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Shaimaa Mahmoud Heider
Cairo University
Eman Tawfik
Capital University
Zeinab Ashour Shedeed
Capital University
Zeinab k. Taha
Cairo University
Mohamed Soliman
Capital University
Marwa Mohamed Soliman
m.soliman85@yahoo.com

Agricultural Research Center

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Morphological, Physiological, anatomical, biochemical, and molecular responses of *Aglaonema commutatum* 'Silver Queen' to foliar application of whey extract

Shaimaa Mahmoud Heider[1], Eman Tawfik[2], Zeinab Ashour Shedeed[2], Zeinab k. Taha[3], Mohamed Soliman[2], Marwa Mohamed Soliman[4,*]

[1] Faculty of Agriculture, Cairo University, Ornamental Horticulture Department, Giza, Egypt. shaimaa.heider@gmail.com, shaimaa.heider@agr.cu.edu.eg.

[2] Botany and Microbiology Department, Faculty of Science, Capital University, Cairo, Egypt; emantawfik@science.Capu.edu.eg, drzeinabashour@gmail.com, prof.msoliman@yahoo.com

[3]Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza 12613, Egypt. Zaynab.taha@agr.cu.edu.eg

[4]Botanical Gardens Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. m.soliman85@yahoo.com, marwa.soliman@arc.sci.eg

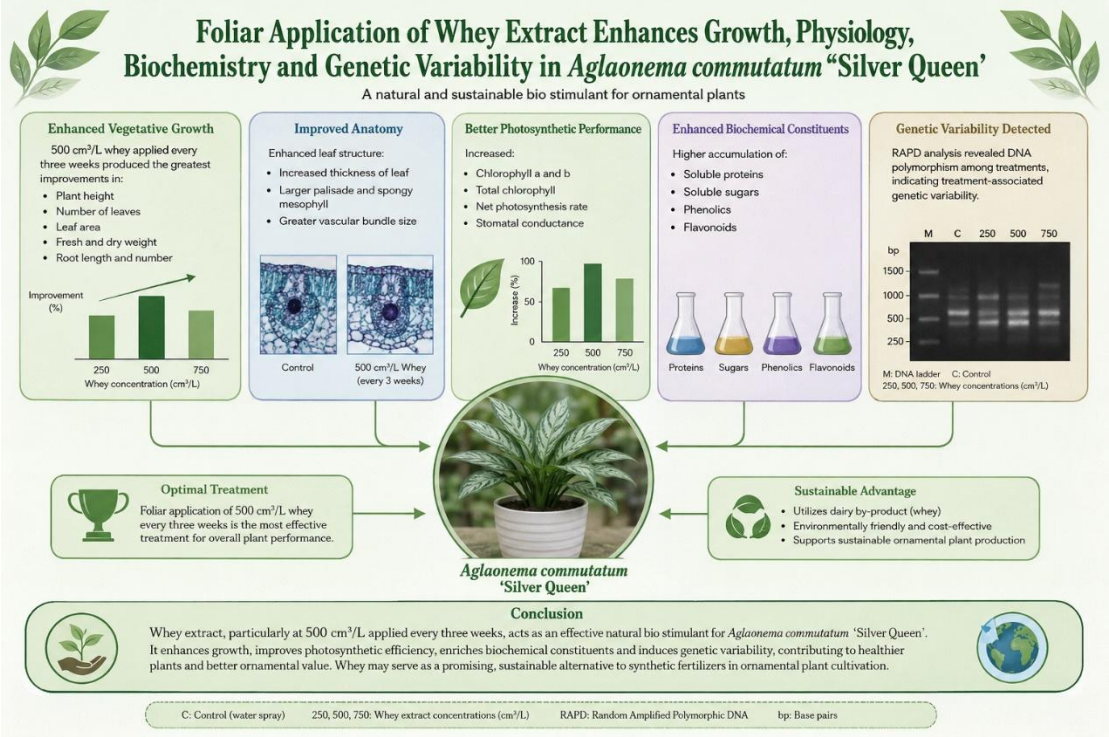
*Corresponding Author: Botanical Gardens Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. m.soliman85@yahoo.com, marwa.soliman@arc.sci.eg, ORCID: 0000-0001-9895-3405

Abstract

Aglaonema commutatum 'Silver Queen' is a common ornamental foliage plant valued for its adaptability and aesthetic appearance. This study investigated the effects of whey extract applied as a foliar spray at different concentrations and application intervals on growth, anatomy, photosynthetic performance, biochemical constituents, and genetic variability of Aglaonema silver plants. The experiment was conducted under greenhouse conditions using three whey concentrations (250, 500, and 750 cm^3/L) applied at one-, two-, and three-week intervals. Growth traits, anatomical characteristics, photosynthetic parameters, soluble proteins, soluble sugars, phenolics, flavonoids, and molecular markers were evaluated. The results demonstrated significant improvements in vegetative growth, root development, chlorophyll content, and leaf anatomy in response to whey application. The treatment of 500 cm^3/L whey applied every three weeks produced the most favourable responses in several growth, anatomical, and photosynthetic traits, whereas 750 cm^3/L whey applied every three weeks resulted in the highest accumulation of soluble proteins, soluble sugars, phenolics, and flavonoids. RAPD analysis revealed detectable polymorphism among treatments, indicating treatment-associated genetic variability. The findings suggest that whey extract can serve as an environmentally friendly bio stimulant for enhancing the physiological and biochemical performance of Aglaonema silver plants.

***Keywords*:** *Aglaonema commutatum*; whey extract; bio stimulants; ornamental plants; photosynthesis; phenolic compounds; flavonoids; RAPD-PCR.

Graphical Abstract.



Introduction

The increasing demand for sustainable agricultural practices has stimulated considerable interest in the utilization of natural bio stimulants and agro-industrial by-products as environmentally friendly alternatives to conventional fertilizers. Among these by-products, whey represents one of the most abundant residues generated by the dairy industry worldwide. It is estimated that approximately 8–9 tons of whey are produced for every ton of cheese manufactured, creating substantial environmental challenges if not properly utilized [21]. Consequently, the agricultural application of whey has received increasing attention as an effective approach for waste recycling while simultaneously improving plant productivity [7].

Whey is characterized by a high nutritional value and contains a wide range of biologically active constituents, including proteins, amino acids, lactose, vitamins and essential mineral elements such as potassium, calcium, magnesium and phosphorus [10,1]. These compounds play important roles in plant metabolism and may contribute to enhanced nutrient uptake, improved physiological performance and stimulation of plant growth. Previous studies have demonstrated that whey application can improve soil fertility, stimulate microbial activity and promote plant development under different cultivation conditions [19, 14, 15,12]. Furthermore, the presence of amino acids and bioactive compounds in whey may positively influence photosynthetic efficiency, carbohydrate metabolism and antioxidant defense systems in plants [7].

Plant bio stimulants have attracted considerable scientific interest because of their ability to enhance plant growth and productivity through mechanisms that extend beyond simple nutrient supply. These substances may improve chlorophyll synthesis, increase photosynthetic

activity, promote root development and stimulate the production of secondary metabolites involved in plant defense and stress tolerance. Secondary metabolites, particularly phenolic compounds and flavonoids, are important components of the plant antioxidant system and contribute significantly to protection against oxidative stress and environmental challenges [16, 17]. Therefore, the identification of natural compounds capable of enhancing the accumulation of these metabolites is of great importance for improving plant performance and quality.

Aglaonema commutatum 'Silver Queen', commonly known as Chinese evergreen, is one of the most popular ornamental foliage plants cultivated worldwide. The species is highly valued for its attractive silver-green leaves, adaptability to indoor environments and relatively low maintenance requirements [9]. The commercial value of Aglaonema largely depends on vegetative growth, leaf quality and ornamental appearance. Therefore, improving growth characteristics and physiological performance represents an important objective for commercial production systems.

Although several studies have investigated the agricultural applications of whey, limited information is available regarding its influence on ornamental foliage plants, particularly Aglaonema species. Moreover, the effects of whey extract on photosynthetic activity, antioxidant metabolites, anatomical characteristics and molecular variation in Aglaonema plants remain largely unexplored. Understanding these responses may contribute to the development of sustainable cultivation practices capable of improving both plant quality and productivity.

Therefore, the present study was conducted to evaluate the effects of different concentrations of whey extract and spraying intervals on vegetative growth, leaf anatomy, photosynthetic performance, soluble proteins, soluble sugars, phenolic compounds and flavonoids in *Aglaonema commutatum* 'Silver Queen'. In addition, molecular analyses based on TFL1 amplification and RAPD-PCR markers were employed to investigate treatment-associated molecular variation among plants subjected to different whey extract applications.

Materials and methods

Experimental procedure and plant material

This experiment was conducted during seasons of 2022-2023 in Horticulture Research Institute, Agricultural Research Centre, Giza. This study aimed to evaluate the impact of whey treatments using as foliar usage on growth of aglaonema silver queen plant (*Aglaonema commutatum*). Aglaonema silver plant was brought from Horticulture Research Institute which were planted with tissue culture technique to Botanical Gardens Department, Horticulture Institute, Agriculture Research Centre, Giza, Egypt. Experiment carried out under Botanical Gardens greenhouse conditions from 1st February to the end of the year for adaptation, and from 1st March to 30th November 2023 the plants were sprayed (whey application). The plants were sprayed using hand-held sprayer and the used volume of the solution was maintained just to cover the whole plant foliage completely. the plants were sprayed once every one week, two

weeks and three weeks with (250, 500 and 750 cm^3/L) of whey concentrate. The samples were collected month later.

Experimental designs and treatments

The experiment was carried out in a randomized complete block design (RCBD) with 3 replicates each replicate has 4 plants. The treatment was as follows:

- Recommended dose of NPK as control
- 250 cm^3/L of whey concentrate, every week, 2 weeks and 3 weeks.
- 500 cm^3/L of whey concentrate, every week, 2 weeks and 3 weeks.
- 750 cm^3/L of whey concentrate, every week, 2 weeks and 3 weeks.

Preparation and analysis of whey extracts

The whey extract was provided from Dairy science department, Faculty of agriculture, Cairo university, Giza, Egypt. Whey was prepared, using tape water, at three rates of 250, 500, and 750 cm^3/L. The chemical analysis was performed to estimate the nutritive value of the extract. The metabolic compounds estimated were the percentage of protein content, ash, fats, carbohydrates (lactose), the acidity of lactic acid, and pH of the extract. The estimated minerals were Na, K, Ca, P, Mg, Fe, Zn and Cu.

Vegetative characters:

A random sample of each replicate was taken for data. the data were recorded as follows: Plant height (cm), Number of leaves, Number of branches, Number of flowers, leaves fresh weight/ plant (g), leaves dry weight/ plant (g), root length (cm), root fresh weight/ plant (g), root dry weight/ plant (g), chlorophyll content, leaf area.

Effect of Whey extract on plant anatomy

Leaves were taken one month post-treatment at 1st January 2024 from *Aglaonema commutatum* plants that had been sprayed with various treatments for anatomical studies. Leaves were killed and fixed for at least 48 h using formalin, acetic acid, and 70% ethyl alcohol. After fixation, the materials were washed in 50% ethyl alcohol, dried through a regular butyl alcohol series and embedded in paraffin wax. Cross sections were cut on a 20-µm-thick rotary microtome. Sections were stained with crystal erythrosine violet before mounting in Canada balsam and fixing the cover slips. Microtechnique procedures were carried out according to (Nassar and Al-Sohar 1998). Slides were examined microscopically and photographed using a micrometre lens and five readings per slide were averaged.

Stimulatory and inhibitory effect of Whey extract on some essential metabolic activities:

1- Photosynthetic activity measurement

A portable photosynthetic system (LCpro-SD, ADC BioScientific, Hoddesdon, UK) fitted with a standard 2*3 cm^2 leaf chamber and LED light source was used to quantify leaf gaseous exchange for control and treated plants (N=3 leaves). The flow rate was fixed at 200 mol s1, and the leaf block temperature was 23 ^oC. The net CO_2 assimilation rate (A), stomatal conductance (gs) and transpiration rate (E) measurements using von Caemmerer and Farquhar's equations. To account for the effects of temperature and humidity on transpiration, the ratio of A to Gs was estimated according to manual instructions.

2- Biochemical analysis

a- Total soluble proteins and soluble sugars

Firstly, 0.2 g of fresh leaves was crushed with 10 ml of 70% ethanol. Secondly, the mixture was centrifuged, and the supernatant was diluted to a known volume. Total soluble proteins were quantified using the Lowry et al. (1951) method. Total soluble sugars were determined using anthrone utilising the Umbriet et al. (1959) technique. A 3 ml sample was heated with a 6 ml anthrone solution (2 g L-1 H_2SO_4).

b- Phenolic and flavonoid compounds

The plant materials were washed, air-dried, crushed, and ground into fine powder by a grinding machine. In a dark container, 50 ml of 99% methanol was added to 5 g of air-dry plant powder for extraction. The samples were incubated in a shaking incubator at 60 ˚C for 48 hours. After the incubation period, the samples were filtered through filter paper No. 1. The filtrate was used to determine the total phenolic compounds content by the method of Savitree et al. (2004). 0.5 ml of each sample was mixed with 2.5 ml of 10 folds diluted Folin-Ciocalteu reagent in a test tube, and 2.0 ml of 7.5% sodium carbonate. The covered tubes were allowed to stand for 30 minutes at room temperature. The blue color intensity was read at 760 nm spectrometrically (Cecil CE. 1010 spectrophotometer). All determinations were performed in triplicate. A standard curve of gallic acid was used for the estimation of the total phenol content of the samples.

3- Genomic DNA Methods

The genomic DNA was isolated from the leaves of the control and treated Aglaonema silver following the protocol of Edwards et al. (1991). Two PCR-based techniques were applied: detection of TFL1 gene and RAPD-PCR molecular marker. The first technique aimed to detect the flowering gene TFL1 in the Aglaonema silver plant. The The TFL1 primers were designed by SnapGene (6.0.2) and the primers data were: forward 5'-TGTCCAGTCCCACAGGCTC-3' and reverse 5'-GTTGACCTCTGGGACTCGG-3'. This detection was estimated depending on the following PCR conditions: initial denaturation at 95^{o}C for 5 min, followed by 35 cycles of 94^{o}C for 1 min, 58^{o}C for 1 min and 72^{o}C for 1 min; then a correction step at 72^{o}C for 10 min. The product was examined on 1% agarose gel against 1kb DNA ladder (NEB) and visualized under UV-transilluminator.

From the previously isolated genomic DNA, the second PCR-based technique (RAPD-PCR marker) was applied to estimate the genetic variation resulted from (type of treatment) effect. The RAPD-PCR programming conditions were as follow: initial denaturation at 95^{o}C for 5 min, followed by 35 cycles of 94^{o}C for 1 min, the annealing was for 1 min (the temperature for each primer was illustrated in table #) and 72^{o}C for 1 min; then a correction step at 72^{o}C for 10 min. Seven primers were applied, but only four primers gave reproducible banding pattern. The data of the RAPD primers applied were illustrated in table (7). The product was examined on 1.6% agarose gel against 100bp DNA ladder (NEB) and visualized under UV-transilluminator. The band scoring was analyzed via BioRad Quantity One (4.6.2) software.

Statistical analysis

The treatments were applied in a randomized complete block design with three replications. The data were initially subjected to analysis of variance ANOVA to determine the presence of significant differences among treatments. Mean comparisons were performed using Tukey significant difference test at the 5% probability level, following Tukey 1949. Simple correlation coefficients among the studied traits were calculated according to the procedures described by Snedecor and Cochran[20]. Cluster heatmap analysis was carried out using Euclidean distance as a measure of dissimilarity, based on the methodology outlined by Everitt [5]. All statistical analyses were performed using R software[11]. The ggplot2 package was used for correlation analysis, whereas the pheatmap package was employed to generate the cluster heatmap. Principal component analysis PCA was conducted using the factoextra, ggrepel, and ggplot2 packages.

Results

The chemical analysis for the whey extract was performed to identify all its nutritive components. The metabolites and minerals estimated and illustrated as: Protein (0.82 ± 0.03%), Acidity as lactic acid (0.4%), Fat (0.5 ± 0.02%), Ash (0.48 ± 0.02%), Carbohydrate (lactose) (4.79 ± 0.11%), pH (4.68), Na (5.34 ±0.09), Ca (0.15 ±0.003), Mg (1.93 ±0.004), Zn (0.16 ±0.032), K (15.22 ±0.08), P (0.10 ±0.002), Cu (0.02 ±0.001) and Fe (0.05 ±0.001).

Data showed in Table (2) that there was a significant difference in all vegetative characteristics (plant height, leaves number/plant, branches number/plant, flowers number/plant, leaves fresh and dry weight/plant) affected by different concentrations of whey on Aglaonema plants. The data indicated that the plants sprayed with whey at 250 cm^3/L sprayed every week gave the highest plants (45.92 cm) while plants received 750 cm^3/L sprayed every three weeks gave the lowest plants (38.50 cm), although there are no significant differences between spraying at 250 cm^3/L and 500 cm^3/L.

While, spraying 500 and 750 cm^3/L whey every three weeks gave the maximum leaves number (17.17 and 17.00) and number of flowers (3.17 and 2.50). the maximum branches number/plant (2.83) was given from spraying 500 cm^3/L every one week while there are no significant differences between this value and the values of spraying 500 cm^3/L every two or

three weeks. Leaves fresh weight/plant and dry weight/plant (g) gave the highest values (132.99 and 13.22 g).

On the other hand, the least values of vegetative growth characters obtained from plants did not receive any whey treatments (control) (37.17) for plant height, (13.50) for Number of leaves / plants, no branches or flowers/plant, (42.68 g) for leaves fresh weight/plant and (4.63 g) for leaves dry weight/plant.

Data illustrated in Table (3) indicated that spraying plants 500 cm^3/L every three weeks gave the best results compared to control. It gave in root length 32.75 cm, root fresh weight 68.86 g and root dry weight 10.40 g, while control gave root length 21.25 cm, root fresh weight 35.26 g and root dry weight 5.66 g. The highest chlorophyll values were obtained from 750 cm^3/L of whey spraying solution in one, two and three weeks which gave (48.40, 47.45 and 48.20), respectively. Leaf area gave maximum value when sprayed with the highest concentration of the solution 750 cm^3/L every three weeks (104.39 cm^3) compared to control which gave (51.34 cm^3).

Table 2. Effect of spraying different whey solution concentration on plant height (cm), leaves number/plant, branches number/plant, flowers number/plant, leaves fresh and dry wight/plant (g) of *Aglaonema commutatum* plants in certain spraying times.

Treatments	Spraying Intervals	Plant height (cm)	Leaves number /plant	Branches number /plant	Flowers number /plant	Leaves fresh weight (g)	Leaves dry weight (g)
Control	No spraying	37.17c	13.50b	0.00b	0.00b	42.68f	4.63c
Whey extract 250 cm^3/L	Once/week	45.92a	15.50ab	0.33b	0.00b	54.50ef	7.55bc
	Once/ 2 weeks	41.83abc	16.33a	2.67a	2.00a	108.73abc	11.29ab
	Once/ 3 weeks	44.67ab	15.33ab	0.5b	0.00b	73.72def	7.98bc
Whey extract 500 cm^3/L	Once/week	44.50ab	15.50ab	2.83a	0.00b	123.39ab	8.90abc
	Once/ 2 weeks	43.50abc	16.83a	2.67a	0.00b	98.36bcd	9.82ab
	Once/ 3 weeks	41.67abc	17.17a	2.50a	3.17a	132.99a	13.22a
Whey extract 750 cm^3/L	Once/week	38.50bc	13.50b	0.67b	0.00b	87.11cd	8.01bc
	Once/ 2 weeks	39.67abc	15.33ab	1.50ab	0.00b	84.76cde	7.33bc
	Once/ 3 weeks	39.00abc	17.00a	1.67ab	2.50a	101.22abcd	7.10bc

Table 3. Effect of spraying different whey solution concentration on root length (cm), root fresh and dry wight/plant (g), chlorophyll and leaf area of *Aglaonema commutatum* plants in certain spraying times.

Treatments	Spraying Intervals	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Chlorophyll	Leaf area (cm^3)
Control	No spraying	21.25bc	35.26c	5.66abc	25.95e	51.34b
	Once/week	18.00c	40.10bc	5.19abc	31.30de	100.50a

Whey extract 250 cm^3/L	Once/ 2 weeks	22.75bc	69.10a	9.19ab	33.10cd	83.13a
	Once/ 3 weeks	23.00bc	53.49ab	8.14abc	26.95e	81.14a
Whey extract 500 cm^3/L	Once/week	21.25bc	56.88ab	3.40c	37.85bc	98.22a
	Once/ 2 weeks	23.00bc	57.14ab	9.30ab	41.55b	98.32a
	Once/ 3 weeks	32.75a	68.86a	10.40a	41.35b	86.08a
Whey extract 750 cm^3/L	Once/week	22.50bc	51.66abc	6.74abc	48.40a	86.93a
	Once/ 2 weeks	26.00ab	41.86bc	4.64bc	47.45a	94.24a
	Once/ 3 weeks	29.00ab	59.90a	8.52abc	48.20a	104.39a

Table (4) and fig (1) shows the anatomical measurements of *Aglaonema commutatum* plant leaves sprayed with whey at concentrations of 250, 500, and 750 cm^3/L every one, two, and three weeks.

It was observed in the treatments that were sprayed with whey in its three concentrations every week that the concentration of 500 cm^3/L gave the highest results in terms of anatomical characteristics (thickness of the upper and lower epidermis, thickness of the mesophyll tissue, thickness of the lamina, thickness of the midvein, thickness of the vascular bundle and thickness of the xylem, compared to the two concentrations of 250 and 750 cm^3/L, where the results of 500 cm^3/L concentration were (22.32, 32.33, 155.45, 192.99, 850.66, 51.45 and 18.45 µm), respectively.

The spraying treatments every two weeks with a concentration of 500 cm^3/L recorded a higher result compared to the other two concentrations, as it recorded (20.57, 25.60, 165.32, 192.25, 700.70, 54.5 and 15.30µm) for the same previous characteristics, respectively.

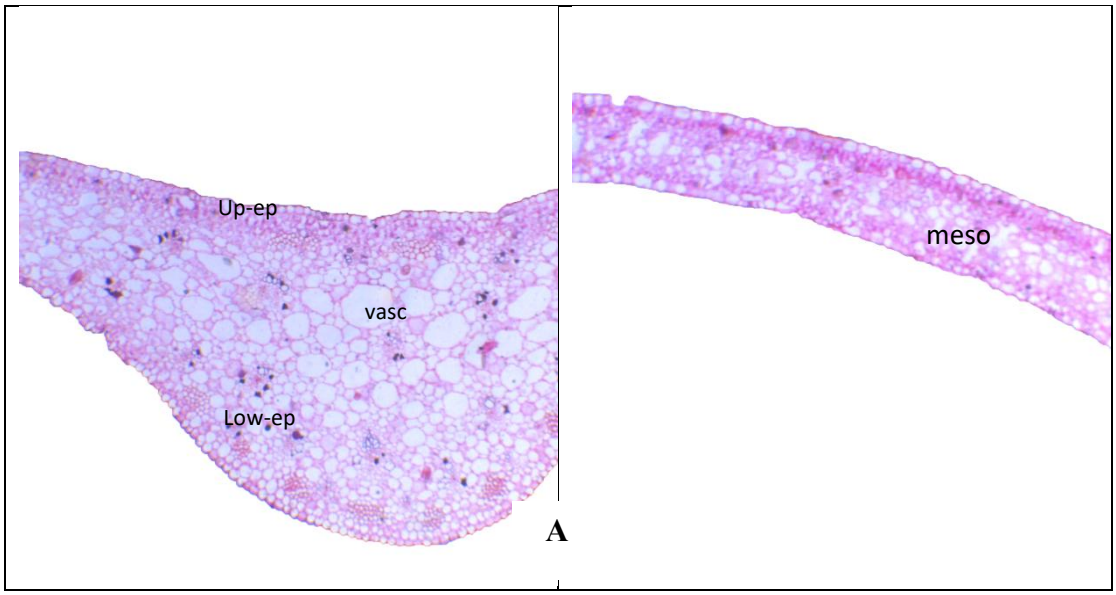
It was also noted that the concentration of 500 cm^3/L that was sprayed every 3 weeks also gave the best results compared to the other two concentrations and the control for all anatomical traits, as it was recorded (18.74, 27.21, 194.98, 230.08, 516.83, 49.37 and 17.78µm), with the former attributes, respectively.

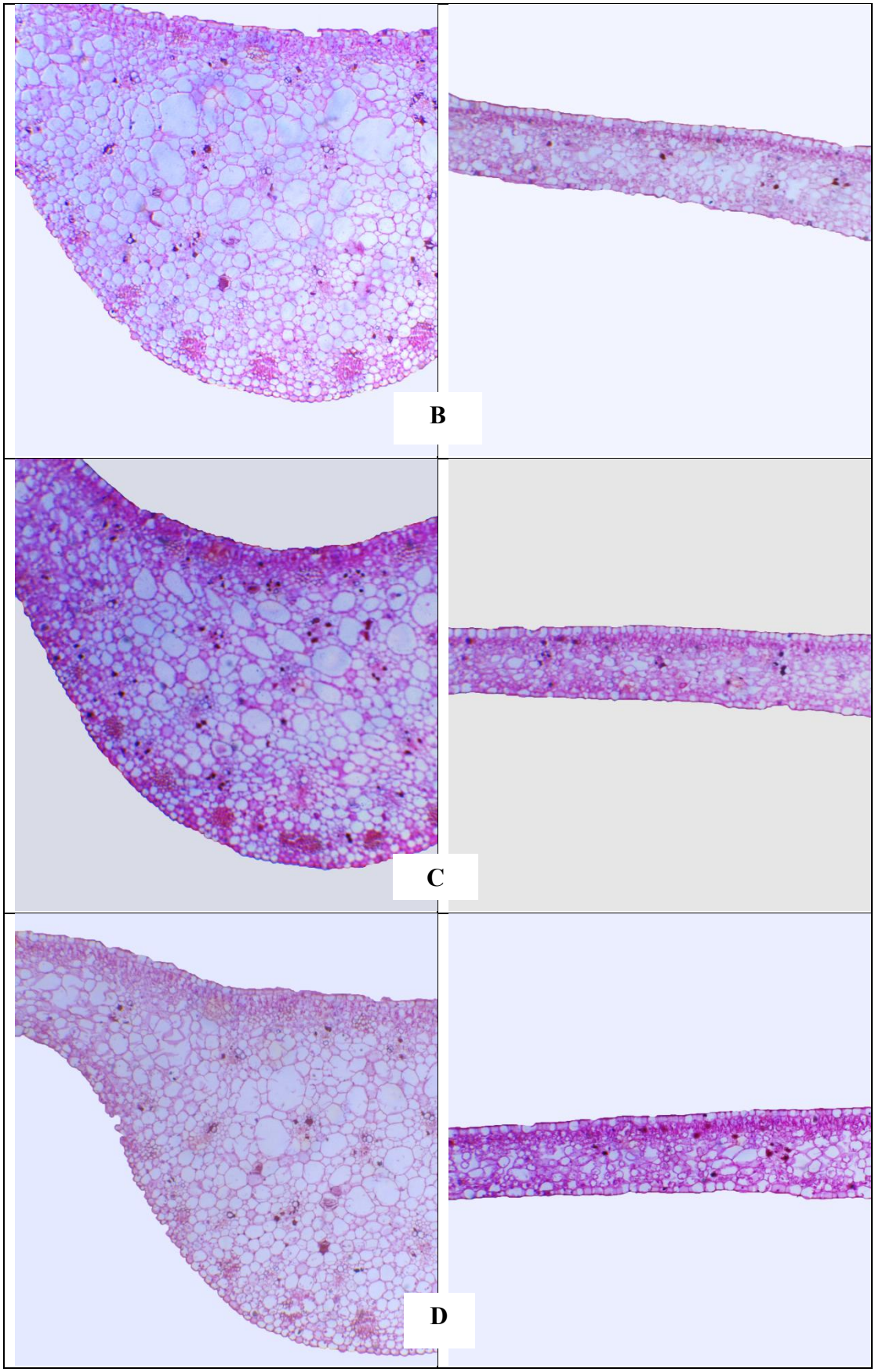
When comparing the spraying at a concentration of 500 cm^3/L every one, two or three weeks, it was found that the measurements are close, and when comparing the treatments with regard to the anatomical and morphological measurements of the plants, we recommend spraying the *Aglaonema commutatum* plants with whey at a concentration of 500 cm^3/L every 3 weeks for best results.

Table 4. The anatomical measurements of *Aglaonema commutatum* plant leaves sprayed with whey at concentrations of 250 cm^3/L, 500 cm^3/L, and 750 cm^3/L every one, two, and three weeks.

Treat-ments	Spraying Intervals	Upper epider thick	Lower epider thick	Meso-phyll	Lamina thickness	Midvein thick-ness	Vascu-lar bundle thick	Xylem thick-ness
Control	No spraying	15.60d	16.64e	126.80e	183.96e	325.17 h	26.74f	9.50h
Whey extract 250 cm^3/L	Once/ week	18.83bc	28.16bc	151.14cd	190.35cd	785.33 b	46.52d	14.50f
	Once/ 2 weeks	15.37d	16.96e	128.65e	165.20f	561.32 e	36.13e	14.54f
	Once/ 3 weeks	17.37cd	16.88e	194.36a	185.20e	517.50 g	34.02e	16.33d
Whey extract 500 cm^3/L	Once/ week	22.14a	32.33a	155.45bcd	192.99b	850.66 a	51.45b	18.59a
	Once/ 2 weeks	20.57ab	25.60d	165.32b	192.25bc	700.70 c	54.50a	15.30e
	Once/ 3week	18.74bc	27.21cd	194.98a	230.08a	516.83 g	49.37bc	17.78b
Whey extract 750 cm^3/L	Once/ week	21.78ab	29.98b	148.48d	189.68d	780.40 b	48.73cd	16.46cd
	Once/ 2 weeks	15.90cd	18.66e	162.35bc	190.86bcd	582.10 d	27.07f	17.06c
	Once/ 3 weeks	10.43e	10.42f	185.45a	228.48a	527.42 f	49.22bc	10.50g
Mean		17.67	22.28	161.30	194.91	614.74	42.38	15.06

Microscopic Anatomy of the leaf:





Details: Up-ep, upper epidermis; mesol, mesophyll; low-ep, lower epidermis; vasc, vascular bundle

Fig (1) Transverse sections of the leaf blade developed at the median portion of the main stem of the *Aglaonema commutatum* plant. Control (A), 1 week (500 cm^3/L) (B), 2 weeks (500 cm^3/L) (C), 3 weeks (500 cm^3/L) (D)

Morphological and anatomical cluster analysis

Figure 2 showed the two ways cluster heat map in this study showed a visual representation of the spraying of whey on the morphological and anatomical parameters of Aglaonema plants. This was carried out to groups into sub-clusters based on their similarities and to assess the contribution of each parameter in response to whey application. The results of the hierarchical clustering analysis show that control and (250 cm^3/L every one week and three weeks) are separated from other treatments showing that they have the lowest impact on the measured parameters. While (500 and 750 cm^3/L every three weeks) and (250 cm^3/L every two weeks) gave the highest values. In the other way of the hierarchical cluster analysis the traits were distributed according to the analysis into three groups. The first contains most of the vegetative and anatomical characters. The latter is the most correlated traits. The most related traits were plant height, upper epider thick, lower epider thick and xylem thickness. The flowers number was related to root length. Leaf area was related to the midvein thickness.

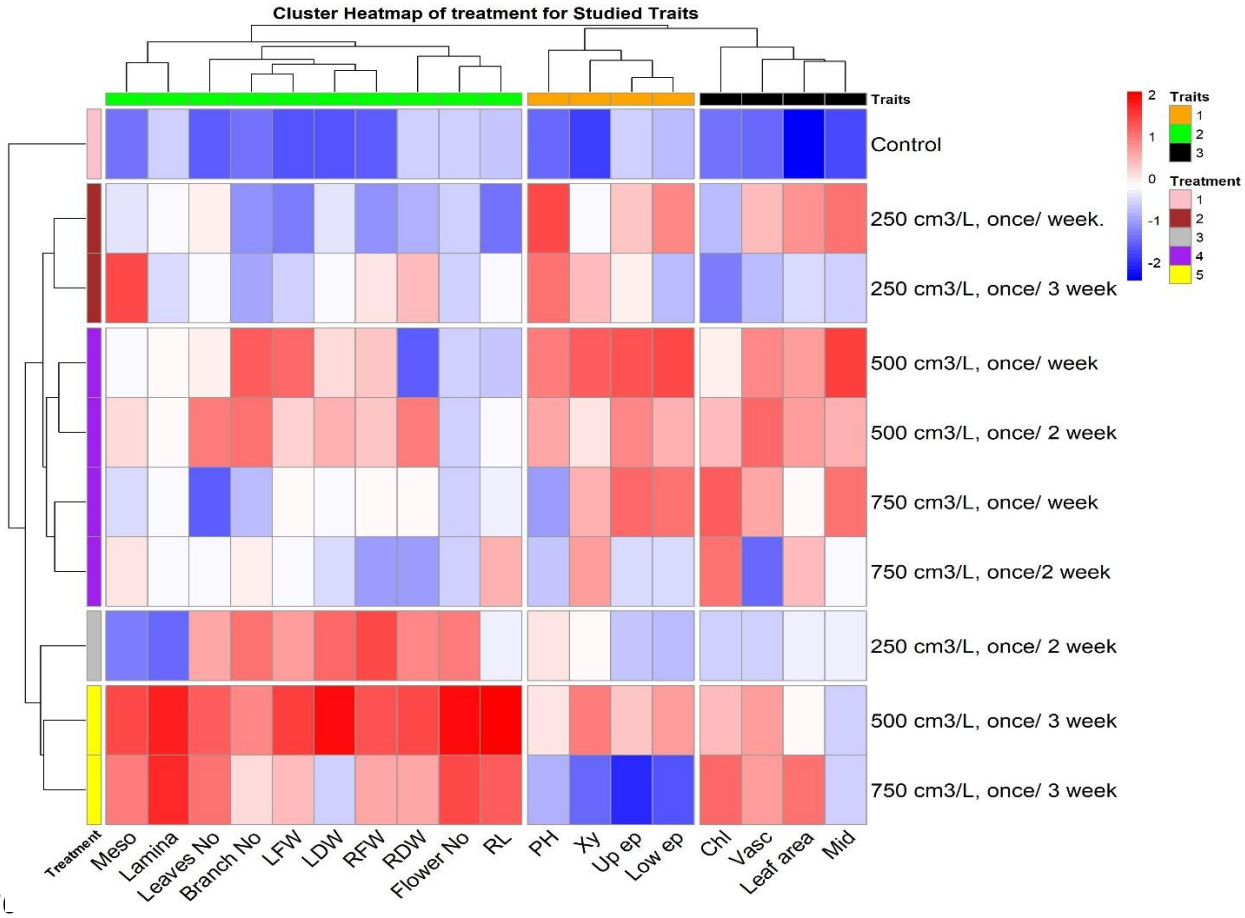


Fig. 2 Cluster heatmap of Aglaonema silver under treatments and studied traits.

Where plant height (PH), leaves No., branches No., flowers No., leaves fresh weight (LFW), leaves dry weight (LDW), root length (RL), root fresh weight (RFW), root dry weight (RDW), chlorophyll (Chl), leaf area, upper epider thick (Up ep), lower epider thick (Low ep), mesophyll (Meso), lamina thickness (Lamina), midvein thickness (Mid), vascular bundle thick (Vasc), and xylem thickness (Xy).

Biochemical analysis

There were significant differences soluble sugars and proteins content among the different treatments of whey extracts at $p<0.05$ as illustrated in Table 5. Increasing the time intervals for spraying for each treatment led to an increase in soluble sugars and soluble proteins. The highest values for soluble sugars and proteins were obtained at the application of the 750 cm^3/L of the whey as shown in Table 1, while the lowest content of soluble sugars and proteins were obtained at 250 cm^3/L of whey extract every week and 750 cm^3/L every week, respectively. The antioxidants compounds (phenolics and flavonoids) significantly increased by increasing the whey extract to reach their maximum values every 3 weeks for each concentration compared to control illustrated in Table 5.

The photosynthetic rate was affected by the different whey concentrations illustrated in Table 6. In the current study, the increase of whey extract leads to photosynthetic rates (A) except whey extract 500 cm^3/L every 3 weeks. This might be ascribed to photosynthesis being slowed by stomatal closure caused by a decrease in intracellular CO_2 concentration (Ci). The highest rate of photosynthetic activity was obtained at 500 cm^3/L of whey extract every 3 weeks where a maximum stomatal conductance and intracellular CO2 (Ci).

Table 5: Effect of different concentrations of Whey and spraying intervals on the soluble protein (mg g^{-1} f wt), soluble sugar (µg g^{-1} f wt), total phenolic compounds (mg g-1 d wt), and flavonoids content in Aglaonema silver leaves.

Treat-ments	Spraying Intervals	Soluble pro-teins	Soluble sug-ars	Total phenol-ics	Total flavo-noids
Control	No spraying	18.60±0.25	20.22±1.16b	44.48±4.4	26.2±0.3
Whey extract 250 cm^3/L	Once/week	11.13±1.7e	13.45±0.45f	36.50±3.60	31.10±0.95
	Once/ 2 week	21.45±0.87d	15.25±0.11e	52.38±2.20	43.80±1.08
	Once/ 3week	37.45±4.74b	17.48±0.29d	101.4±0.40	82.37±1.01
Whey extract 500 cm^3/L	Once/week	27.80±0.45c	16.73±0.18d	70.48±0.40	34.78±0.41
	Once/2week	29.45±0.08c	14.44±0.35e	87.80±4.10	66.34±1.09
	Once/3week	28.33±0.37c	13.24±0.24f	137.7±2.40	119.3±0.91
Whey extract 750 cm^3/L	Once/week	34.54±0.68b	12.11±0.62	73.60±3.10	51.53±1.69
	Once/2week	31.00±1.16c	18.94±0.79	94.03±0.04	71.08±2.20
	Once/3week	43.60±1.70a	23.99±0.9	139.0±2.33	111.1±1.71

Table 6: Effect of different concentrations of Whey and spraying intervals on the photosynthetic rate (A), stomatal conductance (gs), transpiration rate the ratio A/gs, and substomatal intercellular CO_2 in Aglaonema silver leaves.

Treat-ments	Spraying Intervals	A	gs	E	A/gs	Ci
Control	No spraying	3.79±0.04^{b}	9.59±0.13^{b}	0.345±0.004^{c}	792.6±3.7	111.1±1.71^{b}
Whey extract 250 cm^3/L	Once/ week	3.85±0.48^{c}	4.45±0.07^{f}	0.158±0.001^{f}	478.5±414.0^{c}	31.10±0.95^{h}
	Once/ 2 weeks	2.56±0.64^{c}	3.36±0.01^{g}	0.126±0.001^{g}	741.3±54.9^{b}	43.80±1.08^{g}
	Once/ 3 weeks	1.90±0.02^{c}	8.84±0.24^{c}	0.379±0.001^{b}	218.6±88.7^{d}	82.37±1.01^{c}
Whey extract 500 cm^3/L	Once/ week	1.94±0.83^{c}	7.05±0.05^{d}	0.127±0.001^{g}	186.3±4.10^{e}	34.78±0.41^{f}
	Once/ 2 weeks	1.18±0.18^{d}	4.79±0.08^{f}	0.317±0.005^{d}	138.0±35.0^{f}	66.34±1.09^{e}
	Once/ 3 weeks	4.49±0.25^{a}	10.2±0.15^{a}	0.432±0.036^{a}	177.0±6.07^{e}	119.3±0.91^{a}
Whey extract 750 cm^3/L	Once/ week	1.25±0.43^{d}	6.07±0.55^{e}	0.189±0.001^{e}	792.6±3.70^{a}	51.53±1.69^{f}
	Once/ 2 weeks	0.94±0.57^{d}	2.74±0.04^{h}	0.126±0.001^{g}	800.0±49.0^{a}	71.08±2.20^{d}
	Once/ 3 weeks	0.91±0.05^{d}	1.28±0.05^{g}	0.033±0.001^{h}	741.0±54.0^{b}	26.20±0.30^{i}

Detection of TFL1 gene

The PCR was applied for TFL1 gene and nearly a single band was detected at 1050bp for the control and treated samples. The gel banding pattern was illustrated in Figure 3. The band intensity differs from treatment to other and this could be due to the variation in the TFL1 flowering gene levels which is affected by variable treatments. Some treatments (as in 2 and 6) have some changes in DNA sequence that lead to formation of some other bands surrounding the essential TFL1 gene band.

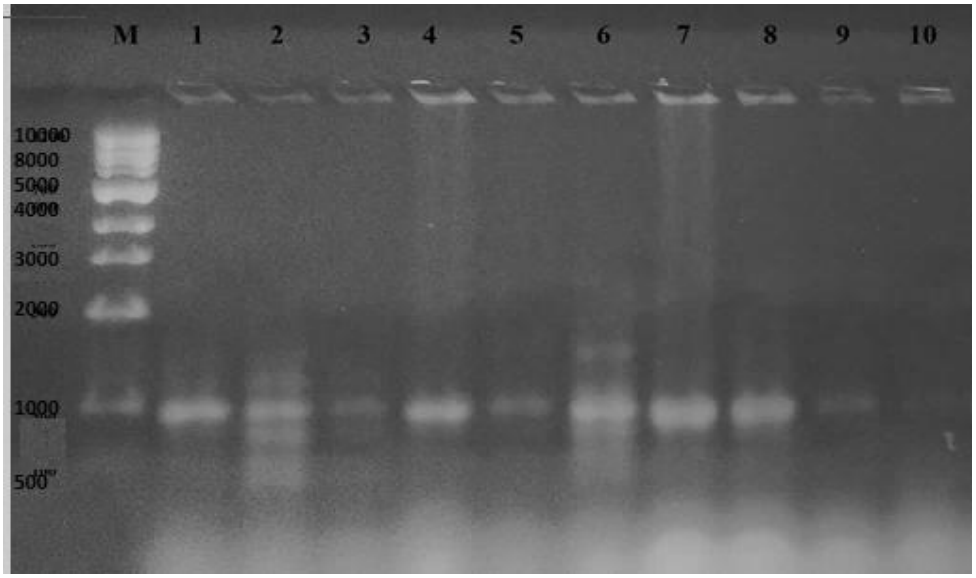


Fig. (3) The gel banding pattern of TFL1 gene in Aglaonema silver treated lines. (M: 1kb DNA ladder, 1: control, 2: 250 cm^3/L whey extract for 1 week, 3: 250 cm^3/L whey extract for 2 weeks, 4: 250 cm^3/L whey extract for 3 weeks, 5: 500 cm^3/L whey extract for 1 week, 6: 500 cm^3/L whey extract for 2 weeks, 7: 500 cm^3/L whey extract for 3 weeks, 8: 750 cm^3/L whey extract for 1 week, 9: 750 cm^3/L whey extract for 2 weeks, 10: 750 cm^3/L whey extract for 3 weeks).

Using RAPD-PCR on Aglaonema silver plants can provide valuable insights into the genetic diversity of this ornamental species. This information is crucial for plant breeding programs, conservation efforts, and understanding the genetic basis of traits such as colour variation and adaptability. The total number of bands was 32 with 16 polymorphic bands. These bands resulted in polymorphism percentage of 50.62%. All these data were represented in Figure 4 and illustrated in Table 7.

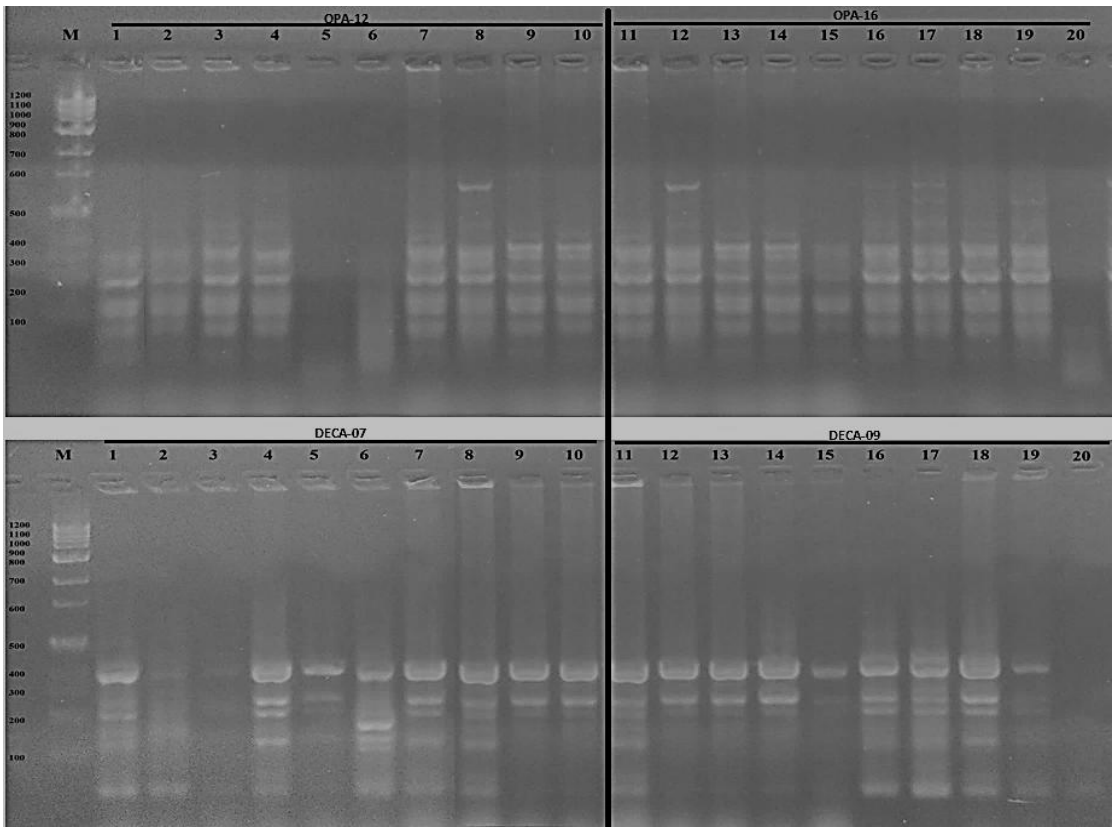


Fig. 4 The gel banding pattern of RAPD-PCR of Aglaonema silver with variable treatments (M: 100 bp DNA ladder, 1: control, 2: 250 cm^3/L whey extract for 1 week, 3: 250 cm^3/L whey extract for 2 weeks, 4: 250 cm^3/L whey extract for 3 weeks, 5: 500 cm^3/L whey extract for 1 week, 6: 500 cm^3/L whey extract for 2 weeks, 7: 500 cm^3/L whey extract for 3 weeks, 8: 750 cm^3/L whey extract for 1 week, 9: 750 cm^3/L whey extract for 2 weeks, 10: 750 cm^3/L whey extract for 3 weeks).

Table (7) The decamer primers data applied for RAPD-PCR analysis of different treatments of Aglaonema silver

No.	Primers names	Primer sequence	Tm °C	GC%	Polymorphic band	Total bands	Polymorphism %
1	OPA-12	5'-TCGGCGATAG-3'	34	60	4	7	57.14
2	OPA-16	5'-AGCCAGCGAA-3'	34	60	4	10	40
3	Deca-7	5'-CCGCCCGGAT-3'	45	80	3	7	42.85
4	Deca-9	5'-TGGCCCCGGT-3'	47.4	80	5	8	62.50
Total					16	32	50.62

Discussion

The present study demonstrated that foliar application of whey extract significantly influenced the morphological, anatomical, physiological, biochemical and molecular characteristics of *Aglaonema commutatum* 'Silver Queen'. The magnitude of the response depended on both whey concentration and spraying interval, indicating that the effectiveness of whey extract is closely related to the availability of nutrients and bioactive compounds supplied to the plant. Similar beneficial effects of whey application on plant growth and physiological performance have previously been reported by [7, 1].

The improvement observed in vegetative growth parameters, including plant height, leaf number, branch formation and biomass accumulation, may be attributed to the rich nutritional composition of whey. Whey contains proteins, amino acids, lactose and several essential mineral nutrients, including potassium, calcium, magnesium and phosphorus, which are required for normal plant growth and metabolic activities [10 ,1]. These nutrients contribute to enhanced cell division, protein synthesis and enzymatic activity, leading to improved vegetative development. The superior performance recorded in plants treated with 500 cm^3/L whey extract every three weeks suggests that moderate concentrations may provide sufficient nutrients without causing metabolic imbalance.

Root growth was also significantly enhanced by whey treatments. The greatest root length, fresh weight and dry weight were obtained in plants treated with 500 cm^3/L whey extract every three weeks. Enhanced root development improves nutrient and water uptake efficiency, which consequently supports shoot growth and biomass production. Similar

observations were reported by [12], who found that whey application positively influenced plant growth through improved nutrient availability and utilization.

The anatomical investigations revealed substantial modifications in leaf structure following whey application. Increases in epidermal thickness, mesophyll development, lamina thickness and vascular tissue dimensions were observed, particularly under the 500 cm^3/L whey treatment. Such anatomical improvements are generally associated with enhanced photosynthetic capacity and more efficient transport of water and assimilates within plant tissues. Increased mesophyll thickness may improve light interception and carbon fixation, whereas enlarged vascular tissues facilitate translocation of water, nutrients and photosynthates throughout the plant [8].

Photosynthetic performance was significantly affected by whey extract application. The highest photosynthetic rate, stomatal conductance and intercellular CO_2 concentration were observed in plants treated with 500 cm^3/L whey extract every three weeks. These findings suggest that moderate whey concentrations enhance leaf physiological activity and gas exchange efficiency. The observed stimulation may be related to improved chlorophyll content and increased availability of nitrogen-containing compounds required for photosynthetic enzymes and proteins. Similar positive effects of nutrient-rich bio stimulants on photosynthetic performance have been reported by [7, 8]. In addition, improved photosynthetic efficiency following organic bio stimulant application has been associated with enhanced chloroplast function, increased carbon assimilation and greater stomatal regulation efficiency, ultimately resulting in higher biomass production and plant vigor [13, 2].

The biochemical analyses demonstrated that whey extract significantly increased soluble protein and soluble sugar contents in treated plants. The highest values were recorded under 750 cm^3/L whey treatment applied every three weeks. The increase in soluble proteins may be attributed to the availability of amino acids and nitrogenous compounds present in whey, which support protein biosynthesis and metabolic activity [10, 1]. Likewise, the accumulation of soluble sugars may reflect enhanced photosynthetic carbon assimilation and improved carbohydrate metabolism resulting from better nutritional status.

Phenolic compounds and flavonoids were also markedly enhanced by whey treatments. The highest concentrations were detected in plants treated with 750 cm^3/L whey extract every three weeks. Phenolics and flavonoids constitute important components of the plant antioxidant defense system and contribute to protection against oxidative stress and environmental challenges [16, 17]. The increased accumulation of these compounds suggests that whey extract may stimulate secondary metabolic pathways and improve the antioxidant capacity of Aglaonema plants. Similar increases in phenolic compounds following the application of natural bio stimulants have been reported by [2], who indicated that bio stimulants can enhance antioxidant metabolism and stimulate the biosynthesis of protective secondary metabolites. Furthermore, [13] reported that bio stimulants promote plant resilience through

activation of physiological and biochemical pathways associated with antioxidant production and stress tolerance.

The cluster heatmap analysis further supported the positive influence of whey extract on plant performance. Treatments involving 500 cm^3/L and 750 cm^3/L whey applied every three weeks were grouped together and associated with the highest values of most vegetative and anatomical parameters. This pattern confirms the existence of a strong relationship between anatomical development and vegetative growth, indicating that structural improvements in leaf tissues contributed directly to enhanced plant performance.

Molecular analysis of the TFL1 locus confirmed the presence of the target sequence in both control and treated plants. Although differences in amplification profiles were observed among treatments, conventional PCR does not directly measure gene expression; therefore, additional transcript-based analyses would be required to determine whether whey application affects TFL1 expression. The present findings should therefore be interpreted as evidence of amplification pattern variation rather than direct changes in gene expression [4].

RAPD-PCR analysis revealed detectable polymorphism among the investigated samples, with a total polymorphism percentage of 50.62%. These results indicate treatment-associated molecular variability among plants exposed to different whey concentrations and spraying intervals. RAPD markers have been widely used for evaluating genetic diversity and molecular variation in ornamental species and have proven effective in distinguishing closely related Aglaonema genotypes [3]. The observed polymorphism may reflect genomic responses associated with long-term exposure to different nutritional treatments.

Overall, the present study demonstrated that whey extract can function as an effective natural bio stimulant for *Aglaonema commutatum* ‘Silver Queen’. Among the tested treatments, foliar application of 500 cm^3/L whey extract every three weeks was the most effective for improving growth, anatomical development and photosynthetic performance, whereas 750 cm^3/L whey extract every three weeks was superior for enhancing soluble proteins, soluble sugars and antioxidant metabolites. These findings highlight the potential of whey as a sustainable agricultural input capable of improving ornamental plant quality while simultaneously contributing to the valorization of dairy industry by-products.

Conclusion

The present study demonstrated that foliar application of whey extract significantly affected the growth, anatomical structure, photosynthetic activity, biochemical constituents and molecular characteristics of *Aglaonema commutatum* ‘Silver Queen’. The responses varied according to both whey concentration and application interval, indicating that treatment efficiency depends on the interaction between these two factors.

Among the tested treatments, the application of 500 cm^3/L whey extract every three weeks proved to be the most effective in enhancing overall plant performance. This treatment

resulted in superior vegetative growth, root development, anatomical characteristics and photosynthetic activity, suggesting that moderate whey concentrations provide optimal conditions for plant growth and physiological functioning. In contrast, the application of 750 cm^3/L whey extract every three weeks produced the highest levels of soluble proteins, soluble sugars, phenolic compounds and flavonoids, indicating a strong stimulatory effect on primary and secondary metabolism.

The anatomical improvements observed in treated plants, including increased mesophyll development, vascular bundle dimensions and leaf tissue thickness, further support the beneficial role of whey extract in enhancing nutrient transport and photosynthetic efficiency. In addition, RAPD-PCR analysis revealed detectable polymorphism among treatments, suggesting the occurrence of treatment-associated molecular variation.

Overall, the findings indicate that whey extract can be utilized as an environmentally friendly and cost-effective bio stimulant for ornamental plant production. The use of whey not only improves plant growth and quality but also represents a sustainable approach for recycling dairy industry by-products. Further studies involving gene expression analysis and long-term physiological evaluations are recommended to better understand the mechanisms underlying plant responses to whey application.

Declarations:

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Authors' contributions: S.M.H. and M.M.S. designed the experiment, evaluated, collected the data and wrote it. E.T, Z.A.S AND M.S. studied the stimulatory and inhibitory on the essential metabolic activities and wrote it. Z.K.T. studied the effect of plant anatomy and wrote it. All authors reviewed the manuscript.

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