Phyton-
International Journal of
Experimental Botany

Tech Science Press

http://doi.org/10.32604/phyton.2026.083745

ARTICLE

Growth, Nutrient Accumulation, and Root Architecture Responses of Cucumber Plants to Different Graphene Oxide Concentrations

Zejin Zhang[1,2,#], Zhengnan Yan[3,#], Xiangyu Ding[3], Weiming Ren[3], Yarong Zhang[3], Haoxu Shen[3], Jinxiu Song[4], Na Lu[5], Ying Liang[1,2] and Li Tang[1,2,*]

[1]Horticulture Research Institute, Sichuan Academy of Agricultural Sciences, Chengdu, China
[2]Sichuan Province Engineering Technology Research Center of Vegetables, Pengzhou, China
[3]College of Horticulture, Qingdao Agricultural University, Qingdao, China
[4]College of Agricultural Engineering, Jiangsu University, Zhenjiang, China
[5]Center for Environment, Health and Field Sciences, Chiba University, Kashiwa, Chiba, Japan
[*]Corresponding Author: Li Tang. Email: tangli-999@163.com
[#]These authors contributed equally to this work

Received: 10 April 2026; Accepted: 09 May 2026; Published: 27 May 2026

ABSTRACT: Graphene oxide (GO) has shown great potential in agricultural applications, however, its concentration-dependent effects on cucumber (*Cucumis sativus* L.) growth, nutrient absorption, and root architecture remain unclear. In the present study, a hydroponic experiment was conducted with different GO concentrations (0.5, 1.0, and 2.0 mg L^{-1}) and setting the non-GO treatment (0.0 mg L^{-1}) as the control for cucumber plants (cv. Qingbaizao). The results showed that low to moderate concentrations (0.5–1.0 mg L^{-1}) significantly promoted cucumber growth, increased shoot and root biomass, enhanced the accumulation of nitrogen, phosphorus, and potassium, and optimized root architecture by increasing cellulose and hemicellulose content. In contrast, high GO concentrations (2.0 mg L^{-1}) exhibited significant inhibitory effects, reducing plant growth indicators, inhibiting nutrient accumulation, particularly in shoots, damaging root structure, and leading to obvious damage to root apical vascular bundles and disrupted the structural integrity of root tips. Further analysis revealed that the regulatory effect of GO on cucumber growth was closely related to its influence on root uptake capacity and root architecture optimization, as differentially expressed genes in 'GO and Control' comparison was remarkably enriched in phenylpanoid biosynthesis. This study systematically explores the concentration-dependent responses of cucumber growth, nutrient accumulation, and root architecture to GO, clarifies the suitable concentration range of GO for cucumber growth promotion, and provides theoretical basis and technical reference for the rational application of GO in cucumber cultivation.

KEYWORDS: Cucumber; graphene oxide; cellulose content; root architecture; nitrogen content

1 Introduction

Nanomaterials have demonstrated remarkable prospects and application value in numerous fields, including biomedicine [1,2], electronics [3], food quality monitoring [4], and environmental remediation [5] in recent years. In addition, nanomaterial-based technologies have been increasingly utilized in protected horticulture, including applications in fertilizers [6], pesticides [7], and drought resistance [8]. Furthermore, nano-enabled agrochemicals, such as nano-pesticides and nano-fertilizers, are gradually being integrated into agricultural production practices, providing new possibilities for the sustainable agriculture.

Graphene has emerged as a highly promising nanomaterial over the past few years [9,10]. As an allotrope of carbon, it exhibits excellent chemical and mechanical stability, a large specific surface area, and low biotoxicity [11]. In addition, recent studies have indicated that oxidized nanomaterials, including graphene oxide (GO, a key derivative of graphene), exert certain effects on the growth of horticultural plants, which are dependent on specific conditions [12,13]. For example, previous studies have found that GO has significant impacts on plant morphology and physiological characteristics [14–16]. Specially, 25–100 mg L^{-1} GO application inhibited root length and fresh weight accumulation in rapeseed seedlings, accompanied by a reduction in abscisic acid content. Moreover, exposure to 50 mg L^{-1} GO significantly modified the gene expression profiles involved in plant hormone metabolism [14]. However, the mean numbers of leaves and roots formed in strawberry *in vitro* cultures increased as GO concentration ranged from 2.5 to 5.0 mg L^{-1}, whereas no significant differences were detected when GO concentration was further ranged from 5.0 to 10.0 mg L^{-1} [17]. These findings demonstrate that different concentrations of GO exert distinct effects on crops, with obvious interspecific differences among plant species.

Compared with conventional soil cultivation, soilless culture has been widely adopted in protected agriculture, primarily owing to its distinct advantages such as higher yield, higher fertilizer use efficiency, and improved produce quality [18,19]. Additional auxiliary substances can be incorporated into the nutrient solution to further enhance crop yield and improve product quality. For instance, trace elements (e.g., zinc, iron, and manganese) [20], plant growth regulators (such as gibberellins and indole-3-acetic acid) [21], and bioactive substances (including humic acid and chitosan) [22] are commonly used auxiliary components, which can synergistically promote nutrient absorption of plants and regulate metabolic processes, thereby optimizing crop yield and quality. GO possesses abundant oxygen-containing functional groups, exhibiting excellent dispersibility and hydrophilicity [23]. In contrast to soil cultivation, GO directly interacts with plant roots in hydroponic systems, thereby exerting more pronounced and direct effects.

Cucumber (*Cucumis sativus* L.) is a globally widely cultivated horticultural crop, characterized by its high content of cellulose, abundant vitamins and minerals, and low caloric value [24–26]. Based on the data from the Food and Agriculture Organization of the United Nations, China ranks first in global cucumber production and harvested area [27]. With the continuous advancement of agricultural production modes in China, achieving efficient production of hydroponic cucumbers has become an inevitable research focus. In production practice, excessive irrigation and fertilization are often applied to promote rapid growth and achieve high yields, which consequently leads to low water and fertilizer use efficiencies [28,29]. Therefore, suitable water and fertilizer management strategies are required to guarantee cucumber yield, while simultaneously improving fruit quality and improving the utilization efficiency of water and fertilizer.

With the continuous advancement in the production and application of nanomaterials, the impacts of nanomaterial particles in agricultural production have emerged as unavoidable research topic in the field. As a by-product generated during the fabrication of graphene, research focusing on the application of GO holds crucial significance for industrial production and the efficient utilization of resources. Therefore, the objective of this study was to regulate the concentration of GO in hydroponic nutrient solutions and investigate its effects on plant growth, nutrient elements, and root architecture of cucumber plants. The results are expected to provide novel strategies for improving crop yields and promoting resource utilization efficiency. Moreover, this approach can broaden the application scenarios and consumption demand of the graphene industry, thereby providing a new impetus for the sustainable development of the horticultural industry.

2 Material and Method

2.1 Plant Material

Cucumber (*Cucumis sativus* L. cv. Qingbaizao) seeds were procured from Chengdu Haote Horticulture Co., Ltd., Sichuan Province, China. The seeds were germinated on sponges soaked in deionized water in a growth chamber, with day/night temperatures of 24°C/22°C and relative humidity (RH) maintained at 60–80%. Following a 2-day dark germination period, seedlings were exposed to white LED light with an intensity of 200 μmol m^{-2} s^{-1} and a 16 h d^{-1} photoperiod. Light intensity at the plant canopy was determined using a spectroradiometer (AvaSpec-ULS2048, Avantes Inc., Apeldoorn, The Netherlands).

2.2 Experimental Design

After the first pair of true leaves unfolded (10 d after sowing), cucumber seedlings were transplanted into 10 L individual tanks containing nutrient solution [30] in a greenhouse on 16 May 2025 at the Sichuan Academy of Agricultural Sciences, Chengdu, China (104.06° E, 30.67° N). Graphene oxide (Suzhou Tanfeng Graphene Technology Co., Ltd., Suzhou, China) was sequentially added to the nutrient solution at various dosages to achieve final concentrations of 0 mg L^{-1}, 0.5 mg L^{-1}, 1.0 mg L^{-1}, and 2.0 mg L^{-1}, respectively. The lateral sheet size of GO ranged from 0.2 to 10 μm, with a predominant distribution of 0.5–5 μm. The oxygen content was approximately 20%, representing the oxidation degree of the material. The GO exhibited a positive Zeta potential. When supplemented into the nutrient solution, GO maintained good dispersion and homogeneous distribution, with no precipitation or obvious aggregation observed. An aeration tube was installed in each tank, and aeration was conducted for 1 min every 2 h to enhance dissolved oxygen in the nutrient solution. The nutrient solution was renewed every 8 d. All physiological and biochemical indices were determined on 3 June 2025. Each treatment consisted of three replicates, with 10 cucumber plants per replicate.

2.3 Growth Measurement

2.3.1 Plant Growth Parameter

The shoot and root fresh weights of cucumber plants were determined using an electronic analytical balance (FA1204B; BioonGroup, Shanghai, China). Shoots and roots were first fixed at 105°C for 3 h in a forced-air oven, then dried to constant weight at 80°C for 72 h, after which their dry masses were recorded. The specific leaf weight = leaf dry weight/leaf area.

2.3.2 Photosynthetic Characteristics

Fully expanded mature leaves were randomly selected for measurement of photosynthetic parameters using a portable photosynthesis system (LI-6400, LI-COR Biosciences, Lincoln, NE, USA). Measurements were performed with an LED red-blue leaf chamber. The light intensity inside the leaf chamber was set at 800 μmol m^{-2} s^{-1}, leaf temperature at 25°C, and CO_2 concentration at 400 μmol mol^{-1}, respectively.

2.3.3 Nutrient Accumulation Analysis

Nitrogen (N) and phosphorus (P) concentration were determined using the Kjedahl method [31], the vanadium-molybdenum blue method [32], respectively. Potassium (K) concentration was determined by flame photometry (FP6410, Jingke Analytical Instruments, Shanghai, China) [33]. Nutrient accumulation was calculated as the product of nutrient concentration and corresponding dry biomass.

2.3.4 Brightfield Microscopy of Cucumber Root

Root tissues of cucumber plants were processed for structural observation via the safranin-fast green staining protocol for plant histology. The samples were observed using brightfield microscope (SWE-CX63, Servicebio, Wuhan, China).

2.3.5 RNA Sequencing and Data Processing

There were three replicate samples from 0 mg L^{-1} (control) and 0.5 mg L^{-1} GO for transcriptomic measurements. Total RNA was extracted using TRIzol reagent (Invitrogen Life Technologies) and qualified using a NanoDrop spectrophotometer (Thermo Scientific). Three μg of high-quality RNA was used for mRNA enrichment, cDNA synthesis, and library construction following standard Illumina protocols, with fragment selection and purification performed using the AMPure XP system (Beckman Coulter). The final library was quantified and sequenced on the NovaSeq 6000 platform (Illumina) by Shanghai Personal Biotechnology Co., Ltd. High-quality clean reads were aligned to the reference genome of *Cucumis sativus* (http://www.cucurbitgenomics.org/organism/20, accessed on 8 July 2025).

2.3.6 Root Structural Carbohydrates in Cucumber

The contents of cellulose and hemicellulose in cucumber roots were determined according to the principle of Van Soest detergent fiber analysis [34,35].

2.4 Statistical Analysis

Statistical comparisons among treatments were performed using one-way analysis of variance (ANOVA) with SPSS 19.0 (IBM, Inc., Chicago, IL, USA), followed by the least significant difference (LSD) test at $p < 0.05$. All data are presented as mean ± standard deviation (SD) from three replicates. To identify differentially expressed genes (DEGs) significantly enriched in specific metabolic pathways and functional categories, functional enrichment analyses were carried out using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway databases, according to the method of Li et al. [36]. DEGs identified were filtered for |log2FoldChange| > 1 and significance p-value < 0.05.

3 Results

3.1 Effects of Different Concentrations of Graphene Oxide on Morphology and Photosynthetic Characteristics of Cucumber Plants

The morphology of cucumber plants was remarkably affected by different concentrations of GO (Table 1). The plant height and specific leaf weight of cucumber plants showed an initial increase followed by a subsequent decrease with increasing GO concentrations. These two parameters reached their maximum values at 0.5 mg L^{-1} GO, and no remarkable differences were exhibited between the 0.5 mg L^{-1} and 1.0 mg L^{-1} treatments. In addition, no remarkable differences were showed in leaf number of cucumber plants among all treatments.

No remarkable differences were exhibited in photosynthetic characteristics of cucumber plants among all treatments, including net photosynthetic rate and transpiration rate (Table 2).

Table 1: Effects of different concentrations of graphene oxide on morphology of cucumber plants.

Treatments (mg L^{-1})	Plant Height cm	Leaf Number	Specific Leaf Weight mg cm^{-2}
0.0	86.0 ± 8.5^{ab}	9.0 ± 0.8^{NS}	0.253 ± 0.009^{b}
0.5	91.6 ± 6.9^{a}	9.0 ± 0.5^{NS}	0.301 ± 0.032^{a}
1.0	86.3 ± 6.2^{ab}	8.5 ± 0.9^{NS}	0.280 ± 0.027^{a}
2.0	84.3 ± 6.2^{b}	9.0 ± 0.5^{NS}	0.224 ± 0.018^{c}
p-value	0.042	0.923	0.037

Difference lowercase letters within one column indicate significant difference at the level of $p < 0.05$ by the least significant difference test. NS indicates no significant difference.

Table 2: Effects of different concentrations of graphene oxide on photosynthetic characteristics of cucumber plants.

Treatments (mg L^{-1})	P_n μmol m^{-2} s^{-1}	T_r mmol m^{-2} s^{-1}	C_i μmol mol^{-1}	G_s mol m^{-2} s^{-1}
0.0	21.17 ± 2.92^{NS}	8.28 ± 1.76^{NS}	318 ± 12^{NS}	0.80 ± 0.25^{NS}
0.5	18.00 ± 2.16^{NS}	8.21 ± 1.21^{NS}	329 ± 6^{NS}	0.77 ± 0.15^{NS}
1.0	20.68 ± 3.34^{NS}	8.52 ± 1.86^{NS}	314 ± 5^{NS}	0.72 ± 0.21^{NS}
2.0	21.64 ± 2.47^{NS}	9.60 ± 1.20^{NS}	323 ± 2^{NS}	0.90 ± 0.16^{NS}
p-value	0.098	0.110	0.101	0.149

P_n, net photosynthetic rate; T_r, transpiration rate, C_i, intercellular CO_2 concentration, G_s, stomatal conductance. NS within one column indicates no significant difference at the level of $p < 0.05$ by the least significant difference test.

3.2 Effects of Different Concentrations of Graphene Oxide on Biomass Accumulation of Cucumber Plants

Significant differences were found in biomass accumulation of cucumber plants treated by different concentrations of GO (Table 3). Shoot fresh weight and root fresh weight of cucumber plants increased with the concentrations of GO and subsequently decreased with the high value in the treatment of 0.5 mg L^{-1} GO. The shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight of cucumber plants grown under 0.5 mg L^{-1} GO increased by 24.3%, 24.8%, 25.0%, and 30.3%, compared with the control treatment. In addition, the biomass accumulation of cucumber plants showed a quadratic correlation with GO concentration (Fig. 1).

Table 3: Effects of different concentrations of graphene oxide on biomass accumulation of cucumber plants.

Treatments (mg L^{-1})	Shoot Fresh Weight (kg per Plant)	Shoot Dry Weight (g per Plant)	Root Fresh Weight (kg per Plant)	Root Dry Weight (g per Plant)
0.0	0.103 ± 0.011^{b}	8.82 ± 1.02^{b}	0.044 ± 0.004^{b}	0.76 ± 0.07^{b}
0.5	0.128 ± 0.012^{a}	11.01 ± 0.78^{a}	0.055 ± 0.005^{a}	0.99 ± 0.08^{a}
1.0	0.120 ± 0.017^{a}	10.26 ± 1.69^{a}	0.052 ± 0.006^{a}	0.98 ± 0.11^{a}
2.0	0.095 ± 0.020^{b}	8.12 ± 1.74^{b}	0.040 ± 0.004^{b}	0.78 ± 0.07^{b}
p-value	0.013	0.012	0.002	0.003

Difference lowercase letters within one column indicate significant difference at the level of $p < 0.05$ by the least significant difference test.

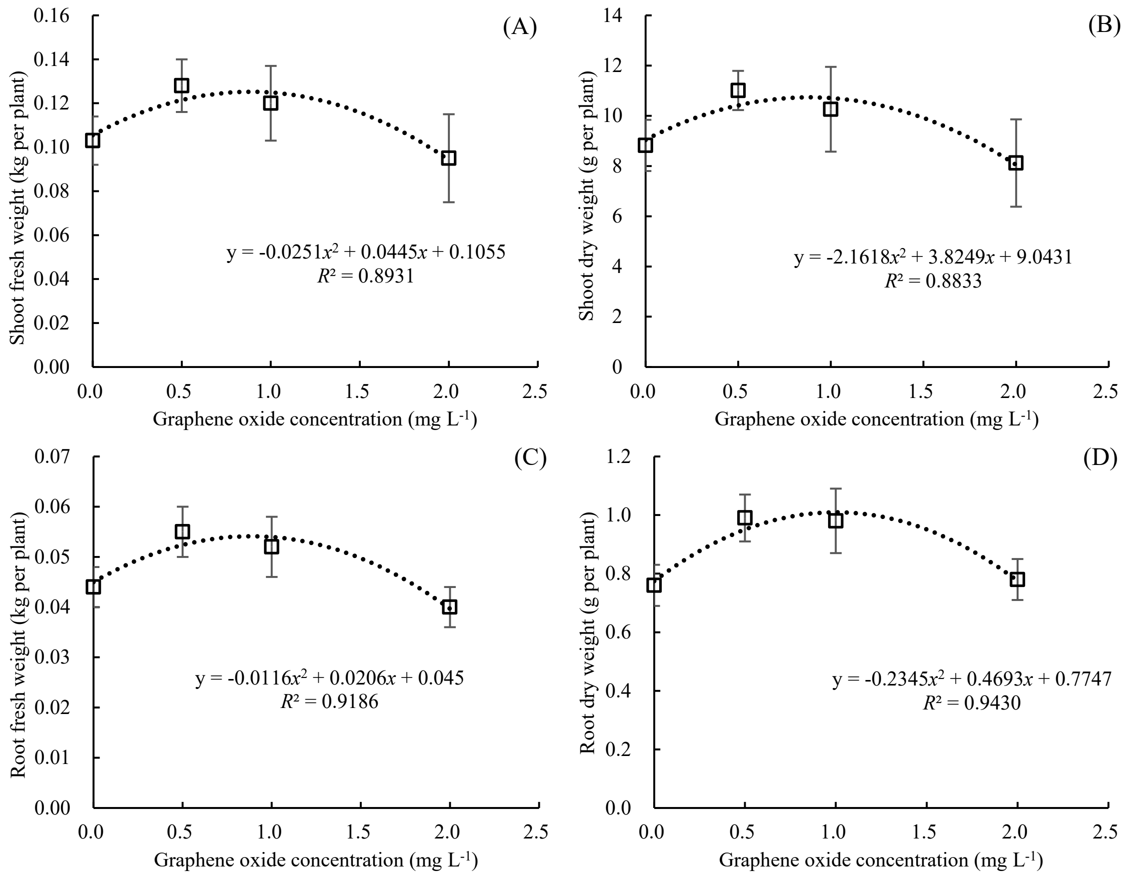

Figure 1: Regressions between different concentrations of graphene oxide (GO) and (**A**) shoot fresh weight, (**B**) shoot dry weight, (**C**) root fresh weight, and (**D**) root dry weight of greenhouse-grown cucumber plants.

3.3 Nutrient Accumulation of Cucumber Plants under Different Concentration of Graphene Oxide

GO exerted a dose-dependent effect on N, P, and K accumulation in cucumber shoots and roots (Table 4). Compared with the control, low GO concentrations (0.5–1.0 mg L^{-1}) significantly promoted nutrient accumulation, with the maximum increases in shoot N (0.5 mg L^{-1}), shoot P and K (1.0 mg L^{-1}), and root K (1.0 mg L^{-1}). In contrast, high GO concentration (2.0 mg L^{-1}) markedly inhibited nutrient accumulation, with shoot N, P, and K contents decreasing to levels significantly lower than those in the low-dose groups and even comparable to the control. However, no remarkable differences were exhibited in root N, P, and K contents in cucumber between the 0.5 and 1.0 mg L^{-1} GO treatments.

Table 4: Effects of different concentrations of graphene oxide concentrations on nutrient accumulation of cucumber plants.

Treatments (mg L^{-1})	Nutrient Elements of Shoot (g per Plant)			Nutrient Elements of Root (g per Plant)		
	Nitrogen	Phosphorus	Potassium	Nitrogen	Phosphorus	Potassium
0.0	0.400 ± 0.015^c	0.083 ± 0.002^b	0.582 ± 0.027^c	0.044 ± 0.003^b	0.011 ± 0.001^b	0.073 ± 0.001^c
0.5	0.502 ± 0.022^a	0.085 ± 0.002^b	0.606 ± 0.025^b	0.057 ± 0.004^a	0.015 ± 0.003^a	0.084 ± 0.002^b
1.0	0.489 ± 0.015^b	0.101 ± 0.003^a	0.661 ± 0.020^a	0.057 ± 0.008^a	0.013 ± 0.004^a	0.094 ± 0.004^a
2.0	0.378 ± 0.012^d	0.078 ± 0.005^c	0.533 ± 0.012^d	0.045 ± 0.003^b	0.013 ± 0.003^a	0.071 ± 0.002^c
p-value	0.000	0.000	0.000	0.000	0.000	0.000

Difference lowercase letters within one column indicate significant difference at the level of $p < 0.05$ by the least significant difference test.

3.4 Effects of Different Concentrations of Graphene Oxide on the Root Microstructure of Cucumber Plants

Different concentrations of GO exerted significant effects on the anatomical structure of cucumber roots (Fig. 2). Our results revealed that GO treatment markedly increased the number of root cortical cells. Specifically, the intercellular spaces of cortical parenchyma cells exhibited an obvious enlargement trend in the 2.0 mg L^{-1} GO treatment. Treatment with 0.5 mg L^{-1} GO effectively promoted vascular bundle development and enhanced its material transport potential. Further observation of longitudinal root apical sections showed that root apical vascular bundles developed well under low-concentration (0.5 mg L^{-1}) and medium-concentration (1.0 mg L^{-1}) GO treatments, with a significantly higher differentiation degree than that in the control group (CK). In contrast, high-concentration (2.0 mg L^{-1}) GO treatment caused obvious damage to root apical vascular bundles and disrupted the structural integrity of root tips.

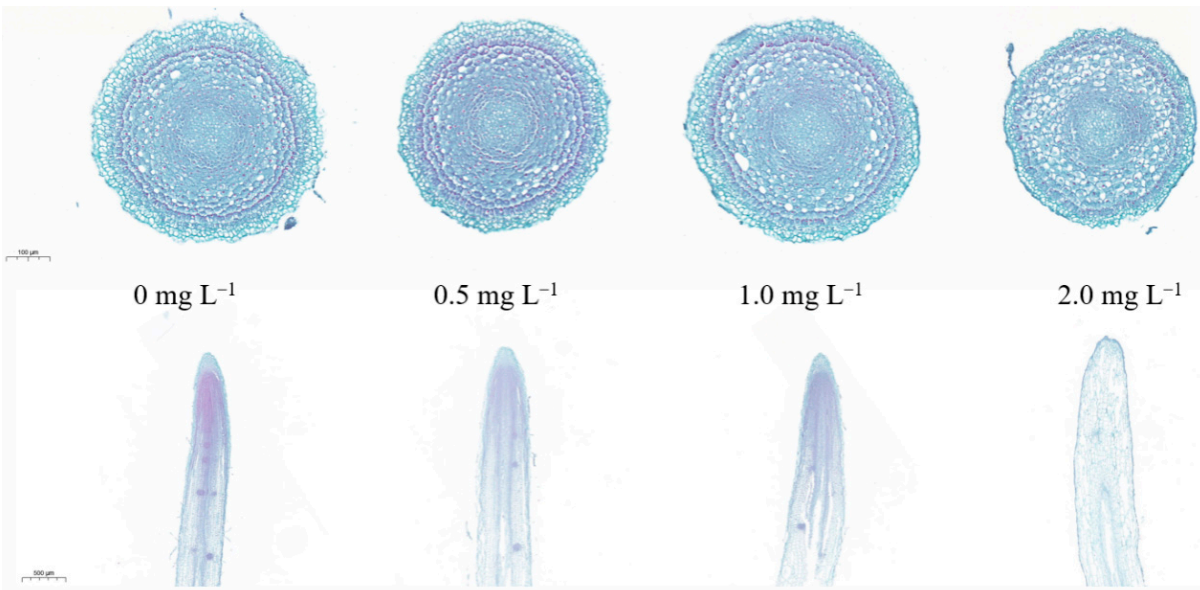

Figure 2: Effects of different concentrations of graphene oxide (GO) on the anatomical structure of cucumber roots. Transverse section of cucumber root in the upper layer and longitudinal section of cucumber root tip in the lower layer.

3.5 Transcriptome Profiling of Cucumber Roots under Different Concentrations of Graphene Oxide

In cucumber roots, GO treatment induced 240 upregulated and 81 downregulated genes (Fig. 3A). Based on KEGG annotation, the differentially expressed genes (DEGs) identified in the GO versus Control comparison were significantly enriched in pathways related to cysteine and methionine metabolism, phenylpropanoid biosynthesis, and the biosynthesis of various secondary metabolites (Fig. 3B). Moreover, GO enrichment analysis revealed that the genes responsive to GO treatments were predominantly enriched in the cell component category (extracellular region), the molecular function category (endopeptidase inhibitor activity), and the biological process category (response to stress), respectively (Fig. 3C).

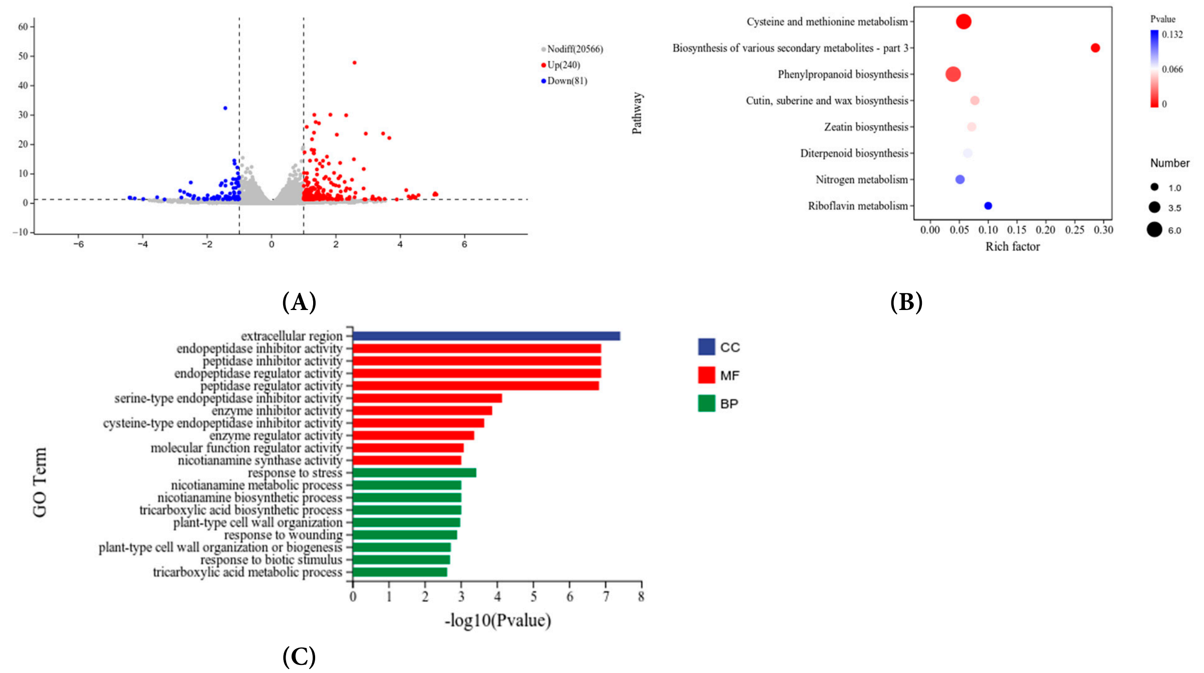

Figure 3: Transcriptome profiles of leaves in cucumber plants under control and graphene oxide (GO) treatments. (**A**) Volcano plot of DEGs between control and GO. (**B**) Pathway enrichment analysis of different expression genes (DEGs) for control and GO treatments. The color of the point represents p, and the size of the point represents the number of enriched DEGs. (**C**) GO analysis of DEGs for control and GO treatments. BP, CC, and MF represent biological processes, cell components, and molecular functions, respectively.

3.6 Effects of Different Concentrations of Graphene Oxide on Structural Carbohydrate Contents of Cucumber Plants

Cellulose and hemicellulose content of cucumber plants were significant affected by different concentrations of GO concentrations (Fig. 4). The cellulose and hemicellulose content of cucumber plants increased with the increased concentration of GO. The highest value of cellulose and hemicellulose content were observed in cucumber plants grown under 0.5 mg L^{-1} and 1.0 mg L^{-1} of GO, respectively. However, cellulose content did not differ significantly between cucumber plants grown under 0.5 and 1.0 mg L^{-1} GO.

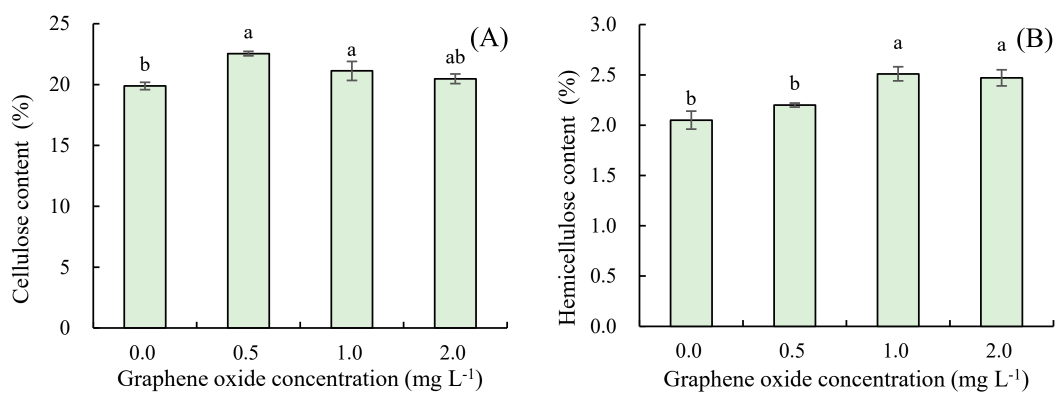

Figure 4: Effects of different concentrations of graphene oxide (GO) concentrations on (**A**) cellulose content and (**B**) hemicellulose content of cucumber root. Different lowercase letters indicate significant differences at the $p < 0.05$ level according to the least significant difference (LSD) test.

4 Discussion

With the continuous expansion of nanomaterial applications in horticulture, the biological effects of their derivatives have garnered increasing research attention [37,38]. Multi-walled carbon nanotubes (MWCNTs) at 10–50 mg L^{-1} promoted seed germination and chlorophyll content in carrot [39]. In addition, carbon-based nanomaterials (e.g., carbon nanotubes) can enter plant tissues via stomata, with no obvious inhibition on plant growth and even promoted nutrient transport at low concentrations, whereas high concentrations disrupt photosynthetic electron transport, demonstrating the concentration-dependent effects of carbon nanomaterials [40]. Similarly, our results indicated that different concentrations of GO significantly influenced the morphology and biomass accumulation of cucumber plants (Tables 1 and 3). The optimal growth of plants was achieved at 0.5 mg L^{-1} concentration of GO, with higher GO concentrations exerted no significant effects on the growth of cucumber plants as compared with those grown under control, indicating that GO regulates the leaf morphology and biomass accumulation of cucumber plants in a concentration-dependent manner. Similar trends were observed in *Stevia rebaudiana* Bertoni [16], maize seedlings [41], and lettuce [42].

Previous studies have revealed species-specific and concentration-dependent effects of GO on photosynthesis-related parameters. For instance, GO treatment decreased chlorophyll a content and disturbed light reactions in bryophytes (*Hypnum plumaeforme*) and impaired chlorophyll structure and biomass accumulation in algae (*Chroococcus* sp.) [43], whereas appropriate GO concentrations promoted photosynthesis in the stress-tolerant species Iris pseudacorus [44]. Similarly on sweet maize, it even alleviated nicosulfuron-induced photosynthetic inhibition in a sensitive cultivar [45]. Furthermore, GO was found to enhance photosynthetic electron transport and light energy conversion efficiency [46]. In the present study, GO exerted no significant effects on photosynthetic characteristics in cucumber leaves, but significantly increased leaf thickness at GO concentrations ranging from 0.5–1.0 mg L^{-1}, suggesting a species-specific adaptive response that may contribute to improved light capture and structural stability without altering pigment levels.

The plant root system is the key carrier for plants to absorb water and mineral nutrients from the medium, and it also undertakes functions such as anchoring and supporting the plant and regulating physiological signals [47,48]. Its structural and functional status directly determines the growth and development level and stress resistance of plants [49,50]. In addition, previous studies indicated that well-developed and vigorous roots can effectively support the vegetative and reproductive growth of plants in the subsequent growth period, which is conducive to promoting yield formation and quality accumulation of vegetable crops [51,52]. For instance, a significantly positive correlation was observed between yield characteristics and root traits such as total root length, root surface area, and root dry weight in grafted tomato plants [53]. Duque et al. [54] further suggested that early-stage root phenotypic traits are closely associated with yield potential and thus should be integrated into sweetpotato ideotype breeding. Our study indicated that cucumber plants developed more vigorous root systems as well as enhanced nutrient elements of plants at the optimal concentration (Fig. 2 and Table 4). Our results indicated that peak values for N, P, and K were observed in the GO concentration range of 0.5–1.0 mg L^{-1}, which were higher compared with those grown under control treatment (Table 4). Similarly, a previous study indicated that the N, P, and K content of leaves in mungbean plant increased initially and then decreased with increasing GO levels from 50 to 250 mg L^{-1}, however, the highest NPK contents under GO treatments showed no significant difference relative to the control [55]. In addition, a previous study indicated that GO affected nitrogen accumulation in tomato crops in a concentration-dependent manner, stimulating growth at low doses but inhibiting it at high doses [40]. Nitrogen uptake depends tightly on nitrate transporters, membrane-localized proteins that play a pivotal role in mediating nitrate uptake across root cell membranes, their transmembrane transport, and subsequent redistribution throughout the entire plant [56,57], this phenomenon was consistent with our transcriptome results, indicating that the nitrogen metabolism pathway was significantly enriched in the KEGG analysis (Fig. 3). The increased NPK contents in shoots and roots of cucumber plants suggest that GO application effectively promotes the uptake and translocation of N, P, and K, thereby improving the mineral nutrition status of plants at suitable GO concentration. The enhanced nutrient accumulation facilitates key physiological processes such as nitrogen metabolism, energy metabolism and osmotic adjustment, thereby supporting plant growth and development [58]. However, the present study did not track nutrient concentrations over time. Future work that includes such time-course measurements would be valuable to differentiate the respective contributions of altered bioavailability and genuine physiological stimulation to the observed increases in nutrient uptake. In this study, the regulatory mechanism of GO exposure on root anatomical structure and morphogenesis of cucumber plants was explored via root cross-section preparation, microscopic observation, and enrichment analysis (Fig. 2). Through anatomical structure analysis of cucumber roots, our study clarified the dose-dependent effects of GO on cucumber root development. Specifically, low and medium concentrations of GO improved root structure and material transport capacity by increasing the number of cortical cells and optimizing vascular bundle differentiation, thereby providing a structural basis for plant nutrient absorption and growth. In contrast, high concentrations of GO exerted toxic stress on root tip tissues, causing vascular bundle damage and structural disruption, which further inhibited normal root development. Similarly, Zhao et al. [59] indicated that the thickness of the periderm and the number of peridermal cell layers of alfalfa decreased as the GO concentration increased. This result corroborates the "low-promotion and high-inhibition" effect of GO in plants, and its underlying mechanism may be associated with the induction of root cell proliferation by low concentrations of GO, while high concentrations of GO trigger oxidative stress and cell damage. The optimization of root structure serves as an important structural basis for GO to promote cucumber growth, and the toxic effects of high concentrations provide critical references for determining the safe application concentration of GO in agricultural production. In addition, Li et al. [60] reported that exposure to 0.1–10 mg L^{-1} GO suppressed both adventitious root elongation and lateral root formation in tissue-cultured 'Gala' apple plantlets. However, 0.1 and 1.0 mg L^{-1} GO significantly increased adventitious root number and rooting rate compared with the control [60]. Furthermore, Zhu et al. [57] demonstrated that GO exposure suppressed the expression of genes associated with root transport and impaired root structural integrity, thereby interfering with the development and function of root xylem. Collectively, the effects of GO on plant root structure are concentration-dependent. Appropriate concentrations of GO can optimize root structure and nutrient uptake and transport capacity by regulating root cell proliferation, lignin synthesis, and vascular bundle development. In contrast, high concentrations of GO trigger root stress responses, damage root structural integrity, and ultimately impair the normal physiological functions of roots.

The most significantly enriched pathways from KEGG enrichment analysis of DEGs were those involved in the biosynthesis of various secondary metabolites, as well as cysteine and methionine metabolism and phenylpropanoid pathway (Fig. 3). The significant enrichment of the phenylpropanoid biosynthesis pathway, combined with the significantly increased cellulose and hemicellulose contents in cucumber roots, indicates that the treatment promotes cell wall polysaccharide biosynthesis by regulating phenylpropanoid metabolism. This regulatory effect contributes to the enhanced root structural integrity and physiological activity observed in our study, highlighting the critical role of phenylpropanoid metabolism in mediating root growth and development in response to exogenous stimuli.

5 Conclusion

Our study demonstrates that GO exerts a concentration-dependent dual effect on cucumber growth, nutrient uptake, and root architecture. Low to moderate GO concentrations (0.5–1.0 mg L^{-1}) promote cucumber growth by enhancing nitrogen, phosphorus, and potassium accumulation and optimizing root architecture via increasing cellulose and hemicellulose content, while high GO concentration (2.0 mg L^{-1}) induces phytotoxicity, inhibits plant growth and nutrient uptake, and damages root structure. The regulatory role of GO in cucumber growth is associated with its modulation of root nutrient uptake capacity, root architecture, nutrient transporter gene expression, and root microenvironment. Our study clarifies the suitable GO concentration range for cucumber growth promotion and provides a technical reference for the rational application of GO in cucumber cultivation. Future research will explore the long-term effects of GO on cucumber fruit performance and conduct transcriptomic analysis of high-concentration treatments. This will further elucidate the dose-dependent regulatory mechanisms and provide more comprehensive theoretical support for the application of nanomaterials in protected vegetable production.

Acknowledgement: Not applicable.

Funding Statement: This work was completed with financial support from Key Research and Development Program of Sichuan Province: Vegetable Breeding Project; Sichuan Innovation Team (SCCXTD-2024-5).

Author Contributions: The authors confirm contribution to the paper as follows: Methodology: Zejin Zhang, Zhengnan Yan, Ying Liang, Na Lu, and Li Tang; data curation: Zejin Zhang, Zhengnan Yan, Xiangyu Ding, and Haoxu Shen; software: Zejin Zhang, Zhengnan Yan, Xiangyu Ding, Weiming Ren, and Yarong Zhang; investigation: Zejin Zhang and Ying Liang; conceptualization: Zejin Zhang, Zhengnan Yan, Na Lu, Jinxiu Song, Ying Liang, and Li Tang; writing—original draft: Zejin Zhang, Zhengnan Yan, Xiangyu Ding, and Haoxu Shen; writing—review and editing: Zejin Zhang, Zhengnan Yan, Ying Liang, and Li Tang; supervision: Jinxiu Song, Na Lu, Ying Liang, and Li Tang; funding acquisition: Zejin Zhang, Ying Liang, and Li Tang; resources and project administration: Zejin Zhang and Li Tang. All authors reviewed and approved the final version of the manuscript.

Availability of Data and Materials: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Iqbal MW, Riaz T, Mahmood S, Bilal M, Manzoor MF, Ahmad Qamar S, et al. Fucoidan-based nanomaterial and its multifunctional role for pharmaceutical and biomedical applications. Crit Rev Food Sci Nutr. 2024;64(2):354–80. [CrossRef].
2. Zhu K, Sun W, Ti C, Li Y. Achievements applications and future of nanomaterials in cancer immunotherapy. Int Immunopharmacol. 2025;161:115022. [CrossRef].
3. Bai F, Wang Z, Liu X, Zhao J, Zhai Z, Sun C. Synthesis and application of lead iodine-based perovskite nanomaterials. Chem Phys Lett. 2025;860:141776. [CrossRef].
4. Zou Y, Shi Y, Wang T, Ji S, Zhang X, Shen T, et al. Quantum dots as advanced nanomaterials for food quality and safety applications: a comprehensive review and future perspectives. Comp Rev Food Sci Food Safe. 2024;23(3):e13339. [CrossRef].
5. Zakir O, Ait-Karra A, Khardazi S, Idouhli R, Khadiri ME, Dikici B, et al. Recent progress in nanomaterials for water treatment: a comprehensive review of adsorption, photocatalytic, and antibacterial applications. J Water Process Eng. 2025;72:107566. [CrossRef].

6. El-Shal RM, El-Naggar AH, El-Beshbeshy TR, Mahmoud EK, El-Kader NIA, Missaui AM, et al. Effect of nano-fertilizers on alfalfa plants grown under different salt stresses in hydroponic system. Agriculture. 2022;12(8):1113. [CrossRef].
7. Yu K, Wang Q, Tian J, Lu H, Zhao P, Pan J. Eco-friendly nanocellulose composites for stabilizing nanoemulsion insecticides: enhancing efficacy and weather resistance. Carbohydr Polym. 2026;375:124693. [CrossRef].
8. Ioannou A, Gohari G, Papaphilippou P, Panahirad S, Akbari A, Dadpour MR, et al. Advanced nanomaterials in agriculture under a changing climate: the way to the future? Environ Exp Bot. 2020;176:104048. [CrossRef].
9. Zhao Y, Ma Y, Zhou R, He Y, Wu Y, Yi Y, et al. Highly sensitive electrochemical detection of paraoxon ethyl in water and fruit samples based on defect-engineered graphene nanoribbons modified electrode. J Food Meas Charact. 2022;16(4):2596–603. [CrossRef].
10. Hassanpouraghdam MB, Mehrabani LV, Khoshmaram L, Rasouli F. Amelioration of the growth and physiological responses of *Capsicum annum* L. via quantum dot-graphene oxide, cerium oxide, and titanium oxide nanoparticles foliar application under salinity stress. Sci Rep. 2025;15:467. [CrossRef].
11. Ghulam AN, dos Santos OAL, Hazeem L, Pizzorno Backx B, Bououdina M, Bellucci S. Graphene oxide (GO) materials—applications and toxicity on living organisms and environment. J Funct Biomater. 2022;13(2):77. [CrossRef].
12. Yang Y, Zhao Y, Wang M, Meng H, Ye Z. Mechanistic analysis of ecological effects of graphene nanomaterials on plant ecosystems. Asia-Pacific J Chem Eng. 2020;15(S1):e2467. [CrossRef].
13. Rafiq H, Aftab ZEH, Akram W, Anjum T, Mirza FS, Yousuf Z, et al. A biological evaluation and molecular docking insight on green synthesized graphene oxide nanoparticles mediated growth promotion in mungbean. Sci Hortic. 2023;318:112097. [CrossRef].
14. Cheng F, Liu YF, Lu GY, Zhang XK, Xie LL, Yuan CF, et al. Graphene oxide modulates root growth of *Brassica napus* L. and regulates ABA and IAA concentration. J Plant Physiol. 2016;193:57–63. [CrossRef].
15. Guo X, Zhao J, Wang R, Zhang H, Xing B, Naeem M, et al. Effects of graphene oxide on tomato growth in different stages. Plant Physiol Biochem. 2021;162:447–55. [CrossRef].
16. Rafiq MT, Ahmad Sajid Z, Khilji SA. Graphene oxide nanoparticle-assisted promotion of stevioside, rebaudioside A, and selected biochemical attributes in *Stevia rebaudiana* bertoni. Scientifica. 2024;2024:6693085. [CrossRef].
17. Pang WQ, Lai CS, Mad' Atari MF, Pandian BR, Mohamad Ibrahim MN, Tan ST, et al. Effect of graphene oxide nanoparticles on *in vitro* growth of *Fragaria* x *Ananassa* (Cameron Highlands white Strawberry) and evaluation of genetic stability using DAMD and ISSR markers. Plant Physiol Biochem. 2023;204:108104. [CrossRef].
18. Mohamed TMK, Gao J, Abuarab ME, Kassem M, Wasef E, El-Ssawy W. Applying different magnetic water densities as irrigation for aeroponically and hydroponically grown strawberries. Agriculture. 2022;12(6):819. [CrossRef].
19. Ali Lakhiar I, Yan H, Syed TN, Zhang C, Ali Shaikh S, Rakibuzzaman M, et al. Soilless agricultural systems: opportunities, challenges, and applications for enhancing horticultural resilience to climate change and urbanization. Horticulturae. 2025;11(6):568. [CrossRef].
20. de Lima BM, Noboa CS, de Lima FM, da Costa Mello S, Purquerio LFV, Sala FC. Agronomic biofortification with zinc in hydroponically cultivated lettuce. Aust J Crop Sci. 2023;17(02):195–205. [CrossRef].
21. Vetrano F, Moncada A, Miceli A. Use of gibberellic acid to increase the salt tolerance of leaf lettuce and rocket grown in a floating system. Agronomy. 2020;10(4):505. [CrossRef].
22. Zhuo L, Tu Y, Xu Y, Peng Y, Liang J, Li Z, et al. Improvement of germination and growth of tomato seeds under phenolic acid stress by humic acid-medium and trace element compounds. Sci Hortic. 2026;355:114557. [CrossRef].
23. Compton OC, Nguyen ST. Graphene oxide, highly reduced graphene oxide, and graphene: versatile building blocks for carbon-based materials. Small. 2010;6(6):711–23. [CrossRef].
24. Wang Y, Ma G, Du X, Liu Y, Wang B, Xu G, et al. Effects of nutrient solution irrigation quantity and downy mildew infection on growth and physiological traits of greenhouse cucumber. Agronomy. 2020;10(12):1921. [CrossRef].
25. Yan H, Ma J, Zhang J, Wang G, Zhang C, Akhlaq M, et al. Effects of film mulching on the physiological and morphological parameters and yield of cucumber under insufficient drip irrigation. Irrig Drain. 2022;71(4):897–911. [CrossRef].

26. Yan H, Deng S, Zhang C, Wang G, Zhao S, Li M, et al. Determination of energy partition of a cucumber grown Venlo-type greenhouse in southeast China. Agric Water Manag. 2023;276:108047. [CrossRef].
27. FAO. FAOSTAT database. 2025 [cited 2025 Mar 7]. Available from: https://www.fao.org/faostat/en/#data.
28. Grewal HS, Maheshwari B, Parks SE. Water and nutrient use efficiency of a low-cost hydroponic greenhouse for a cucumber crop: an Australian case study. Agric Water Manag. 2011;98(5):841–6. [CrossRef].
29. Rasool G, Guo X, Wang Z, Ali MU, Chen S, Zhang S, et al. Coupling fertigation and buried straw layer improves fertilizer use efficiency, fruit yield, and quality of greenhouse tomato. Agric Water Manag. 2020;239:106239. [CrossRef].
30. Liang Y, Yang R, Sadras VO, Wang Y, Tang L, Wang Z, et al. Fulvic acid promotes cucumber (*Cucumis sativus* L.) growth and nitrogen use efficiency under moderate nitrogen deficit in soilless culture. J Agric Food Res. 2026;27:102786. [CrossRef].
31. Ihnat M. AOAC official method 960.52 microchemical determination of nitrogen: Micro-Kjeldahl method. In: Latimer GW, editor. Official methods of analysis of AOAC INTERNATIONAL. New York, NY, USA: Oxford University Press; 2023.
32. Kitson RE, Mellon MG. Colorimetric determination of phosphorus as molybdivanadophosphoric acid. Ind Eng Chem Anal Ed. 1944;16(6):379–83. [CrossRef].
33. da Silva IJS, da Silva MM, da Silva R, De França EJ, da Silva MJ, Kato MT. Microwave-assisted digestion for multi-elemental determination in beans, basil, and mint by ICP OES and flame photometry: an eco-friendly alternative. Food Chem. 2025;481:143970. [CrossRef].
34. GB/T 20806-2006. Determination of neutral detergent fiber in feedstuffs. Beijing, China: Standardization Administration of China; 2016. (In Chinese with English Abstract).
35. NY/T 1459-2007. Determination of acid detergent fiber in feedstuffs. Beijing, China: Ministry of Agriculture of the PRC; 2007. (In Chinese with English Abstract).
36. Li Y, Tian Q, Wang Z, Li J, Liu S, Chang R, et al. Integrated analysis of transcriptomics and metabolomics of peach under cold stress. Front Plant Sci. 2023;14:1153902. [CrossRef].
37. Yang Y, Zhang R, Zhang X, Chen Z, Wang H, Li PCH. Effects of graphene oxide on plant growth: a review. Plants. 2022;11(21):2826. [CrossRef].
38. Zhan Q, Ahmad A, Arshad H, Yang B, Chaudhari SK, Batool S, et al. The role of reduced graphene oxide on mitigation of lead phytotoxicity in *Triticum aestivum* L. plants at morphological and physiological levels. Plant Physiol Biochem. 2024;211:108719. [CrossRef].
39. Park S, Ahn YJ. Multi-walled carbon nanotubes and silver nanoparticles differentially affect seed germination, chlorophyll content, and hydrogen peroxide accumulation in carrot (*Daucus carota* L.). Biocatal Agric Biotechnol. 2016;8:257–62. [CrossRef].
40. Seyed Mehdi T. Chapter 2—uptake of nanomaterials by plants and translocation within plants. In: Kanchan V, Nitin K, Agbaje L, editors. Nanomaterial-plant interactions: microbiome and nano-cross-talk. Cambridge, MA, USA: Academic Press; 2024. p. 19–41. [CrossRef].
41. Zhang X, Huang W, Kong D, Guo W, Sun H. Regulation of photosynthetic characteristics carbon and nitrogen metabolism and growth of maize seedlings by graphene oxide coating. Sci Rep. 2025;15(1):2763. [CrossRef].
42. Gao M, Chang X, Yang Y, Song Z. Foliar graphene oxide treatment increases photosynthetic capacity and reduces oxidative stress in cadmium-stressed lettuce. Plant Physiol Biochem. 2020;154:287–94. [CrossRef].
43. Lin X, Chen L, Hu X, Feng S, Huang L, Quan G, et al. Toxicity of graphene oxide to white moss *Leucobryum glaucum*. RSC Adv. 2017;7(79):50287–93. [CrossRef].
44. Zhou Z, Li J, Li C, Guo Q, Hou X, Zhao C, et al. Effects of graphene oxide on the growth and photosynthesis of the emergent plant iris pseudacorus. Plants. 2023;12(9):1738. [CrossRef].
45. Wang S, Wang X, Liu Y, Sun G, Kong D, Guo W, et al. Regulatory effect of graphene on growth and carbon/nitrogen metabolism of maize (*Zea mays* L.). J Sci Food Agric. 2024;104(3):1572–82. [CrossRef].
46. Liu Z, Lu Q, Zhao Y, Wei J, Liu M, Duan X, et al. Ameliorating effects of graphene oxide on cadmium accumulation and eco-physiological characteristics in a greening hyperaccumulator (*Lonicera japonica* Thunb.). Plants. 2023;13(1):19. [CrossRef].

47. Tunio MH, Gao J, Shaikh SA, Lakhiar IA, Qureshi WA, Solangi KA, et al. Potato production in aeroponics: an emerging food growing system in sustainable agriculture forfood security. Chil J Agric Res. 2020;80(1):118–32. [CrossRef].
48. Wang Y, Xue X, Schagerl M, Hu X, Srinuanpan S, Rehman OU, et al. Combined application of microalgae and plant growth-promoting rhizobacteria: synergistic effects in improving soil health and crop root disease resistance. Appl Soil Ecol. 2026;218:106684. [CrossRef].
49. Fujii K. Plant strategy of root system architecture and exudates for acquiring soil nutrients. Ecol Res. 2024;39(5):623–33. [CrossRef].
50. Farooq M, Rafique S, Zahra N, Rehman A, Siddique KHM. Root system architecture and salt stress responses in cereal crops. J Agron Crop Sci. 2024;210(6):e12776. [CrossRef].
51. Bano Z, Kiran A, Imran A, Wakeel A. Root system architecture modulates nitrogen uptake and use efficiency, influencing grain yield in rice. Sci Rep. 2026. [CrossRef].
52. Gallegos-Cedillo VM, Nájera C, Signore A, Ochoa J, Gallegos J, Egea-Gilabert C, et al. Analysis of global research on vegetable seedlings and transplants and their impacts on product quality. J Sci Food Agric. 2024;104(9):4950–65. [CrossRef].
53. Bayındır S, Kandemir D. Root system architecture of interspecific rootstocks and its relationship with yield components in grafted tomato. Gesunde Pflanz. 2023;75(2):329–41. [CrossRef].
54. Duque LO, Hoffmann G, Pecota KV, Yencho GC. Early root architectural traits and their relationship with yield in *Ipomoea batatas* L. Plant Soil. 2025;510(1):677–96. [CrossRef].
55. Mirza FS, Aftab ZEH, Ali MD, Aftab A, Anjum T, Rafiq H, et al. Green synthesis and application of GO nanoparticles to augment growth parameters and yield in mungbean (*Vigna radiata* L.). Front Plant Sci. 2022;13:1040037. [CrossRef].
56. Abouelsaad I, Weihrauch D, Renault S. Effects of salt stress on the expression of key genes related to nitrogen assimilation and transport in the roots of the cultivated tomato and its wild salt-tolerant relative. Sci Hortic. 2016;211:70–8. [CrossRef].
57. Zhu YX, Weng YN, Zhang SY, Liu LJ, Du ST. The nitrate uptake and growth of wheat were more inhibited under single-layer graphene oxide stress compared to multi-layer graphene oxide. Ecotoxicol Environ Saf. 2022;247:114229. [CrossRef].
58. Yan Z, Cao X, Bing L, Lin D, Cheng F, Wang K, et al. Assessment of the growth and quality of pepper seedlings under the combinations of daily light integral and nitrogen concentration. Hortic Environ Biotechnol. 2025;66(2):331–46. [CrossRef].
59. Zhao S, Wang W, Chen X, Gao Y, Wu X, Ding M, et al. Graphene oxide affected root growth, anatomy, and nutrient uptake in alfalfa. Ecotoxicol Environ Saf. 2023;250:114483. [CrossRef].
60. Li F, Sun C, Li X, Yu X, Luo C, Shen Y, et al. The effect of graphene oxide on adventitious root formation and growth in apple. Plant Physiol Biochem. 2018;129:122–9. [CrossRef].