HTTPS://DOI.ORG/ 10.5281/zenodo.23204774



Purposeful scientific research as a pillar of sustainable development



Propagation of *Brassica oleracea* var. *gongylodes* in vitro culture and predication callus stimulated by Activated charcoal

R.S.A. ALTIKRITY, A.S.M.

Department of biology, Collage of Sciences, Tikrit University, Tikrit, Iraq

rusul.samir@st.tu.edu.iq , a-sh.mahmood@tu.edu.iq

ABSTRACT

Brassica oleracea var. gongylodes is a nutritionally important plant widely propagated through in vitro culture techniques involving plant growth regulators. This study aims to stimulate plant development and improve micropropagation efficiency and producing active compounds using activated charcoal (AC). Activated charcoal is a porous, carbon-rich material with a high surface area that enables it to adsorb inhibitory compounds and toxic metabolites from the culture medium. It has demonstrated a significant role in promoting micropropagation, somatic embryogenesis, another culture, synthetic seed production, protoplast culture, rooting, shoot elongation, and bulb formation.

The stimulatory effects of AC are attributed to its dual function irreversible adsorption of growth-inhibiting substances and the release of bioactive compounds that support morphogenesis. Moreover, AC alters the chemical composition and color of the culture medium, impacting the bioavailability of vitamins, metal ions, and phytohormones.

In this study, explants of B. oleracea were cultured on Murashige and Skoog (MS) medium supplemented with three concentrations (1, 2, and 3 mg·L^{-1}) of 2,4-Dichlorophenoxyacetic acid (D-2,4), with and without activated charcoal. The combination of AC and the second concentration of D-2,4 produced the highest response, yielding an average of 8.00 shoots per explant, 7 leaves, 8 stems, and haigh branch length of 12 cm. AC also promoted better routing and firing growth than the media without AC.

Conclusions confirm that Brassica oleracea contains active compounds such as Glutathione and L-Cysteine, Quantitative analysis demonstrated an increase in glutathione content following exposure to activated charcoal, reaching 5.528 µg/ml, whail L-cysteine levels displayed a marked decrease in treated samples, measuring 20.877 µg/ml. The treatment with activated charcoal significantly modulates the biosynthesis and accumulation of critical antioxidant compounds, namely glutathione and L-cysteine, in *Brassica oleracea* tissues.

Keywords: *Brassica oieracea*, Activated charcoal, callus. D-2,4

Introduction

White cabbage (Brassica oleracea var. gongylodes) is a low-growing biennial plant belonging to the Brassicaceae family, found in various regions around the world. It is primarily cultivated for its edible tuber that develops at the lower part of the stem (1). The internal flesh of the tuber remains consistently white regardless of the stem's external color, which can vary between white, red, or green. Like maximum cabbages, white cabbage is valued for its excessive dietary and healing properties and producing active important compounds, due to its rich content of nutritional fiber, vitamin C, and quite a number other vitamins (A, B1, B2, B5, B6, and E), critical minerals along with potassium, calcium, magnesium, zinc, and iron, in addition to antioxidants recognized for his or her anticancer activities.

Unlike some varieties of bite cabbage, as a sharp taste, *B. Oleresia* shows. Gongylodes have a delicate and mild taste, which are probably responsible for high levels of hydrophilic amino acids. Lowering starch, sugar and sugar, especially fructose and glucose is also plentiful. Due to the short vegetative period and low temperature (especially in purple cultivation) and other crosses dried to the relative drought conditions, the cabbage can be grown in a less favorable environment, making it a viable alternative for economically large Brasica crops.

Comparative analyses between white cabbage and radish (*Raphanus sativus*) have demonstrated that white cabbage contains higher total levels of free amino acids and essential amino acids, highlighting its superior nutritional value. Furthermore, cabbage stems exhibit the highest concentrations of glucosinolates such as glucobrassicin and glucoraphanin among sixteen identified types, compounds which are important nutritionally and have been linked to cancer chemoprevention through their hydrolysis products like sulforaphane isothiocyanate. In addition, the peroxydage enzyme content suggests its ability as an alternative commercial source for these enzymes [1].

Extensive research has focused on the metabolism of phenol compounds and glucosinolates in white cabbage, examining their antioxidants and antimicrobial capacity, anthocyanin biosynthesis and plant wallpaper and different alicitor effects on growth, dividend and quality. Transcriptomic and hormonal studies have also explored the mechanisms underlying stem tuber formation.

In plant tissue culture, in vitro regeneration provides convincing evidence of cellular pluripot. Under controlled physical and chemical conditions, a large number of vegetation clones can be produced by growing plant cells, tissues and organs [2]. Nevertheless, micropagation techniques face challenges, including accumulation of preventive compounds in the cultural media and reduce cellular discrimination capacity. Active litter (AC) is widely recognized as an effective additive in tissue culture media, which improves the growth of plants of phenol compounds and toxic metals released by cultivated tissues, reducing their preventive effect on developing cells [3]. In addition, the AC culture stimulates organogenesis and physical embryogenesis by increasing the bioavailability of nutrients and plant vegetables in the medium [4].

Previous studies have shown that the involvement of active coal in the cultural media increases shoots and root formation, promotes voice boost and stimulates various morphological reactions in many plant species, especially when 2.4-diklorphinoccic acid (2,4-D) [5] is used in combinations with development regulations. Construction at these findings is the aim of the current study to evaluate the effect of active coal on micropaging of *Brasica Oleresia* was. Gongilods by examining the interaction between D,2-4 active charcoal and different concentrations in the cultural medium.

Materials and Methods

Seed Sterilization

Brassica -Olesia seeds were placed on the surface to eliminate potential microbial contaminants before in vitro culture. The seeds were originally rinsed three times with sterile distilled water (SDW) to remove the

surface of the surface. They then drowned in 90% ethanol for 2 minutes to get the initial disinfection and it was the best period for sterilization. Then the seeds were treated with sodium hypochlorite solution (NAOCL), which was diluted in ratio 1: 3 (volume/volume) with SDW and was gently stimulated in a sterile Erlenmeyer -Colbe for 2 minutes. Then the seeds were rinsed twice with SDW to remove remaining disinfectants and dried in the air on sterile filter paper under a lamina airflow cabinet for a few minutes from cultural initiation.

Plant Growth Conditions

The sterilized fetus of Brasica Oleresia was grown by Murashij and Scog (MS) solid medium [6]. The culture medium was adjusted to a pH of 5.7–5.9 and solidified with 8 g/L agar. Media were sterilized via autoclaving at 121 °C under a pressure of 1.4 kg/cm² for 14 minutes. During the first seven days of germination, the cultures were maintained under controlled conditions in a growth chamber.

The growth chamber was set to a photoperiod of 16 hours light and 8 hours dark, using a combination of daylight and warm white, fluorescent tubes (LF-40 W, 1:1 ratio), delivering photo synthetically active radiation (PAR) at an intensity of 200 µmol m^{-2} s^{-1}. The temperature was maintained at 24 ± 1 °C with a relative humidity of approximately 70%.

After adequate seedling development, shoot tips and nodal segments (approximately 2.0 cm in length) were excised using a sterile scalpel and transferred to fresh MS media supplemented with varying concentrations of 2,4-dichlorophenoxyacetic acid (D-2,4) as follows:

- 0.125 mg/L
- 0.250 mg/L
- 0.500 mg/L
- A hormone-free medium served as the control treatment.

Following a 7-day incubation period under standard growth conditions, the explants (including control and elicitor-treated samples) were harvested and air-dried at room temperature. Dried tissues were then finely ground using a sterile porcelain mortar and pestle.

For metabolite extraction, 5 g of powdered tissue from each treatment was subjected to Soxhlet extraction using 99% absolute ethanol as the solvent. Extracts were collected in round-bottom flasks and concentrated by rotary evaporation at 45 °C under reduced pressure using a Heidolph Laborota 4000 rotary evaporator. The dried residues were stored at 4 °C until analysis. Quantitative and qualitative analyses of bioactive compounds were performed using High-Performance Liquid Chromatography (HPLC; Shimadzu, Japan).

Elicitation Using Activated Charcoal

The dual action of AC—adsorbing inhibitors and releasing bioactive substances—is well acknowledged, and it can enhance some changing like media composition changes due to AC presence. Activated charcoal (AC) was utilized as a chemical elicitor to enhance the physiological and morphogenic responses of *Brassica oleracea* explants. A concentration of 1 mg/L AC was prepared by dissolving the charcoal in 70% ethanol and thoroughly incorporated into the Murashige and Skoog (MS) culture medium prior to solidification. Both treated explants and untreated control groups (each in triplicate) were incubated under standardized conditions of complete darkness at 24 ± 1 °C for a period of 5 to 7 weeks. This elicitation strategy aimed to investigate the influence of AC on shoot regeneration and developmental responses, particularly its interaction with auxin-type plant growth regulators under in vitro culture conditions.

Results and Discussion

The findings of the present study demonstrated that the growth and morphogenic responses of *Brassica oleracea* explants were significantly influenced by the application of the auxin 2,4-dichlorophenoxyacetic acid (2,4-D), particularly at a concentration of mg/L 0.125. In addition to fyo -hormonal remedies, Alication

was used using Active Coal (AC), which reveals a collective interaction between AC and endogenous or outlined plant development regulators.

Fytohormones are recognized as an essential modulator in plant tissue cultural systems, playing an important role in regulating cell division, increase, discrimination and organogenesis. Oxinary applications such as 2,4-D have been shown to inspire formation of lime, increase the increase in shooting and promote rotining under in vitro conditions [7]. In this study, incorporated 2,4-D into the cultural medium, stimulated effectively explicit development, and supports cellular reprogramming and well-written role hormones in morphogenesis.

In addition, active coalalikitor activity helped modify inhibitory substances, modify medium structure or provide hormone accessibility to improve exploration. Joint use of oxin and active charcoal in vitro. Regeneration provides a promising approach to optimization of the regeneration protocol for *Oleresia*.

Elicitation of *Brassica oleracea* Explants (Shoot Tips and Leaves) Using Activated Charcoal and Phytohormonal Interaction

The in vitro propagation of *Brassica oleracea* explants, specifically shoot tips and leaves, demonstrated a significant improvement in morphogenic responses, including the number of regenerated shoots, shoot length, and number of developed leaves. Application of the synthetic auxin 2,4-dichlorophenoxyacetic acid (D-2,4) showed a dose-dependent effect on explant development.

Among all treatments, explants exposed to mg/L 0.125 of D-2,4 exhibited the most pronounced response, recording an average of 7.0 shoots per explant, a shoot length of 7.9 cm, and 7.0 leaves per explant. In comparison, the control group produced 5.0 shoots per explant, a shoot length of 6.9 cm, and 5.0 leaves per explant, confirming the stimulatory role of low concentrations of D-2,4 in shoot proliferation and elongation (Table 1).

Explant samples treated with mg/L 0.250 D-2,4 showed a slightly reduced response, producing 6.0 shoots per explant with a shoot length of 7.0 cm and 6.0 leaves. These findings suggest that while D-2,4 is effective in promoting shoot induction, optimal concentrations must be carefully determined to avoid inhibitory effects at higher levels.

Regarding leaf explants, results presented in Table 2 indicate that mg/L 0.250 of D-2,4 was the most effective concentration, yielding 6.0 secondary buds, a bud length of 4.5 cm, and 5.0 secondary leaves. Other concentrations, particularly mg/L 0.125 and mg/L 4.0, demonstrated a comparatively reduced effect on leaf regeneration, except for a slight increase in bud length at mg/L 3.2.

These results underline the importance of hormonal balance in tissue culture systems, and highlight the synergistic role of elicitors such as activated charcoal in modulating phytohormonal responses to enhance regeneration efficiency in *B. oleracea*.

Table 1. Elicitation explants with D-2,4 mg. L^{-1} concentrations on regeneration from sterilized explants of *B. oleracea* shoot. tips cultured on the MS solid medium.

D-2,4 0.125 (mg. L-1)	**Shoots Nr.**	**Shoots L. (cm)**	**Leaves Nr.**
0.0	A 5.0	A 6.9	A 5.0
1.0	A 7.0	A 7.9	A 7.0
2.0	A 6.0	A 7.0	A 6.0
3.0 P.Valve قيمة	0.0 0.4219 n.s	0.0 0.6071 n.s	0.0 0.6297 n.s

Table 2: Elicitation explan ts interaction with concentration of D-2,4 mg. L^{-1} concentrations on regeneration from sterilized explants of B. oleracea leaves cultured on the MS solid medium.

D-2,4 0.125 (mg. L-1)	Shoots Nr.	Shoots L. (cm)	Leaves Nr.
0.0	A 7	C 7.9	A 7
1.0	B 4.0	A 3.2	B 4.0
2.0	B 6	B 4.5	B 5
3.0 P.Valve قيمة	0.0 <.0001 **	0.0 0.0008 **	0.0 0.0004 **

Extraction of *Brassica oleracea* Shoot Tips and Leaves: Influence of Activated Charcoal and Phytohormonal Interaction

The application of activated charcoal (AC) in combination with varying concentrations of the auxin 2,4-dichlorophenoxyacetic acid (D-2,4) demonstrated significant effects on the morphogenic response of *Brassica oleracea* explants. As shown in Table 3, the incorporation of activated charcoal into the culture medium markedly influenced shoot regeneration parameters, including the number of buds, bud length, and the number of developed leaves.

At a concentration of mg/L1.0 D-2,4, the shoot tip explants exhibited optimal growth performance, producing an average of 8.0 lateral shoots, 5.0 cm bud length, and 8.0 leaves. Similarly, treatment with 2.0 mg/L D-2,4 also resulted in 8.0 buds but with an extended bud length of 9.0 cm and a reduced leaf number of 4.0. These results underscore the dose-dependent influence of D-2,4 when used in conjunction with activated charcoal.

Activated charcoal is known for its high adsorptive capacity and is widely utilized in plant tissue culture for its ability to modulate the medium's chemical composition, remove inhibitory compounds, and improve nutrient availability. In the current study, the controller showed without controlling hormones or charcoal treatment 5.0 buds, 6.9 cm shot length and average values of 5.0 leaves, confirming the favorable role of AC in promoting shooting generation.

Conversely, the application of active charcoal for exploration of the blade in less clear morphogenic reaction resulted. At mg/L1.0 D-2,4, investigators developed 4.0 buds, 3.2 cm bud length and 4.0 leaves. When the concentration was expanded up to mg/l 2.0, investigators produced only 2.0 buds, with a bud of 4.5 cm and 2.0 leaves. These findings suggest that while the AC shot tip can increase the spread of shooting in the tip, its effect on leaf exploration is more variable and is likely to be affected by tissue type and hormonal sensitivity.

In contrast, the application of active charcoal resulted in detection of knives in less pronounced morphogenic reaction in the resulting application. At 1.0 mg/l 2.4- ON, investigators developed 4.0 buds, 3.2 cm bud length and 4.0 leaves. When the concentration was expanded up to mg/l 2.0, investigators produced only 2.0 buds, with a bud of 4.5 cm and 2.0 leaves. These findings suggest that although the AC shot may increase the spread of shoots in the tip, the effect on leaf finds is more variable and is likely to be affected by tissue type and hormonal sensitivity. Its incorporation into regeneration media has been associated with improved somatic embryogenesis and shoot conversion efficiency [8].

Further investigations by [9] evaluated the physicochemical effects of eight different types of activated charcoal on culture media, including free D-2,4 concentration, pH, conductivity, and osmolarity. Their findings revealed that charcoal type significantly impacted embryogenic callus induction, with acid-washed DARCO and USP-grade charcoal promoting ~60% embryogenesis, particularly when small particle sizes

(<38 µm) were used. These small fractions produced up to 70% embryogenic callus, highlighting the relevance of both charcoal composition and particle size in optimizing in vitro culture conditions.

Table 3: Elicitation explants with activated charcoal interaction with concentrations of 2,4-D mg.L^{-} concentrations on regeneration from sterilized explants of *B. oleracea* shoot. tips cultured on the MS solid medium**.**

D-2,4 (mg•L^{-1})	**Shoots Nr.**	**Shoots L. (cm)**	**Leaves Nr.**
0.0	A 5.0	A 6.9	B 5.0
1.0	A 8.0	A 5.0	A 8.0
2.0	A 8.0	A 9.0	A 4.0
3.0 P.Valve قيمة	0.0 0.1372 n.s	0.0 0.4219 n.s	0.0 0.640 n.s

Table 4: Elicitation explants with activated charcoal interaction with concentration of D-2,4 mg. L^{-1} concentrations on regeneration from sterilized explants of B. oleracea leaves cultured on the MS solid medium.

D-2,4 (mg•L^{-1})	**Shoots Nr.**	**Shoots L. (cm)**	**Leaves Nr**
0.0	A 7	A 7.9	A 7
1.0	B 4.0	B 3.2	A 4.0
2.0	A 2.0	A 4.5	A 2.0
3.0 P.Valve قيمة	0.0 0.0983 n.s	0.0 0.0723 n.s	0.0 0.1014 n.s

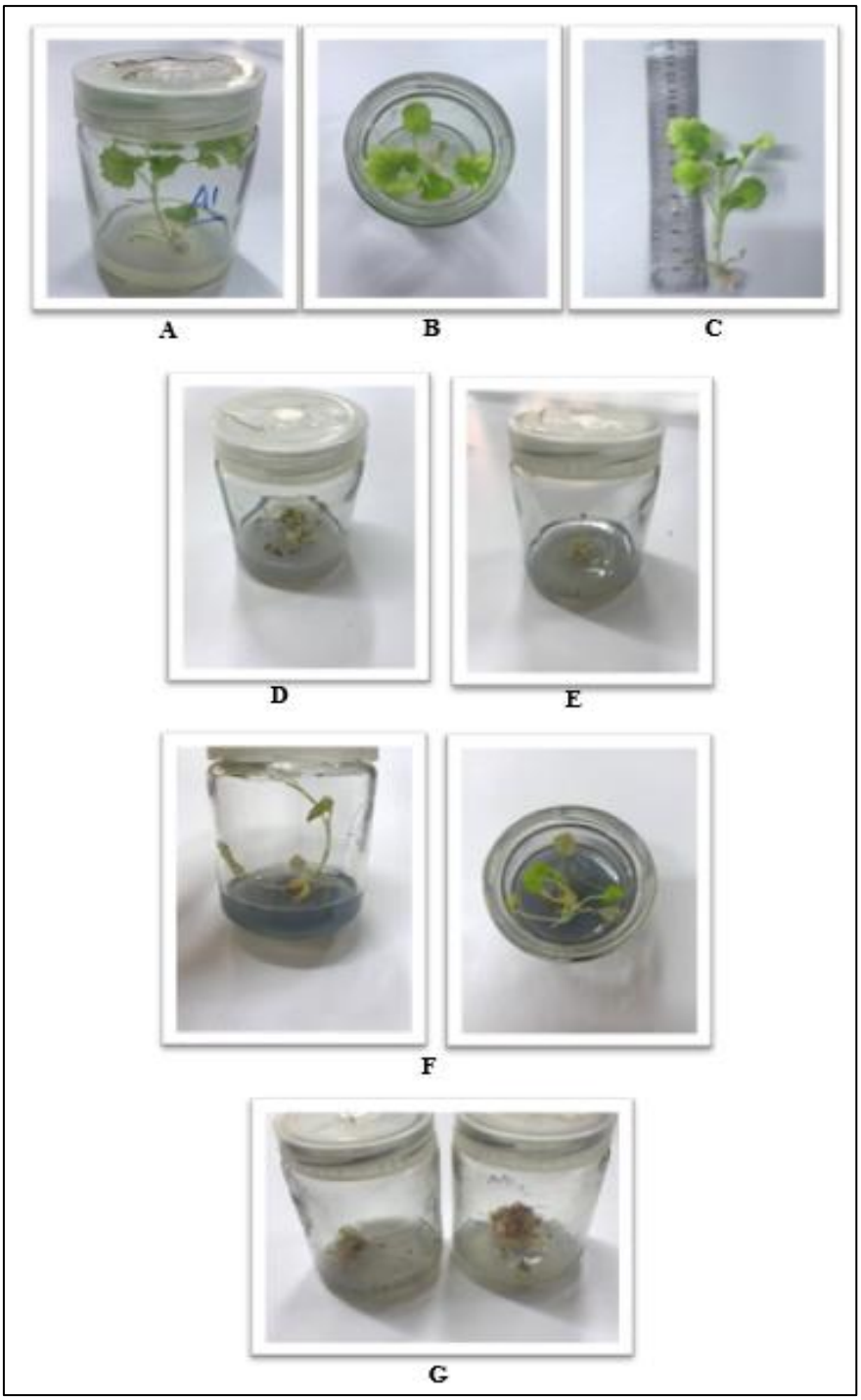


Fig. 1: B. oleracea after 6 weeks of growth in growth chamber in interaction with the concentration of D-2,4 0.125 mg•L^{-1}.

A:B Reaction with D-2,4concentration 0.125 mg•L^{-1}Concentrations of stem grown in medium

C: Illustration of the leg length measurement reaction with D-2,4concentration 0.125 mg•L^{-1}concentrations

D: Activated charcoal and reaction with D-2,4concentration at a concentration of 0.125 mg•$L^{-1}$for leaves.

E: Activated charcoal and reaction with D-2,4concentration 0.125 mg•$L^{-1}$for stems

F: Reaction of activated charcoal with D-2,4concentration at concentrations of 0.250 mg•$L^{-1}$for stems

G: Plant seeds with activated charcoal hormone at a concentration of 0.5 mg•L^{-1}

Statistical Analysis

Statistical analysis was performed using SAS software, version 9, which is widely recognized for its precision and reliability in handling experimental data [10]. The software was employed to conduct both descriptive and inferential statistical procedures relevant to the study objectives.Experimental data were imported into SAS from Excel spreadsheets after thorough verification to ensure the absence of missing or outlier values. Descriptive statistics, including means, standard deviations, and data distribution characteristics, were initially calculated to summarize the dataset. Subsequently, a one-way analysis of variance (ANOVA) was carried out to test for statistically significant differences among treatments. Where significant differences were detected, Duncan's Multiple Range Test was applied to identify specific differences between treatment means at a significance level of $p \leq 0.05$.

Effect of Activated Charcoal on the Accumulation of Antioxidants (Glutathione and L-Cysteine) in *Brassica oleracea*

The present study revealed that treatment with activated charcoal significantly modulates the biosynthesis and accumulation of critical antioxidant compounds, namely glutathione and L-cysteine, in *Brassica oleracea* tissues. Quantitative analysis demonstrated an increase in glutathione content following exposure to activated charcoal, reaching 5.528 µg/ml (Figure 2) compared to 4.660 µg/ml in untreated control samples (Figure 1). This pronounced elevation underscores the positive regulatory influence of activated charcoal on glutathione biosynthesis.

Conversely, L-cysteine levels displayed a marked decrease in treated samples, measuring 20.877 µg/ml (Figure 4) relative to 30.816 µg/ml in the control group (Figure 3). This reduction likely reflects its metabolic consumption as a precursor in glutathione synthesis or diversion into other antioxidant pathways.

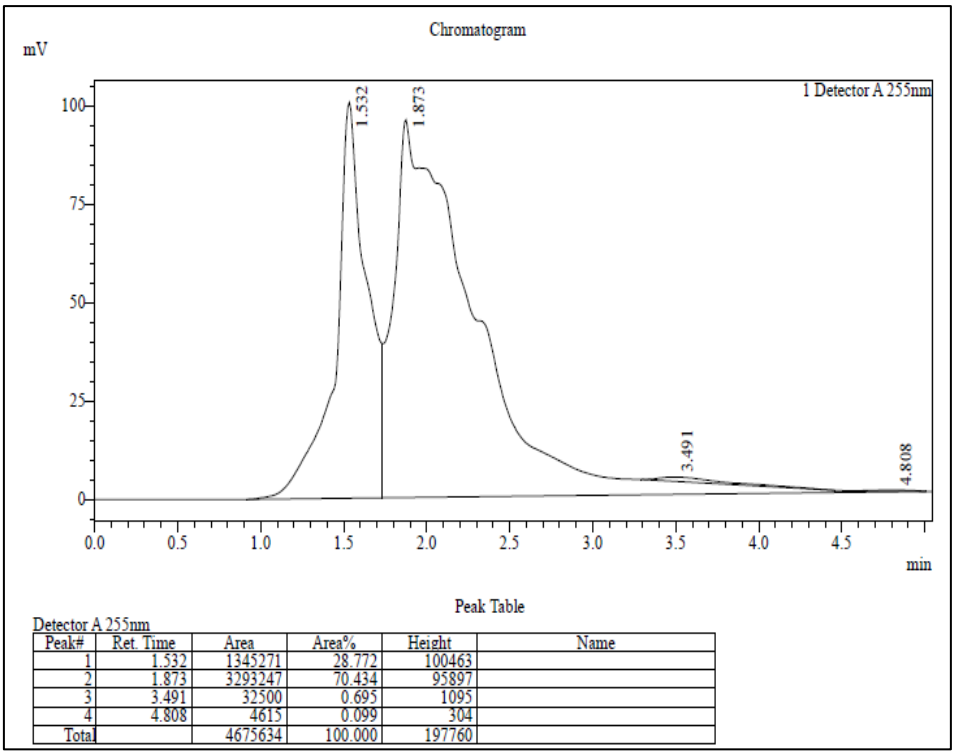
These findings align with prior research indicating that chemical elicitors, including activated charcoal, can modulate secondary metabolite accumulation by inducing physiological stress responses or redirecting metabolic fluxes [11]. The adsorptive properties of activated charcoal are believed to improve the culture medium environment by sequestering growth-inhibitory phenolic compounds and regulating the availability of phytohormones and essential nutrients, thereby promoting secondary metabolism and antioxidant biosynthesis [12].

Glutathione acts as a crucial antioxidant, neutralizes reactive oxygen species [13] and protects the cells of plants from oxidative damage. L-cysteine, as an amino acid driver containing sulfur for glutation synthesis, plays an integrated role in cellular redox homeostasis and stress optimization mechanisms [14].

Emerging evidence further shows that the active litter acts as an inorganic alicitor, antioxidant triggers the reduction of genes involved in defense and phenyl propanoid biosynthesis route, which is important for cellular discrimination and improved stress flexibility [15, 16].

In addition, extensive phytochemical profiles - Sumvesh steroids, terpenoids and other bioactive secondary metabolites - are important to clarify the medical capacity and biochemical reactions received by active coal and other external stimuli in medical plants [17]. Finally plant tissue culture considered an important technique to propagation and stimulation active compounds [18]

Overall, this data highlights not only the versatile role of active coal as a growth amplifier, but also as a powerful alicitor that increases antioxidant accumulation, which improves plant tissue culture efficiency and stress tolerance.

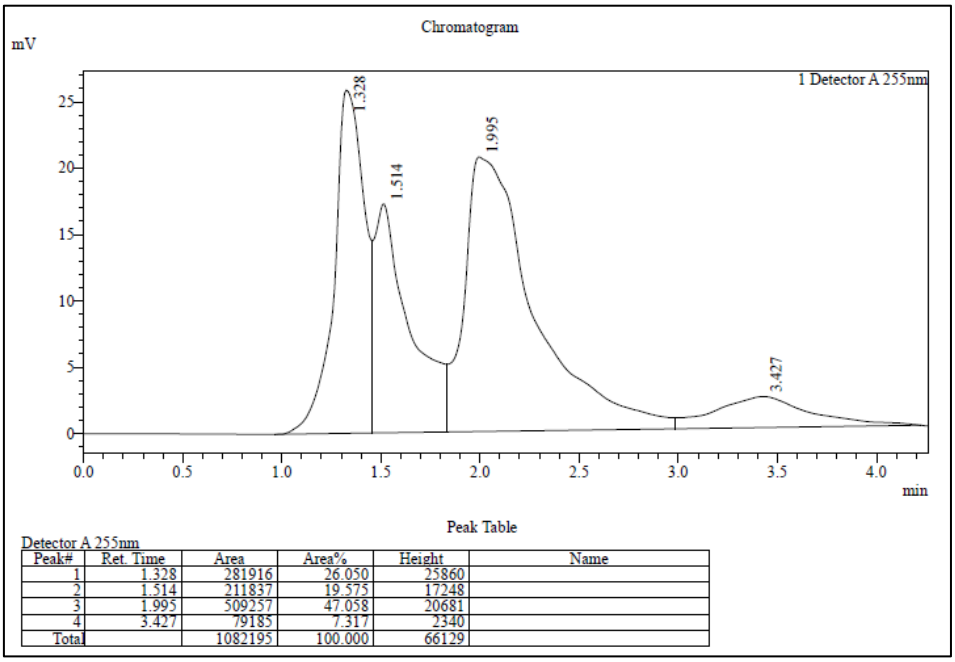


Peak Table

Detector A 255nm

Peak#	Ret. Time	Area	Area%	Height	Name
1	1.532	1345271	28.772	100463	
2	1.873	3293247	70.434	95897	
3	3.491	32500	0.695	1095	
4	4.808	4615	0.099	304	
Total		4675634	100.000	197760	

Fig. 1: Chart of HPLC Active compound Glutathione (High Performance Liquid Chromatography) control.

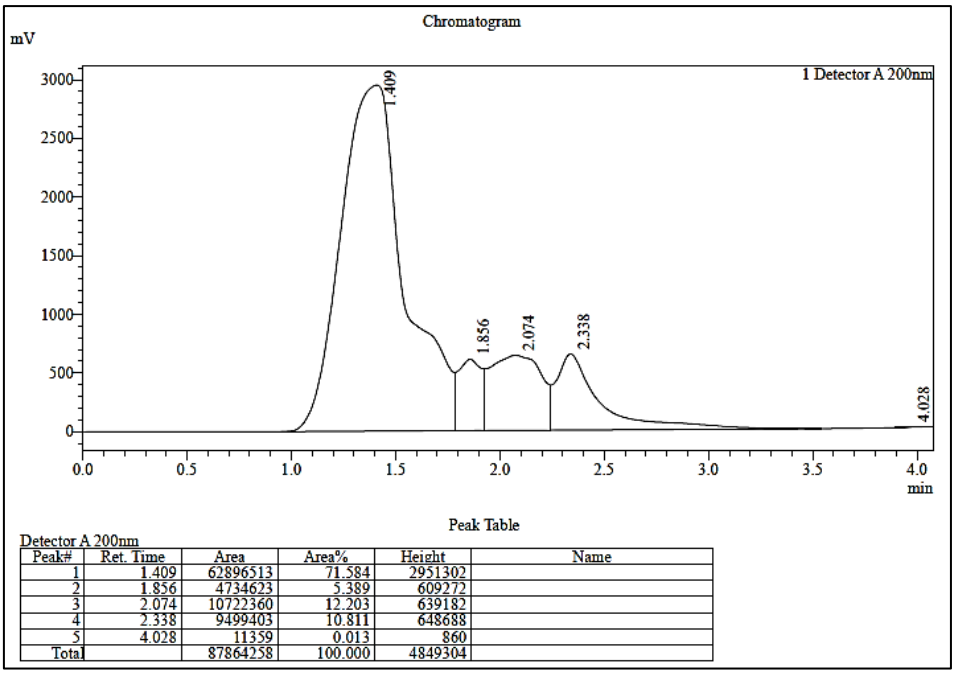


Peak Table

Detector A 255nm

Peak#	Ret. Time	Area	Area%	Height	Name
1	1.328	281916	26.050	25860	
2	1.514	211837	19.575	17248	
3	1.995	509257	47.058	20681	
4	3.427	79185	7.317	2340	
Total		1082195	100.000	66129	

Fig. 2: Schematic of the active compound glutathione (HPLC) after elution with activated charcoal.

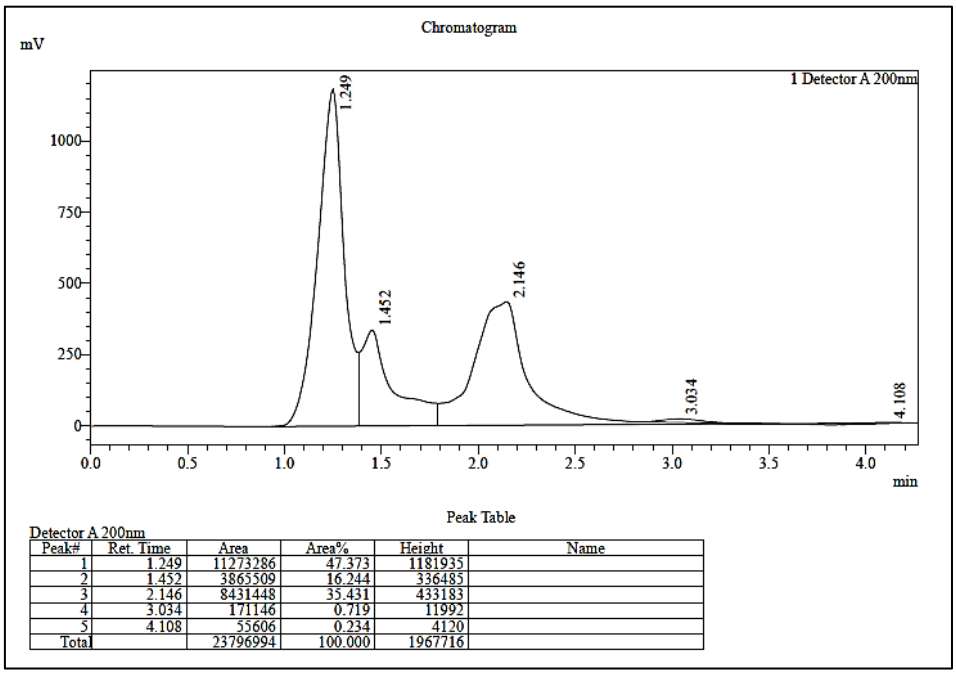


Peak Table

Detector A 200nm

Peak#	Ret. Time	Area	Area%	Height	Name
1	1.409	62896513	71.584	2951302	
2	1.856	4734623	5.389	609272	
3	2.074	10722360	12.203	639182	
4	2.338	9499403	10.811	648688	
5	4.028	11359	0.013	860	
Total		87864258	100.000	4849304	

Fig. 3: Chart of HPLC Active compound L Cysteine (High Performance Liquid Chromatography) control



Peak Table

Detector A 200nm

Peak#	Ret. Time	Area	Area%	Height	Name
1	1.249	11273286	47.373	1181935	
2	1.452	3865509	16.244	336485	
3	2.146	8431448	35.431	433183	
4	3.034	171146	0.719	11992	
5	4.108	55606	0.234	4120	
Total		23796994	100.000	1967716	

Fig. 4. Schematic of the active compound L-cysteine in high-performance liquid chromatography (HPLC) after elution with activated charcoal.

Conclusion

This study demonstrated that activated charcoal at concentrations of 1.0 and mg/L2.0 significantly enhances shoot tip growth from explants. Notably, explants treated with these concentrations exhibited increased leaf numbers, reaching up to 8 leaves per explant. The optimal concentration for inducing the highest average number of buds was identified as 1.0 mg/L of D-2,4, resulting in an average of 8.0 buds per sample, with a maximum bud length of 5.0 cm and 8 leaves per explant. Treatment with activated charcoal combined with mg/L 1.0 D-2,4 produced 4.0 buds per sample, a bud length of 3.2 cm, and 4.0 leaves per explant. However,

at mg/L2.0 activated charcoal, a significant reduction in leaf and branch development was observed, with only 2.0 branches and leaves per explant, compared to the control group which exhibited 7 branches, 7.9 cm branch length, and 7 leaves per explant.

Furthermore, activated charcoal treatment led to a notable increase in glutathione accumulation in *Brassica oleracea*, reaching 5.528 µg/ml compared to 4.66 µg/ml in controls. This suggests a role for activated charcoal in enhancing antioxidant capacity. The study also revealed that L-cysteine concentration decreased to 20.88 µg/ml under activated charcoal treatment, compared to 30.82 µg/ml in controls, indicating complex interactions and possible competitive dynamics between secondary metabolites such as glutathione and L-cysteine.

At the molecular level, activated charcoal appears to promote seedling growth by upregulating genes involved in the phenylpropanoid biosynthesis pathway, which are crucial for cell differentiation and development, as well as genes associated with plant hormone signaling pathways linked to stress resistance. Collectively, these findings highlight the multifaceted role of activated charcoal in modulating physiological and molecular processes that enhance explant growth and resilience.

Conflict of interests: The authors declared no conflicting interests.

Sources of funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contribution: Authors contributed equally in the study.

References

(1) Raspor, M., Motyka, V., Kaleri, A. R., Ninković, S., Tubić, L., Cingel, A., & Ćosić, T. (2021). Integrating the roles for cytokinin and auxin in de novo shoot organogenesis: from hormone uptake to signaling outputs. *International Journal of Molecular Sciences*, *22*(16), 8554.8554.

(2) Zhao, M., Wu, Y., & Ren, Y. (2024). Complete Chloroplast Genome Sequence Structure and Phylogenetic Analysis of Kohlrabi (Brassica oleracea var. gongylodes L.). *Genes*, *15*(5), 550.550.

(3) Al-Taie, G. R. I., Al-Mashea, R. A., Saddon, N., & Al-Anbari, A. K. (2018). A comparative study of the phenolic compounds of some Brassicaceae taxa by high-performance liquid chromatography (HPLC) technique. *Tikrit Journal of Pure Science*, *23*(2), 45-48

[4] Yasmine HJ, Meena AM, Sara FH. Biohistological study of the effect of isolated flavonoids from Brassica Olearacea var. Capitata in the effectiveness of (AST, ALT) and liver tissue structure in alloxan induced diabetic white rats females. Tikrit Journal of Pure Science. 2023;22(5):40-4. https://doi.org/10.25130/tjps.v22i5.767

(5) Danova, K., Stanoeva, J. P., Aneva, I., Alipieva, K., & Stefova, M. (2023). Plant growth regulators and activated charcoal selectively affect phenylethanoid and flavone glycoside accumulation in Sideritis scardica Griseb. tissue culture. *Plants*, *12*(13), 2541.

(6) Classic Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. plant*, *15*, 473-497..

(7) Alsoufi, A. S., Ahmed, Z. S., & Salim, A. M. (2021, April). The efficiency of interaction between cytokines and Auxins in Micropropagation of Chrysanthemum plant (Chrysanthemum indicum L.). In *IOP Conference Series: Earth and Environmental Science* (Vol. 735, No. 1, p. 012048). IOP Publishing..

(8) Al-Qady, N. M. H., & Shaban, R. K. (2020). Physiological and histological effect of Captopril on kidney and the protective role of Brassica nigra seed extract in male rats. *Tikrit J. Pure Sci*, *25*, 27-32..

(9) Sáenz, L., Herrera-Herrera, G., Uicab-Ballote, F., Chan, J. L., & Oropeza, C. (2010). Influence of form of activated charcoal on embryogenic callus formation in coconut (Cocos nucifera). *Plant Cell, Tissue and Organ Culture (PCTOC)*, *100*(3), 301-308.

(10) Cody, R. P., & Smith, J. K. (1987). *Applied statistics and the SAS programming language*. North-Holland Publishing Co..

(11) Gadouche, L., Alsoufi, A. S. M., Pacholska, D., Skotarek, A., Pączkowski, C., & Szakiel, A. (2023). Triterpenoid and steroid content of lipophilic extracts of selected medicinal plants of the Mediterranean region. *Molecules*, *28*(2), 697

(12) Dheeran, P., & Kumar, S. Wastewater and Algal Biorefinery

(13) Noctor, G., Mhamdi, A., & Foyer, C. H. (2014). The roles of reactive oxygen metabolism in drought: not so cut and dried. *Plant physiology*, *164*(4), 1636-1648.

[14] Hell, R., & Bergmann, L. (1990). Sulfur metabolism in higher plants: uptake, assimilation and metabolic fluxes. *Physiologia Plantarum*, 78(4), 601-607.

(15) Vogt, T. (2010). Phenylpropanoid biosynthesis. *Molecular plant*, *3*(1), 2-20.

(16) Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry*, *48*(12), 909-930.

[17] Gadouche, A., et al. (2023). Chemical elicitors and secondary metabolites accumulation in medicinal plants: Mechanisms and applications. *Journal of Plant Biochemistry and Biotechnology*, 32(1), 45-60. https://doi.org/10.1016/j.sajb.2021.10.014

(18) Ahmed, Z. S., Salim, A. M., & Alsoufi, A. S. M. (2021). In vitro micropropagation of Atropa belladonna L. using plant growth regulators. *Plant Cell Biotech Mol Biol*, *22*(3&4), 169-174..