



Optimized 24-Epibrassinolide Application Enhances morpho-physiological traits and Yield in Eggplant (*Solanum melongena* L. var Aretussa)

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ABSTRACT

Epibrassinolide (EBL) regulate plant development and environmental stress responses through complex signaling networks, yet optimization of exogenous 24-Epibrassinolide (EBL) application for commercial vegetable production remains underexplored. We investigated dose- and frequency-dependent effects of foliar 24-epibrassinolide (0, 0.5, and 0.75 μM) applied once (AR1) or twice (AR2) on eggplant (*Solanum melongena* L.) physiological performance, growth, and yield in a 3 × 2 factorial completely randomized design. Eggplant seeds were sown in a cocopeat and perlite growing medium at a ratio of 3:1 and the seedlings were watered daily in greenhouse. Foliar spraying was done in two stages before transplanting (four-leaf stage) in greenhouse and before flowering in farm. After the first foliar spray, the Eggplant seedlings were transplanted to the field and the plants were irrigated every three days throughout the growth period. Optimal EBL treatment (0.75 μM × AR2) elicited coordinated enhancement of photosynthetic capacity and membrane integrity. Chlorophyll metabolism was substantially upregulated, with SPAD values increasing 43%, total chlorophyll content rising 33.3%, and carotenoid accumulation elevated 82.7% compared to controls. Concurrently, cellular membrane stability improved markedly, evidenced by 43% reduction in electrolyte leakage. These physiological modifications translated into accelerated developmental progression 20.7–32.9% increase in vegetative growth and 17.8–21.7% advancement in flowering time culminating in approximately a two-fold increase in yield under the optimal regimen. Our findings demonstrate that strategic EBL application synchronously increased photosynthetic capacity and cellular homeostasis, establishing dual-spray 24-epibrassinolide treatment as an efficacious biostimulant strategy for Solanaceae crop intensification. Elucidating the underlying molecular pathways particularly EBL receptor dynamics and downstream transcriptional cascades—represents a critical next step for optimizing this approach across diverse genotypes and environmental conditions.

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1. Introduction

Global food systems face increasing pressure from climate instability and the need for sustainable intensification, requiring agronomic strategies that improve both productivity and resource-use efficiency (Gruda et al., 2019). Enhancing yield potential and nutritional quality will depend mainly on leveraging plants' intrinsic regulatory networks rather than further increasing external inputs (Gruda et al., 2025b, Gruda et al., 2025a). This shift underscores the importance of bioactive signaling molecules that modulate developmental processes and stress-response pathways (Ikiz et al., 2025, Memeli et al., 2025). Among these regulators, 24-Epibrassinolide are particularly notable for their capacity to coordinate growth, metabolic regulation, and environmental adaptation.

24-Epibrassinolide (EBL) are plant hormones recognized for their essential roles in controlling various aspects of plant growth and development. They participate in seed germination, vegetative and reproductive growth, photosynthetic efficiency, vascular differentiation, and fruit yield and quality (Aryal and Alferez, 2025). Recently, 24-epibrassinolides have attracted considerable attention for their potential to boost vegetable crop productivity. BRs enhance growth traits such as plant height, stem thickness, leaf size, and early flowering, as well as improving fruit set and fruit quality (Bajguz and Hayat, 2009, Yusuf et al., 2016, Vardhini, 2019). Additionally, EBLs increase plants' resistance to abiotic stresses such as drought, salinity, and mechanical damage (Yang et al., 2019, Ahammed et al., 2020).

Eggplant (*Solanum melongena* L.), a key member of the Solanaceae family, is a widely cultivated vegetable prized for its nutritional value, including dietary fiber, antioxidants, and essential vitamins (Edeke et al., 2021). In 2018, global eggplant production reached approximately 55 million metric tons, with China, India, and Egypt contributing nearly 90% of the total output (FAO, 2018). This versatile crop is a dietary staple in regions like South and East Asia and the Mediterranean, where it features prominently in traditional cuisines. Despite its popularity, eggplant production faces challenges from biotic stresses, such as diseases, and abiotic stresses, like drought and nutrient deficiencies, which can limit yield and quality. Improving eggplant growth and productivity through innovative agricultural practices is a critical research focus.

Among the most widely studied and commercially utilized forms is 24-epibrassinolide (EBL), a synthetic analog known for its high biological activity and stability, making it an ideal candidate for agricultural applications. In eggplants, treatments using the analog 24-epibrassinolide have demonstrated notable physiological and morphological improvements. Physiologically, EBL enhances photosynthetic efficiency and cellular defense. For instance, 100 nM EBL (Wu et al., 2015) and 0.1 μ M EBL (Wu et al., 2014a, He et al., 2016) were found to increase chlorophyll concentration in eggplant. This enhancement of photosynthetic pigments is critical for improving overall productivity. Furthermore, EBL plays a role in maintaining cellular integrity; it strengthens cell membranes, typically measured by reduced electrolyte leakage (EL) under optimal or stress conditions (Ahammed et al., 2020). These positive physiological responses translate into enhanced morphological growth and final yield. Wu et al. (Wu et al., 2014b) reported that application of 0.05–0.2 μ M EBL remarkably promoted plant growth in eggplant. Wu et al. (Wu et al., 2016) and He et al. (He et al., 2016) indicated that 0.1 μ M EBL increased plant heights, shoot, and root mass by 23.9%, 78.6%, and 13.8%, respectively in eggplant. Ultimately, these growth and physiological benefits lead to superior fruit output: Wakchaure et al. (Wakchaure et al., 2020) also found that 1 μ M EBL treatment resulted in larger, heavier fruits, leading to higher marketable yields compared to untreated eggplants.

However, recent evidence suggests that application timing and frequency may be equally critical as concentration in determining EBL efficacy. Evidence suggests that multiple sprays during the growing season, especially at key growth and reproductive stages such as flowering and fruit setting, are often more effective than a single application. Yang et al. (Yang et al., 2019) reported that eggplants sprayed twice with EBLs showed 15–20% greater increases in plant height and fruit yield than those sprayed once. This pattern of increased growth and yield has also been observed in other Solanaceae crops, where EBLs enhance fruit size, fruit number, and chlorophyll content, thereby improving photosynthetic efficiency and overall productivity (Hayat et al., 2012). Similarly, EBLs have been shown to improve growth, yield, and physiological traits across many crops. In peppers, they enhanced fruit set, fruit size, and antioxidant levels (Słomnicka et al., 2025). In cucumbers, foliar application of 2 ppm EBL reduced time to first fruit and increased both fruit number and yield (Yadav et al., 2022). In tomatoes, EBL treatment promoted shoot, root, and leaf dry mass, along with higher photosynthetic pigment levels (Maia Júnior et al., 2022). In watermelon, EBLs increased fresh weight and chlorophyll content while lowering oxidative stress markers (Liu et al., 2023). Comparable benefits have also been reported in beans, soybeans, phalsa, and cotton, where EBL improved vegetative growth and yield (El-Bassiony et al., 2012; Perez-Borroto et al., 2022; Vankar et al., 2021; Xu et al., 2025). Additionally, EBL enhanced photosynthetic pigment accumulation in the aquatic plant *Wolffia arrhiza* (Chmur and Bajguz, 2025).

While 24-epibrassinolide (EBL) demonstrates growth-promoting activity across diverse plant species, a critical optimization gap remains regarding the synergistic effects of concentration and application frequency under optimal cultivation conditions. Previous investigations have predominantly examined single-dose responses or stress-mitigation scenarios, neglecting systematic factorial evaluation of concentration \times frequency (C \times F)

interactions that govern BR signaling dynamics and physiological responses. This knowledge deficit constrains the development of evidence-based protocols to maximize EBL-mediated productivity enhancement in high-value vegetable crops, particularly under non-limiting growth conditions, where endogenous EBL homeostasis may respond differently to exogenous supplementation. We hypothesized that a synergistic interaction between elevated EBL concentration and dual application frequency optimizes eggplant performance by coordinating the enhancement of photosynthetic capacity and cellular homeostasis. Specifically, the optimal interaction regime amplifies photosynthetic pigment biosynthesis and membrane integrity, establishing physiological conditions that accelerate developmental progression and maximize reproductive output. This investigation pursued three integrated objectives: (1) systematically evaluate C × F interactions of foliar EBL application to identify the optimal treatment regime maximizing photosynthetic efficiency and cellular integrity in *Solanum melongena* L.; (2) quantify the developmental responses including vegetative growth dynamics and reproductive phenology elicited by optimized EBL treatment under field conditions; and (3) establish a validated, commercially applicable protocol delivering maximum marketable yield for eggplant cultivation systems.

2. Materials and Methods

2.1. Experimental Design and Location

This study aimed to examine the effects of different concentrations of EBL (Sigma- Aldrich Co., United States) [(a control group with no EBL (B₀), a low concentration (BR₁, 0.5 μM), and a higher concentration (BR₂, 0.75 μM)] and spraying frequencies [applied either once (AR₁) or twice (AR₂)] on the growth and physiological responses of eggplant according to a completely randomized design arranged as a 3 × 2 factorial with three replicates and three plant per each replication. For the foliar application of EBL, the solution was prepared in 100% ethanol (absolute ethanol), with a final concentration of 0.01% Tween 20 as a surfactant to aid dispersion and adhesion of the spray on plant surfaces. Control plants were sprayed with distilled water only. The pH of the spray solution was adjusted to 6.5 to ensure optimal stability and effectiveness of the 24-Epibrassinolide (EBL). A total of 200 mL of solution was applied per plant during each spraying event to ensure even coverage of the plant leaves. The first application was made before transplanting the seedlings (at the 2–4 leaf stage in the greenhouse), and the second application was conducted during the pre-flowering stage in the field. The experiment was conducted in the research farm of the Faculty of Agriculture at Shahid Bahonar University of Kerman during the 2023-2024 growing season.

2.2. Experimental Setup

Seeds of Aretussa RZ F1 (Rijk Zwaan) were obtained via Sepahan Rooyesh (Isfahan, Iran). Aretussa RZ F1 is a white-fruited F1 hybrid eggplant variety from Rijk Zwaan. It is known for its high-yielding, attractive, and uniform fruits that are ideal for local fresh markets and specialty, premium, or farm-stand sales. Seeds were sown in a 50-cell tray with a mixture of cocopeat and perlite at a ratio of 3:1 and the seedlings were watered daily in greenhouse. Foliar spraying was done in two stages before transplanting (four-leaf stage) in greenhouse and before flowering in farm. After the first foliar spray, the eggplant seedlings were transferred to the field and the plants were irrigated every three days throughout the growth period. For planting, a distance of 50 cm was considered on the row and one meter between the rows. A plot measuring 100 × 100 square meters was considered for this experiment. Fertility management was carried out in two stages: first at planting and then 45 days later, using a combination of 20 tons per hectare rotted animal manure, 100 kg ha⁻¹ urea (70% at the seedling stage and 30% fruit-setting stage), 150 kg ha⁻¹ potassium sulfate (50% at the seedling stage and 50% fruit-setting stage), and 80 kg ha⁻¹ superphosphate. Weeding was performed as needed to prevent competition with the crop.

2.3. Vegetative Parameters

The following vegetative parameters were measured during the study: plant height, stem diameter, days to flowering, fruit number, fruit length, fruit width, and fruit yield. Plant height was recorded with a standard ruler, while stem diameter, fruit length, and fruit width were measured using a digital caliper. Fruit number and yield were recorded throughout the growing season, and at harvest, yield was determined based on the cultivated area. ha⁻¹.

2.4. Photosynthetic Pigments

To determine the concentrations of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, 0.25 g of fresh leaf tissue was ground in a porcelain mortar with 10 mL of 80% acetone. The mixture was then transferred to Falcon tubes (20 mL) and centrifuged at 3500 rpm for 10 minutes. The absorbance of the supernatant was measured using a T80 UV/VIS spectrophotometer (PG Instruments Ltd., UK). The pigment concentrations were calculated using the following equations from Arnon (Arnon, 1967):

$$\text{Chlorophyll a (mg g}^{-1}\text{ FM)} = [(12.7 \times A_{663}) - (2.69 \times A_{645})] \times (V / 1000 \times W)$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ FM)} = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times (V / 1000 \times W)$$

$$\text{Total Chlorophyll (mg g}^{-1}\text{ FM)} = [(A_{652} \times 1000) / 34.5] \times (V / 1000 \times W)$$

$$\text{Carotenoids (mg g}^{-1}\text{ FM)} = [(7.6 \times A_{480}) - (1.49 \times A_{510})] \times (V / 1000 \times W)$$

where: A = Absorbance at the specified wavelength, V = Volume of acetone used (10 mL), W = Fresh sample mass (0.25 g)

2.5. Greenness Index (SPAD)

The chlorophyll content, expressed as the SPAD index, was measured using a SPAD-502 Chlorophyll Meter (Konica Minolta, Japan). Four leaves from the 3rd and 6th nodes of each plant were selected for measurement.

2.6. Plant Water Relations

The Relative Water Content (RWC) was used to assess the plant's water status. Circular leaf discs (6 mm in diameter) were punched from fresh leaves, and their fresh weight was immediately recorded. The discs were then submerged in distilled water in Petri dishes for 6 hours to ensure full turgidity. Afterward, the discs were blotted dry and weighed again to obtain their turgid weight. The final dry weight was determined after oven-drying the discs at 70°C for 48 hours. The RWC was calculated using the following formula:

$$\text{RWC (\%)} = 100 \times [(\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight})] \text{ (Slavík and Jarvis, 1974).}$$

2.7. Ion Leakage

Electrolyte leakage was measured by placing leaf samples in distilled water and incubating them at 40°C (EC₁) and 100°C (EC₂), as described by Sairam et al. (Sairam et al., 2002). The leakage percentage was then calculated as follows:

$$\text{EL} = (\text{EC}_1/\text{EC}_2) \times 100$$

2.8. Statistical Analysis

Data were analyzed according to a completely randomized 3×2 factorial design with three replicates per treatment. The assumptions of normality and homogeneity of variance were tested before the analysis. Specifically, the Shapiro-Wilk test was used to assess normality, and Levene's test was used to determine homogeneity of variances across treatment groups. Data that met these assumptions were then analyzed using a two-way analysis of variance (ANOVA) to assess the main effects and interactions between the treatments. For significant differences detected by ANOVA ($p \leq 0.05$), LSD test was used for post-hoc comparisons of means. Statistical analysis was performed using SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA). All multivariate analyses, including correlation analysis, heatmap, cluster analysis, and PCA biplot, were performed using Origin Pro 2024b (Northampton, Massachusetts, USA). Before performing the multivariate analyses (PCA and clustering), all variables were standardized (z-score transformation) to ensure that each variable had a mean of 0 and a standard deviation of 1. This was done to account for differences in the variables' scales and to ensure that no single variable dominated the analysis.

3. Results

3.1. Electrolyte Leakage

Electrolyte leakage was significantly affected ($p \leq 0.01$) by 24-Epibrassinolide concentration, application frequency, and their interaction (Table 1). Also, the lowest leakage was observed in the BR₂ × AR₂ treatment, representing a 43% reduction compared with the control. Also, other EBL and AR combinations (BR₂ × AR₁, BR₁ × AR₂, and BR₁ × AR₁) decreased electrolyte leakage in eggplant compared to the control (Table 2).

3.2. Relative Water Content (RWC)

Relative water content was affected significantly ($p \leq 0.01$) with 24-Epibrassinolide application (Table 2). Control plants showed an RWC of 77.6%, while the 0.5 and 0.75 μM treatments raised it to 82.5% and 82.1%, corresponding to 6.3% and 5.8% increases, respectively (Figure 1A). Application frequency also had a significant effect ($p \leq 0.05$), with two sprays improving RWC by approximately 2% (Figure 1B). The interaction effect was nonsignificant.

3.3. SPAD (Chlorophyll Index)

SPAD values were significantly influenced ($p \leq 0.01$) by EBL concentration (Table 1). The control recorded a SPAD value of 42, which increased to 55 and 60 under 0.5 and 0.75 μM treatments, corresponding to 30.9% and 42.9% increases, respectively (Figure 2A). Neither the application frequency nor the interaction effect was significant.

Table 1. The effect of EBL concentrations and application frequency on the physiological characteristics of eggplant

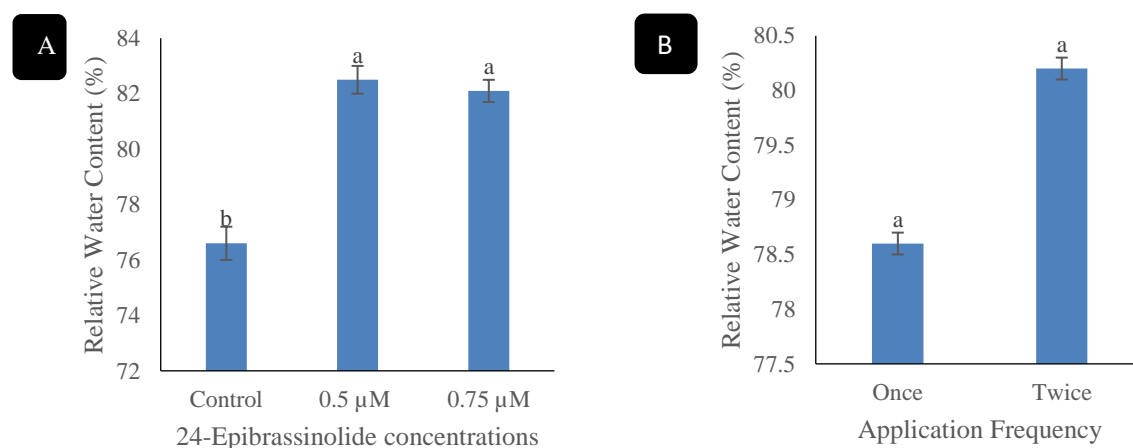
Source of variation	df	Electrolyte leakage (%)	Relative water content (%)	SPAD	Chlorophyll <i>a</i> content (mg.g ⁻¹ FW)	Chlorophyll <i>b</i> content (mg.g ⁻¹ FW)	Total chlorophyll content (mg. g ⁻¹ FW)	Carotenoid content (mg. g ⁻¹ FW)
24-Epibrassinolide (BR)	2	1027**	150.3**	518**	28.3*	4.99*	74**	7.14**
Application Frequency (AR)	1	26.8*	10.8*	8ns	22.2*	0.24ns	32**	0.84ns
BR*AR	2	17.3*	5.7ns	8ns	2.7ns	0.69ns	2ns	0.54ns
Experimental error	12	2.09	1.8	2.5	2.09	0.95	1.0	0.42
Coefficient of variation (CV)	-	4.66	2.33	4.8	12.4	12.8	4.1	11.2

** , * and ns indicate significance at the 1% and 5% levels, respectively, and non-significant ($p > 0.05$) according to LSD test.

Table 2. The interaction of different EBL concentrations and Spraying Frequencies on the number of fruits, yield, and electrolyte leakage of eggplant

Treatment	Electrolyte leakage (%)	Number of fruits per plant	Fruit yield (t ha ⁻¹)
EBL × Application Frequency (AR)	B ₀	41a	3.5d
	BR ₁ × AR ₁	30c	5c
	BR ₁ × AR ₂	35b	6b
	BR ₂ × AR ₁	35b	6.06b
	BR ₂ × AR ₂	21d	7.2a

The same letters in each column and for each mean comparison indicate no significant difference at LSD test .

**Figure 1A-B. Effect of EBL concentrations (A) and application repetition (B) on relative water content in eggplant**

3.4. Chlorophyll *b* content

Chlorophyll *b* was significantly influenced by EBL concentration ($p \leq 0.05$) (Table 1). The control had 6.8 mg.g⁻¹ FW, while 0.5 and 0.75 μM EBL treatments increased it to 7 and 8.5 mg.g⁻¹ FW, corresponding to 2.9% and 25% increases, respectively (Figure 2B). The effects of application frequency and interaction were not significant.

3.5. Chlorophyll *a* content

Chlorophyll *a* content was significantly affected ($p \leq 0.05$) by EBL concentration and application frequency (Table 1). The control recorded $14.5 \text{ mg g}^{-1} \text{ FW}$, while 0.5 and $0.75 \mu\text{M}$ EBL treatments resulted in 17 and $18.8 \text{ mg.g}^{-1} \text{ FW}$, representing 17.2% and 29.7% increases, respectively (Figure 2C). Also, two sprays (AR_2) further enhanced chlorophyll *a* content (Figure 2D).

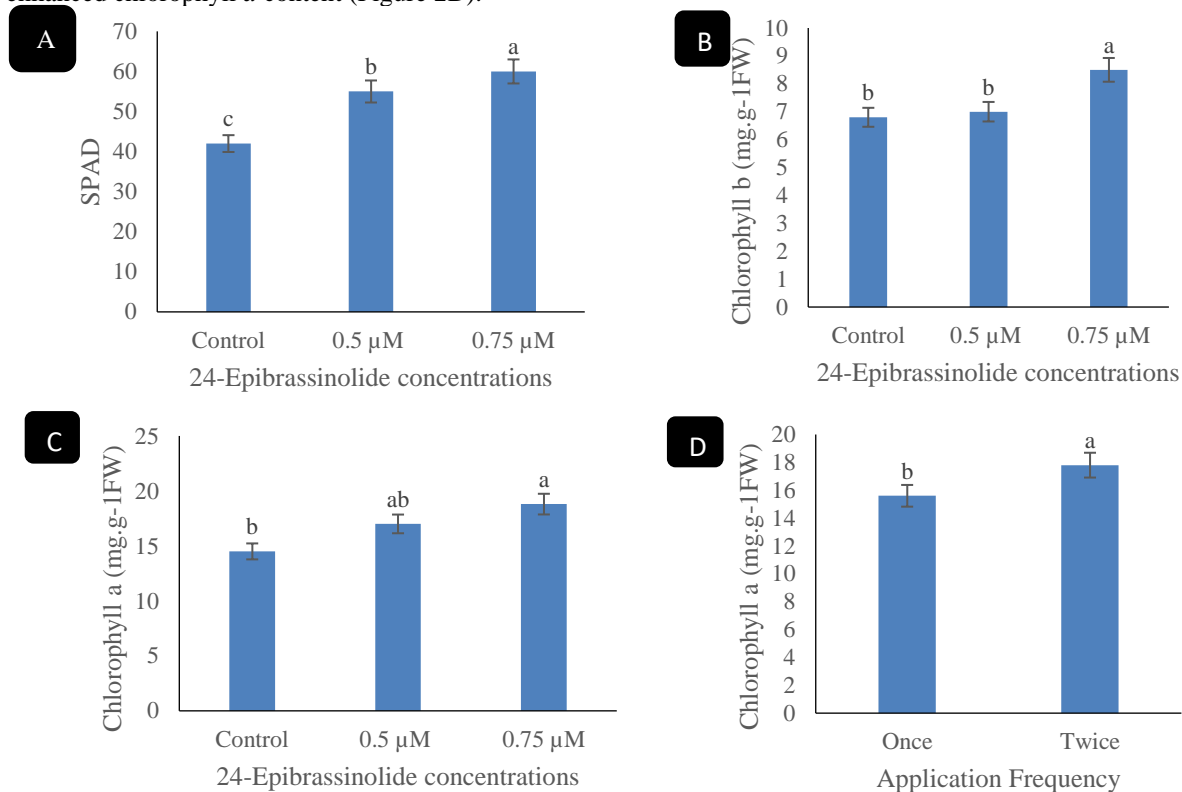


Figure 2A-D. The effect of EBL concentrations on SPAD (A), chlorophyll *b* (B), and *a* (C), and application repetition on chlorophyll *a* content (D) in eggplant

3.6. Total Chlorophyll content

Total chlorophyll content was significantly affected by both EBL concentration ($p \leq 0.01$) and spray frequency ($p \leq 0.01$) (Table 1). The control recorded $21 \text{ mg g}^{-1} \text{ FW}$, while 0.5 and $0.75 \mu\text{M}$ EBL treatments resulted in 24 and $28 \text{ mg.g}^{-1} \text{ FW}$, corresponding to 14.3% and 33.3% increases (Figure 3A). Also, two sprays increased total chlorophyll by 11.3% compared with one spray (Figure 3b).

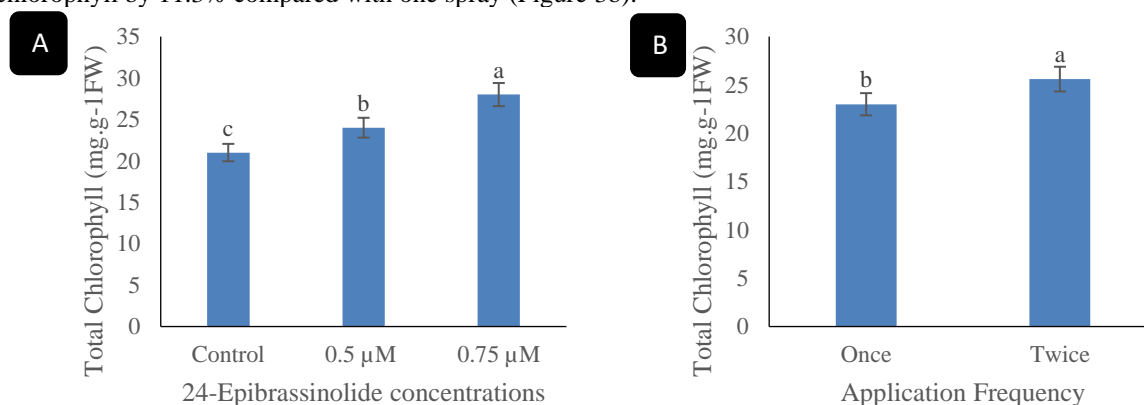


Figure 3A-B. Effect of EBL concentrations (A) and application repetition (B) on total chlorophyll content in eggplant

3.7. Carotenoid Content

Carotenoid concentration was significantly affected ($p \leq 0.01$) by EBL (Table 1). The content rose from $2.6 \text{ mg.g}^{-1} \text{ FW}$ in the control to $4.0 \text{ mg g}^{-1} \text{ FW}$ and $4.75 \text{ mg.g}^{-1} \text{ FW}$ under 0.5 and $0.75 \mu\text{M}$ EBL, representing 53.8%

and 82.7% increases, respectively (Figure 4). Neither application frequency nor the interaction effect was significant.

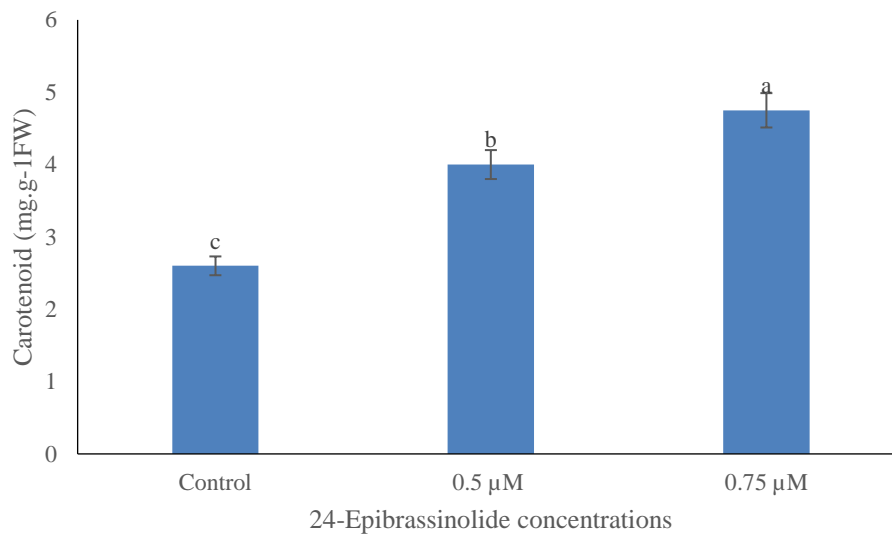


Figure 4. Effect of EBL concentrations on carotenoid content in eggplant

3.8. Plant Height

Application of EBL had a significant effect on plant height ($p \leq 0.01$) (Table 3). In the control (B_0), plants reached an average height of 77.5 cm, while those treated with 0.5 µM (B_1) and 0.75 µM (B_2) attained 93.6 cm and 103 cm, corresponding to 20.7% and 32.9% increases compared with the control (Figure 5A). Application frequency (AR) also had a significant effect ($p \leq 0.05$); plants sprayed twice (AR_2) were 6.2% taller than those sprayed once (AR_1) (Figure 5B); however, the $B \times AR$ interaction was not statistically significant.

Table 3. The effect of EBL concentrations and application frequency on growth and yield traits of Eggplant

Source Variation	of	df	Plant height (cm)	Stem diameter (mm)	Days to flowering	Number of fruits	Fruit length (cm)	Fruit width (cm)	Fruit yield (t.ha ⁻¹)
EBL		2	998.7**	31.5**	334.5**	451.5**	1055**	77.7*	15.1**
Application Frequency (AR)		1	133.3*	4.5ns	8ns	60.5**	206.7ns	80.2*	2.27**
EBL *AR		2	22.3ns	4.5ns	0.5ns	15.5*	51.7ns	13.7ns	0.57**
Experimental error		12	4.6	1.8	1.58	1.7	7.9	3.8	0.18
Coefficient of variation (CV)		-	5.09	10.4	2.8	5.6	5.2	6.5	3.5

** , * and ns indicate significance at the 1% and 5% levels, respectively, and non-significant ($p > 0.05$) according to LSD test.

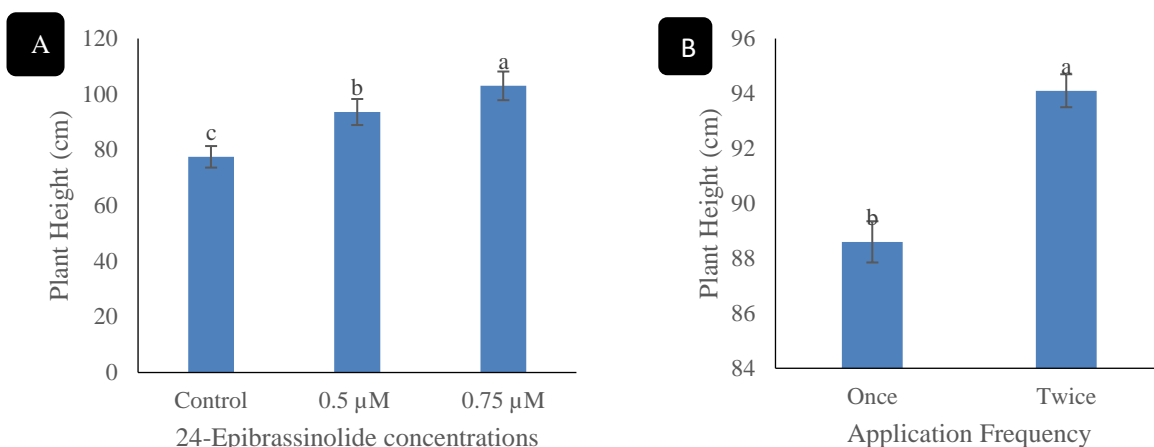


Figure 5A-B. Effect of EBL concentrations (A) and application repetition (B) on eggplant height

3.9. Stem Diameter

Stem diameter responded significantly ($p \leq 0.01$) to EBL application (Table 3). The mean stem thickness increased from 15 mm in the control to 18 mm and 19.5 mm at 0.5 and 0.75 μM, representing 20% and 30% increases, respectively. Neither spray frequency nor the interaction between factors was significant (Figure 6A).

3.10. Days to Flowering

EBL had a significant ($p \leq 0.01$) effect on the number of days required for flowering (Table 3). Control plants flowered after 64.5 days, whereas plants treated with 0.5 μM and 0.75 μM EBL flowered after 53 and 50.5 days, showing reductions of 17.8% and 21.7%, respectively (Figure 6B). Neither application frequency nor the interaction effect was significant.

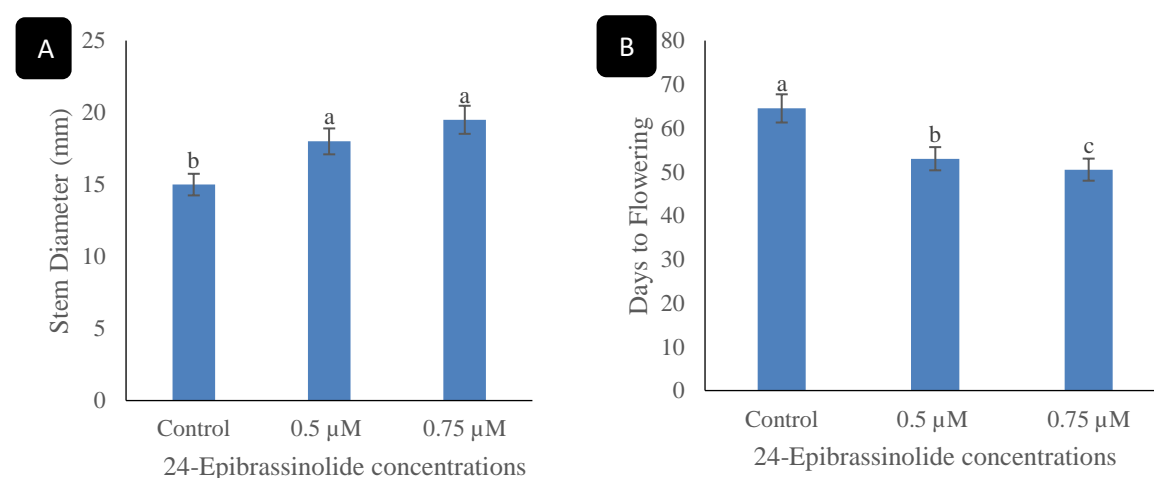


Figure 6A-B. Effect of EBL concentrations on stem diameter (A) and days to flowering (B) in eggplant

3.11. Number of Fruits per Plant

The number of fruits per plant was significantly affected by both EBL concentration and spray frequency ($p \leq 0.01$), as well as their interaction ($p \leq 0.05$) (Table 3). The $BR_2 \times AR_2$ combination produced the highest fruit count (41 fruits per plant), equivalent to a 95% increase over the control. Also, the results showed that $BR_2 \times AR_1$, $BR_1 \times AR_2$, and $BR_1 \times AR_1$ were increased by 40, 40, and 25.7%, respectively, compared with the control (Table 2).

3.12. Fruit Length

EBL concentration had a significant effect on fruit length ($p \leq 0.01$) (Table 3). The control produced fruits with a mean length of 135 μM, while those treated with 0.5 and 0.75 mm reached 155.1 mm and 160 mm, corresponding to 14.9% and 18.5% increases. The interaction effect was not significant (Figure 7A).

3.13. Fruit Width

Both EBL concentration ($p \leq 0.05$) and application frequency ($p \leq 0.05$) had significant effects on fruit width. The control plants produced fruits 55.5 cm wide, while the 0.5 and 0.75 μM treatments increased fruit width to 58.5 cm and 62.6 cm, representing 5.4% and 12.8% gains, respectively (Figure 7B). Spraying twice (AR_2) produced fruits that were 7.6% wider than those from a single application (Figure 7C).

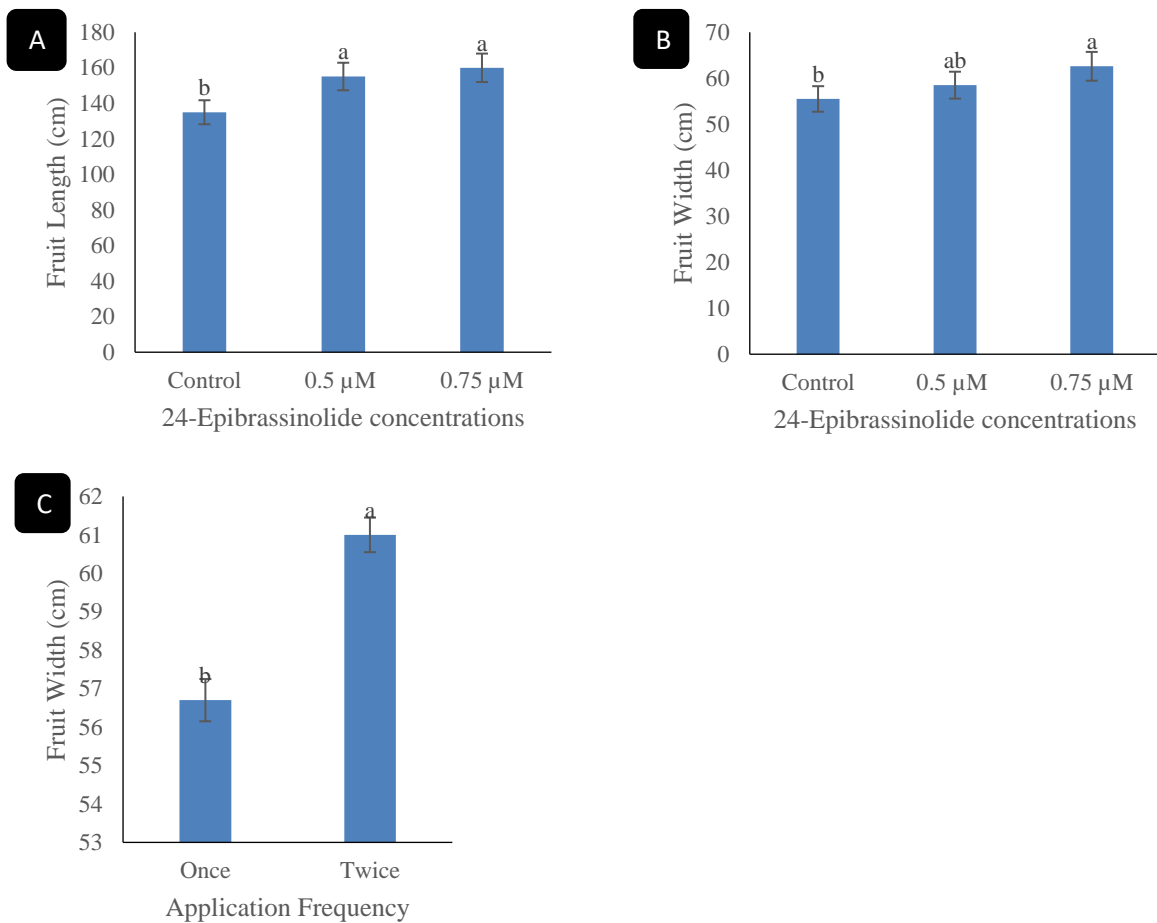


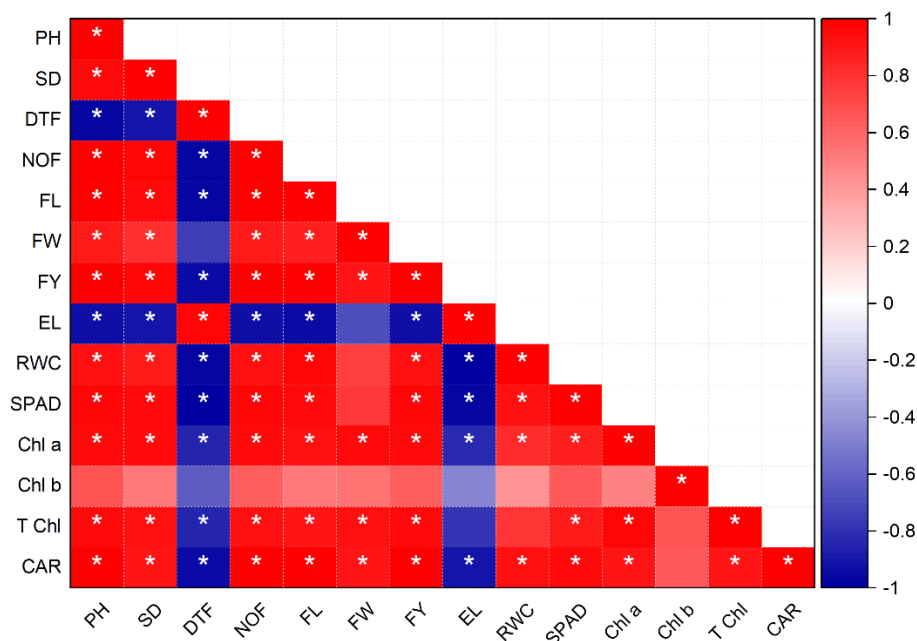
Figure 7A-C- Effect of EBL concentrations on fruit length (A), fruit width (B), and application repetition (C) in eggplant

3.14. Fruit Yield

Fruit yield was highly responsive to brassinosteroid concentration, application frequency, and their interaction ($p \leq 0.01$) (Table 3). The $\text{BR}_2 \times \text{AR}_2$, $\text{BR}_2 \times \text{AR}_1$, $\text{BR}_1 \times \text{AR}_2$, and $\text{BR}_1 \times \text{AR}_1$ combination produced the highest yield, equivalent to a 4.3%, 15.6%, 8.10% and 10.52% increase relative to the control (Table 2).

3.15. Correlation Analysis

The correlation matrix (Figure 8) demonstrates the relationships among growth, yield, and physiological parameters under varying brassinosteroid concentrations and spraying intervals. Strong positive correlations were observed among plant height, stem diameter, number of fruits, fruit length, fruit width, and fruit yield. This indicates that more vigorous vegetative growth contributes directly to higher reproductive performance and yield. Similarly, SPAD readings, chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids showed significant positive correlations, suggesting that pigment accumulation and leaf greenness are closely linked with enhanced photosynthetic activity. Relative water content (RWC) also exhibited strong positive associations with yield-related traits, underscoring the role of improved hydration in sustaining growth and fruit production. In contrast, electrolyte leakage (EL) displayed a pronounced negative correlation with most parameters, especially RWC, pigments, and yield. This relationship indicates that greater membrane damage is detrimental to water status, photosynthetic capacity, and ultimately productivity. Days to flowering (DTF) was also negatively correlated with yield and fruit number, highlighting that earlier flowering promoted by BR application is advantageous for fruit set and harvest potential. Many of these relationships, particularly between RWC and yield or EL and physiological traits, were statistically significant ($p \leq 0.05$), reinforcing their biological importance.



* $p \leq 0.05$

Figure 8. Pearson correlation analysis of varying EBL concentrations and spraying intervals on growth and physiology of eggplant. The variables are the following: plant height (PH), stem diameter (SD), days to flowering (DTF), number of fruits (NOF), fruit length (FL), fruit width (FW), fruit yield (FY), electrolyte leakage (EL), Relative water content (RWC), SPAD, chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (T Chl) and carotenoid (Car).

3.16. The heatmap with hierarchical clustering

The heatmap with hierarchical clustering (Figure 9) provides a clear visual representation of treatment responses. Treatments with the highest BR concentration, applied twice ($BR_2 \times AR_2$), formed a distinct cluster, showing consistently high values for plant height, fruit number, yield, SPAD, and pigment content. In contrast, the control treatments ($B_0 \times A_1$ and $B_0 \times A_2$), grouped at the bottom, exhibited low growth, reduced pigment levels, and high electrolyte leakage. The clustering of yield-related traits with physiological parameters indicates that improvements in chlorophyll, carotenoids, RWC, and SPAD directly contribute to enhanced yield performance.

3.17. Principal Component Analysis (PCA)

The first principal component (PC1) accounts for 89.13% of the total variance, while the second principal component (PC2) explains 5.45%. Together, they capture over 94% of the total variation, indicating that the treatments strongly structure the dataset and that the main differences among treatments are effectively summarized by these two components. Most growth and physiological traits—including plant height (PH), stem diameter (SD), fruit number (NOF), fruit length (FL), fruit width (FW), fruit yield (FY), relative water content (RWC), SPAD, chlorophyll *a*, total chlorophyll, and carotenoids (Car) (Figure 10), cluster positively on the right side of PC1. This suggests that BR application, particularly at higher concentrations, strongly promotes these traits. Chlorophyll *b* (Chl *b*) also loads positively, but more along PC2, indicating it contributes additional variability beyond that captured by PC1. Electrolyte leakage (EL) and days to flowering (DTF) load negatively on PC1, aligning with untreated or low-BR treatments. This confirms that higher EBL concentrations help reduce membrane damage and accelerate flowering in eggplant. Treatments with higher BR concentrations ($0.75 \mu\text{M}$) and repeated applications ($B_2 \times A_2$, $B_2 \times A_1$) are positioned far to the right on PC1, closely associated with yield-related and pigment traits. In contrast, control treatments ($B_0 \times A_1$, $B_0 \times A_2$) cluster to the left, aligning with EL and DTF, which represent stress and delayed flowering. Intermediate treatments ($0.5 \mu\text{M}$ EBL, applied once or twice) are located between the extremes, reflecting moderate improvements in growth and physiology. Overall, the PCA demonstrates that $0.75 \mu\text{M}$ EBL treatment, mainly when applied twice, induces a distinct physiological and agronomic profile characterized by enhanced growth, higher pigment levels, improved water retention, and greater yield. Untreated plants, on the other hand, cluster with stress-related traits such as higher electrolyte leakage and

delayed flowering. This separation emphasizes that the BR application not only promotes growth but also alleviates physiological stress, leading to a comprehensive improvement in eggplant performance.

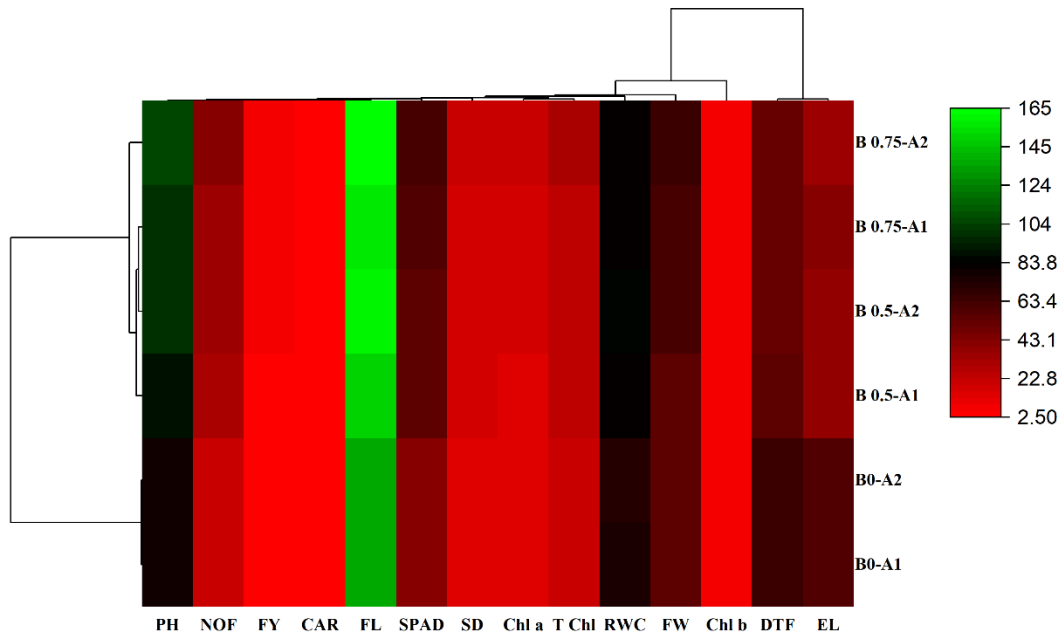


Figure 9. Heatmap diagram of treatments clustering in rows and measured traits in columns with complete linkage clustering methods and the Pearson method for distance measurement of varying EBL concentrations and spraying intervals on the growth and physiology of eggplant. The variables are: Plant Height (PH), Stem Diameter (SD), days to flowering (DTF), number of fruits (NOF), fruit length (FL), fruit width (FW), fruit yield (FY), electrolyte leakage (EL), relative water content (RWC), SPAD, chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (T Chl) and carotenoid (Car).

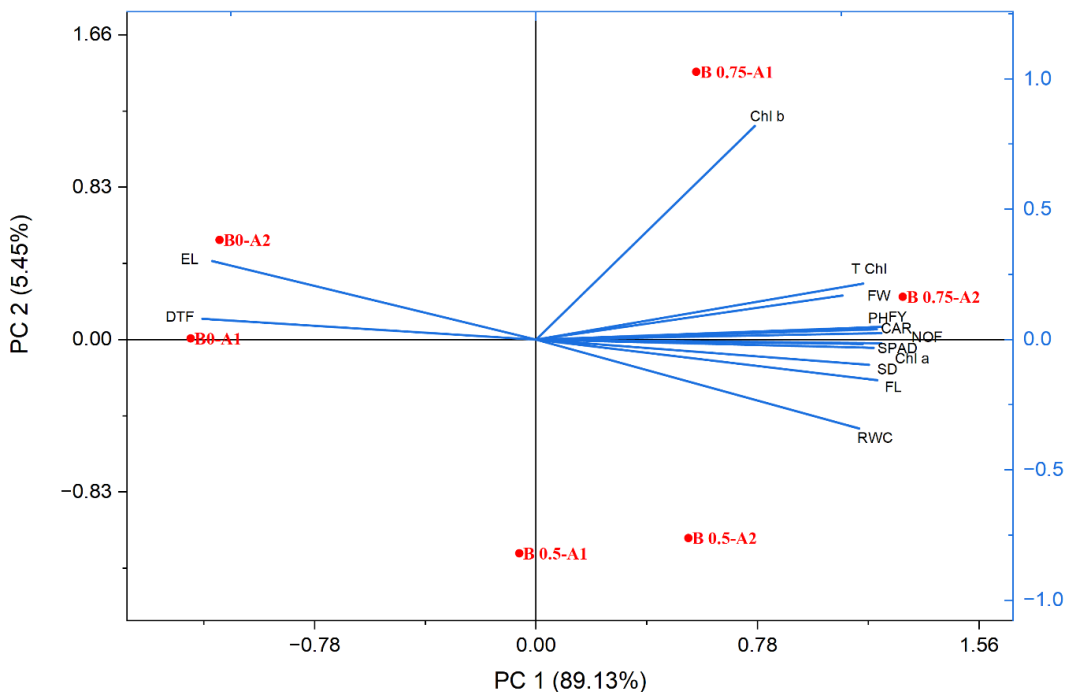


Figure 10. PCA biplot analysis of varying EBL concentrations and spraying intervals on the growth and physiology of eggplant. The variables are: plant height (PH), stem diameter (SD), days to flowering (DTF), number of fruits (NOF), fruit length (FL), fruit width (FW), Fruit Yield (FY), electrolyte leakage (EL), relative water content (RWC), SPAD, chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (T Chl) and carotenoid (Car).

4. Discussion

Epibrassinolide (EBL) are well known for their role in improving stress resilience in plants. In this study, EBL treatment significantly improved electrolyte leakage and relative water content (RWC), indicating that these hormones enhance membrane stability and water retention. The reduction in electrolyte leakage is a strong indication of improved membrane integrity, suggesting that EBLs help to prevent cellular damage under stressful conditions (Yusuf et al., 2016). Similar findings have been observed in vegetable crops such as tomatoes and peppers, where EBL application reduced drought-induced damage and improved water use efficiency (Ahammed et al., 2020, Ahammed et al., 2015). The positive impact of EBLs on stress tolerance is critical, especially in regions prone to water stress or climate variability, as it can help maintain yield stability. EBL effectively reduced oxidative damage by enhancing antioxidant enzyme activity, thereby stabilizing membranes and maintaining cellular homeostasis (Khosravifar et al., 2025). In the present study, it was also found that plants treated with EBL had less electrical leakage, and the relative leaf water content in plants treated with 0.75 μM EBL significantly increased compared to the control treatment, indicating cell membrane stability and reduced water loss in eggplant plants treated with EBL. Also, other positive responses of EBL on plants are mainly due to the preservation of plasma membrane integrity and fluidity, as well as the proper functioning of ion channels, which are supported by the accumulation of compatible solutes stimulated by EBL (Gupta et al., 2017, Wani et al., 2019).

The significant increases in chlorophyll content, including both chlorophyll a and chlorophyll b, observed in this study suggest that EBLs enhance the plant's photosynthetic capacity. EBLs are known to stimulate chlorophyll biosynthesis, and the increase in chlorophyll concentration is indicative of improved light-harvesting efficiency (Yusuf et al., 2016). This enhancement in photosynthesis can directly contribute to increased biomass production and fruit yield. In tomatoes and peppers, similar increases in chlorophyll content were observed following EBL treatment, with corresponding increases in photosynthetic efficiency (Ahammed et al., 2015). The role of EBLs in enhancing photosynthetic pigment synthesis is crucial for improving overall plant health and productivity, especially in high-light environments where photosynthesis is the primary driver of growth. Researchers found that 50-200 nM EBL increased chlorophyll content in eggplant, whereas 400 nM EBL had no significant effect (Wu et al., 2012). Ogwen et al. (Ogwen et al., 2008) suggest that the application of EBL protects PSII against overexcitation, perhaps by preventing loss of thylakoid membrane integrity. EBL application confers clear advantages in plants by enhancing key physiological processes related to photosynthesis and growth (Yang et al., 2019, Ma et al., 2020). EBL treatment increases CO_2 assimilation efficiency and improves the quantum yield of photosystem II (ΦPSII), thereby enhancing light use and carbon fixation. These improvements are accompanied by the upregulation of genes encoding both the small and large subunits of Rubisco, as well as other enzymes involved in the Calvin cycle, resulting in higher Rubisco activity (Xia et al., 2009). By stimulating the synthesis and activation of photosynthetic enzymes, EBL ultimately promotes stronger photosynthetic performance and overall growth in plants such as cucumber (Xia et al., 2009). It has also been reported that EBL application enhanced the accumulation of photosynthetic pigments, increased the rate of photosynthesis, and promoted stomatal aperture, while preserving the structural integrity of chloroplasts (Yang et al., 2019). These results are consistent with our results in this study, as it was shown in the present study that the use of 0.75 μM EBL caused an increase in photosynthetic pigments and greenness index of eggplant plants. The observed increase in carotenoid content following EBL treatment has significant implications for both the nutritional quality and stress tolerance of the plants. Carotenoids affect photoprotection and oxidative stress resistance, and their accumulation in response to EBLs may help protect the plant from photo-oxidative damage (Bajguz and Hayat, 2009). This is particularly important for vegetables such as tomatoes, carrots, and peppers, which are rich in carotenoids, including lycopene and beta-carotene. In vegetables such as tomatoes, studies have shown that EBLs not only increase carotenoid levels but also improve fruit color and flavor, which are essential for consumer acceptance (Meng et al., 2023). These benefits also extend to the plant's ability to cope with environmental stress, contributing to higher yield and quality under less-than-ideal growing conditions.

Brassinosteroids (BRs), a class of plant hormones, have garnered significant attention for their roles in regulating a wide range of physiological processes in plants. These have been shown to enhance vegetative growth, reproductive success, stress tolerance, and photosynthetic efficiency in a range of crops, including vegetables. They are well-documented for their ability to increase plant height by promoting cell elongation and division. The results from this study, in which plant height increased by up to 32.9% in response to a 0.75 μM EBL treatment, are consistent with findings across various vegetable crops. For instance, tomatoes treated with EBLs showed an increase in plant height of up to 25% (Hayat and Ahmad, 2010), and similar results have been reported in peppers and eggplants (Serna et al., 2012, Wakchaure et al., 2020). EBLs mediate these effects by promoting the expression of genes associated with cell wall loosening and auxin transport, which together enhance cell elongation. Additionally, the influence of application frequency was evident in this study, with plants sprayed twice being taller than those sprayed once. This is consistent with other studies, such as one on cucumbers, which demonstrated that repeated BR applications produced a more pronounced growth response than a single spray (Yang et al., 2019).

The repeated application of EBLs may increase the cumulative effect of the hormone on growth, leading to greater vegetative development.

The observed increase in stem diameter with EBL treatment is indicative of enhanced vascular development and cell wall thickening. EBLs have been shown to stimulate the production of lignin and other cell wall components, thereby strengthening plant tissues (Bajguz and Hayat, 2009). The results of this study, in which stem thickness increased by up to 30% with higher EBL concentrations, support this idea. Increased stem diameter is crucial as it indicates a stronger plant structure capable of supporting more substantial fruit development. Research on vegetables such as eggplant and tomato has also shown that EBLs significantly improve stem strength and diameter, enhancing the plant's ability to resist lodging and mechanical damage (Nazir et al., 2021). This is particularly valuable for crops grown in regions with strong winds or heavy fruit loads. Wu et al. (Wu et al., 2012) reported that Application of 50 and 100 nM EBL had a significant positive effect on plant height and root fresh mass, while only 100 nM EBL caused a marked induction of stem diameter and shoot fresh mass in eggplant. One of the most significant outcomes of EBL application was the reduction in days to flowering, with treated plants flowering earlier than control plants. This effect is consistent with the known role of EBL in regulating the transition from the vegetative to the reproductive phase of growth (Li and He, 2020). By modulating the expression of key genes involved in flower induction, such as those related to floral meristem development and flowering time regulation, EBLs can accelerate flowering (Guo et al., 2013). In other studies, tomatoes treated with EBLs flowered earlier, with a reduction in flowering time of up to 20% (Meng et al., 2023). Similarly, in peppers and eggplants, EBL applications have resulted in earlier flowering and fruiting, which could benefit by shortening the overall growth cycle (Ahmad et al., 2025). However, the application frequency did not significantly affect this trait in the present study, suggesting that a single EBL application at a higher concentration may be sufficient to accelerate flowering (Ali et al., 2022). Researchers found that EBL treatment accelerated the emergence of the first female flower and increased the overall number of female flowers along the main stem in monoecious cucumber plants. This enhancement coincided with elevated ethylene production, indicating that the action of EBL in cucumber may be mediated through stimulation of ethylene biosynthesis (Słomnicka et al., 2025). In a separate investigation, the external application of EBL was shown to affect flowering behavior and enhance yield in watermelon (Susila et al., 2010). Comparable responses have also been reported in cucumber, where exogenously applied BRs stimulated vegetative development and floral formation, ultimately leading to higher fruit production (Dhall and Singh, 2016, Yadav et al., 2022). Ali et al. (Ali et al., 2022) reported that the use of 2 μ M EBL increased the number of flowers in strawberry during three foliar spraying periods (100, 115, and 130 days of runner transplantation).

The increase in fruit number observed in this study is one of the most promising results for crop productivity. In the $B_2 \times AR_2$ treatment, fruit number increased by nearly 95% compared to the control. This is consistent with previous research on vegetables, in which tomatoes treated with BRs showed an increase in fruit set of up to 50%, particularly under controlled environmental conditions (Hayat et al., 2012). In cucumbers and peppers, EBLs also enhanced fruit set, with increases ranging from 30% to 60% (Serna et al., 2012, Wakchaure et al., 2020). The observed synergistic effect between EBL concentration and spray frequency is particularly noteworthy. This suggests that repeated applications of higher EBL concentrations may help to ensure sustained or enhanced reproductive output over time. This has practical implications for vegetable growers, as increasing the number of fruits per plant can lead to more efficient land use and higher overall yield. Moreover, the increased fruit number is likely due to improved flowering and fruit set, which EBLs influence through both direct effects on floral initiation and indirect effects on plant health and vigor. The improved pollination success and fruit retention in EBL-treated plants could also contribute to the higher fruit numbers observed. Beyond quantity, fruit quality is a critical determinant of marketable yield. In this study, significant increases in fruit length and fruit width were observed, with the $B_2 \times AR_2$ treatment producing the largest fruits. This is consistent with findings in other crops, where EBLs have been shown to enhance cell expansion and fruit development (Yang et al., 2019). For instance, tomato fruits treated with EBLs were larger, and eggplant fruits were more uniform in size and shape. Furthermore, EBLs can influence fruit nutritional quality, as evidenced by increased chlorophyll content and carotenoid levels in treated plants. Carotenoids are essential antioxidants and contribute to the color and flavor of fruits, particularly in crops like tomatoes and peppers. EBL treatment has been shown to enhance carotenoid biosynthesis in various crops, leading to more vibrant and nutritionally rich fruits (Bajguz and Hayat, 2009). EBL enhances fruit development and yield by increasing fruit size, weight, and overall production. Its positive effects occur under both normal and stress conditions, reflecting its growth-promoting function even in the absence of stress. EBL particularly enhances fruit transverse diameter, which contributes to higher productivity (Maia Júnior et al., 2022). Previous studies across different crops support this role; for example, in unstressed tomato plants, low concentrations of EBL were shown to reduce apical fruit rot while increasing fruit diameter and weight (Riboldi et al., 2019). These improvements were associated with altered calcium distribution within the fruit, promoting more favorable conditions for fruit growth and development.

Conclusion

This study demonstrates that the strategic application of 24-epibrassinolide increases eggplant performance through coordinated physiological and developmental modulation. Optimal EBL treatment significantly raised photosynthetic capacity by elevating chlorophyll and carotenoid content, while strengthening cellular membrane integrity and plant water status. These physiological changes translated into faster vegetative growth and earlier flowering, resulting in approximately a two-fold increase in yield compared to controls. The observed dose- and frequency-dependent responses indicate that dual application of 0.75 μM EBL optimizes metabolic activity and assimilate partitioning toward reproductive structures, positioning EBLs as effective biostimulants for sustainable Solanaceae crop intensification. Future research should clarify the molecular basis of these responses to support precision biostimulant use across genotypes and environments.

Data Availability Statement: The authors declare that data supporting the findings of this study are available in the article. If raw data files are required, they are available from the corresponding author upon reasonable request.

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

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