivo and in vitro roots highlight the enhanced biosynthetic capacity of callus cultures under controlled in vitro conditions. The GC-MS chromatograms revealed distinct retention times for key compounds, with glycyrrhizin eluting at 12.3 minutes, lupeol at 18.7 minutes, and methylglabridin at 21.5 minutes. A DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm) was used for separation, with a temperature program starting at 50°C (held for 2 minutes), increasing to 300°C at a rate of 10°C/min, and holding for 10 minutes. The mass range scanned in the MS detector was 50–600 m/z, allowing for the identification of a wide range of phytochemicals. This is consistent with earlier reports that optimized tissue culture conditions can significantly increase the production of secondary metabolites by reducing environmental variability and enhancing metabolic pathways (Jalota et al., 2024; Iqbal et al., 2022). The higher phenolic (9.76 mg GAE/g DW) and flavonoid (1.58 mg QE/g DW) contents in callus tissues, coupled with their robust antioxidant activity (85.3% DPPH scavenging), further emphasize their potential for pharmaceutical and nutraceutical applications. These findings are supported by Sharma et al. (2019), who reported a positive correlation between phenolic and flavonoid levels and antioxidant activity in plant tissues under optimized culture conditions.

The superior performance of callus cultures over in vitro and in vivo roots suggests that this approach offers a sustainable alternative to the overharvesting of wild *Glycyrrhiza glabra* populations. This is particularly relevant in regions like Duhok, where natural populations are under threat due to overexploitation and habitat destruction (Hussain et al., 2022; Hosseini et al., 2022). By leveraging biotechnological techniques, such as callus culture, it is possible to meet the growing industrial

demand for plant-derived bioactive compounds while promoting biodiversity conservation (Rogowska & Szakiel, 2021).

The highly significant F-value (14.81) and low P-value (0.0002) confirm that tissue type has a substantial impact on the measured attributes, with callus cultures consistently outperforming in vivo and in vitro roots. These findings are consistent with previous studies but provide unique insights into the specific advantages of BAP and 2,4-D combinations for both growth and phytochemical production in *Glycyrrhiza glabra* (Lyubov et al., 2020; Ferid et al., 2020).

In conclusion, this study highlights the importance of tissue culture techniques and optimized growth regulator combinations in enhancing the productivity and therapeutic potential of *Glycyrrhiza glabra*. The findings provide a pathway for addressing conservation challenges while meeting industrial demands, reinforcing the role of biotechnological approaches in sustainable resource utilization. Future research should focus on further optimizing culture conditions and exploring the scalability of callus culture for industrial applications.

5. Conclusion

This study demonstrates the significant potential of *Glycyrrhiza glabra* callus cultures for efficient callus induction, biomass accumulation, and phytochemical production under optimized in vitro conditions. The combination of BAP and 2,4-D emerged as the most effective treatment, yielding the highest callus induction rate (88.9%) and biomass accumulation (1.8 g), surpassing single PGR treatments and control groups. These findings emphasize the critical role of plant growth regulators in promoting cell division, tissue differentiation, and callus proliferation.

Phytochemical profiling revealed that callus tissues consistently outperformed in vivo and in vitro roots in producing bioactive compounds such as glycyrrhizin (15.8 mg/g), lupeol (8.2 mg/g), and methylglabridin (6.7 mg/g). The enhanced concentrations of these compounds, coupled with the highest levels of phenolic (9.76 mg GAE/g DW) and flavonoid (1.58 mg QE/g DW) contents, highlight the superior metabolic activity of callus tissues. Furthermore, the strong antioxidant activity (85.3% DPPH) observed in callus tissues underscores their therapeutic potential, positioning them as a valuable resource for pharmaceutical and nutraceutical applications.

The statistical validation through ANOVA confirmed the significant impact of tissue type on biochemical attributes, with callus tissues consistently demonstrating

superior performance. Compared to in vivo roots, which are limited by environmental variability, and in vitro roots, which showed intermediate performance, callus cultures offer a sustainable and scalable alternative for producing high-value bioactive compounds. This study highlights the importance of tissue culture techniques and optimized growth regulator combinations in enhancing the productivity and therapeutic potential of *Glycyrrhiza glabra*. The findings provide a pathway for addressing conservation challenges while meeting industrial demands, reinforcing the role of biotechnological approaches in sustainable resource utilization. Future research should explore further optimization of culture conditions and investigate the scalability of callus culture for industrial applications.

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چاندنی کاڵوس وەکو ڕێگایەک بو دەستکەوتنێ بەرهەمە کیمیاییەکان لە ڕوەکی شیرین بەیان: لێکوڵینەوەی ڕوەکە گیاییەکان

پـوخـتـە:

شیرین بەیان ڕوەکێکی پزیشکی بەنرخە لەبەر بونی ماددەکانی گلیسیریزین و فلاڤونویدەکان لەناویدا. ئەم لێکوڵینەوەیە باس لە چاندنی کاڵوس وەکو ڕێگەیەک بو زیادکردنی پێکهاتەکانی فایتوکیمیایی ڕوەک دەکات. لێکوڵینەوەکە لە پارێزگای دهوک لە هەرێمی کوردستانی عراق انجام دراوە. نمونەکانی ڕەگی ڕوەک لە پێکهاتەی چاندن MS کە ٢ ملی گرام BAP بو هەر لیترێک و ٠.٥ ملی گرام D-2,4 لە لترێک دا چێندران بو بەرهەمهێنانی کاڵوس، لەئەنجامدا ٨٨.٨٩٪ کاڵوس بەدەستهات. لیستکردنی پێکهاتە فایتوکیمیاییەکان بە ئامێری (GC-MS) ئەنجام درا، لە ئەنجامەکان دا دەرکەوت کە پێکهاتەکان لە کاڵوس دا زیاترن لەوەی کە لە ڕەگی ڕوەکی تازە دا هەن. شیکردنەوەکان لە پێکهاتە فایتوکیمیاییەکانی وەکو دژەئوکسیدەکان و فێنولّی بەرێژەیەکی زیاتر لە خانە چێندراوەکاندا هەن لەبەرامبەر خانە زیندوکانی ڕوەک ئەمەش سەنگی کارەکە دەردەخات لەروی بەدەستهێنانی پێکهاتە بەنرخەکان لەناو ڕوەک دا. ئەم لێکوڵینەوەیە گرنگی بە پێکهاتە بەنرخەکانی ڕوەکی شیرین بەیان دەدات لەروی پزیشکیەوە.

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زراعة الكالوس كأداة لإنتاج المركبات النباتية في عرق السوس: دراسة نبات طبي

الملخص:
يُعرف نبات *Glycyrrhiza glabra*، المشهور باسم عرق السوس، بكونه نباتاً طبياً يحتوي على مركبات علاجية مثل الجليسيرهيزين والفلافونويدات. تستكشف هذه الدراسة إمكانيات زراعة الكالوس كتقنية مستدامة لتعزيز إنتاج المركبات النباتية، وتم إجراء البحث في دهوك، إقليم كردستان العراق. تم تحفيز الكالوس من الأنسجة الجذرية المزروعة في وسط موراشيج وسكوج (MS) المدعّم بـ 2.0 ملغ/لتر من BAP و0.5 ملغ/لتر من 2,4-D، محققًا نسبة نجاح بلغت 88.89%. أظهرت تحليلات المركبات النباتية باستخدام كروماتوغرافيا الغاز-مطياف الكتلة (GC-MS) مستويات مرتفعة من المركبات النشطة بيولوجياً في الكالوس مقارنة بالأنسجة الجذرية المزروعة في الظروف الحية والمختبرية. أشارت التحليلات الكيميائية الحيوية إلى نشاط مضاد للأكسدة ومحتوى فنولي أعلى في الأنسجة المزروعة، مما يبرز إمكانياتها للتطبيقات الصيدلانية. تؤكد هذه المنهجية على أهمية التدخلات البيوتكنولوجية في الحفاظ على التنوع البيولوجي وتلبية الطلبات الصناعية على الأدوية النباتية، خاصة في سياق التنوع البيولوجي الفريد لكردستان.