ISSN: 2690- 912X

Research Article

Journal of Genetic Engineering and Biotechnology Research

Regulatory Role of Plant-Based Biostimulants in Chlorophyll Biosynthesis and Endogenous Hormone Regulation in Plants

Abdullah Al Mamun*, Shabiha Tasbir Rahman and Salman Khan

Department of Biotechnology and Genetic Engineering, Faculty of Biological Science, Islamic University, Bangladesh

*Corresponding Author
Abdullah Al Mamun, Department of Biotechnology and Genetic Engineering, Faculty of Biological Science, Islamic University, Bangladesh.

Submitted: 2025, Mar 05; **Accepted:** 2025, Apr 28; **Published:** 2025, May 12

Citation: Al Mamun, A., Rahman, S. T., Khan, S. (2025). Regulatory Role of Plant-Based Biostimulants in Chlorophyll Biosynthesis and Endogenous Hormone Regulation in Plants. *J Gene Engg Bio Res, 7*(2), 01-05.

Abstract

Plant-based bio-stimulants offer a sustainable approach to enhancing plant growth by influencing physiological processes such as chlorophyll biosynthesis and hormone regulation. This study investigates the effects of aqueous leaf extracts from Justicia gendarussa, Osmunda regalis, and Senna occidentalis on chlorophyll content and endogenous hormone levels in rice (Oryza sativa) plants. Seeds were treated with varying extract concentrations (10%, 20%, and 50%) and analyzed after four weeks. Results showed a significant, dose-dependent increase in chlorophyll a and b content, with J. gendarussa at 100% concentration yielding the highest chlorophyll a (7.21 mg/g) and chlorophyll b (3.23 mg/g) levels. Endogenous hormone levels were also elevated, with J. gendarussa treatment resulting in auxin (12.53 µg/g), gibberellin (9.63 µg/g), and cytokinin (6.57 µg/g) concentrations. ANOVA confirmed the statistical significance of both plant type and extract concentration on chlorophyll and hormone levels ($p < 0.05$). These findings highlight the potential of these natural extracts, particularly J. gendarussa, as eco-friendly bio-stimulants for improving rice plant physiology and promoting sustainable agriculture.

Keywords: Plant-Based Bio-Stimulants, Justicia Gendarussa, Chlorophyll Biosynthesis, Phytohormone, Sustainable Agriculture

1. Introduction

Plants are highly sensitive to their environmental conditions, and their growth and development are significantly influenced by various factors, including light, temperature, water availability, and soil nutrients [1,2]. Among these, the regulation of chlorophyll biosynthesis and the modulation of endogenous hormones are crucial processes that dictate plant health, growth, and overall productivity [3]. Chlorophyll, the green pigment responsible for photosynthesis, is vital for the plant's energy production, while plant hormones, such as auxins, cytokinin, and gibberellins, are key regulators of developmental processes, including cell division, elongation, and differentiation [4,5]. Recent advancements in plant biotechnology have led to the development of bio-stimulants, substances or microorganisms that enhance plant growth, yield, and stress tolerance without being classified as fertilizers or pesticide [6,7]. Plant-based bio-stimulants, derived from natural sources such as seaweed, plant extracts, and microbial formulations, have gained significant attention due to their ability to stimulate various physiological processes in plants, including chlorophyll biosynthesis and hormonal regulation [8,9]. The impact of plant-based bio-stimulants on chlorophyll development and hormone regulation remains an area of active research. Several studies suggest that these bio-stimulants can enhance chlorophyll content, thereby improving photosynthetic efficiency, and they may also modulate the levels of key plant hormones, further influencing growth and stress responses [10,11]. However, the exact molecular mechanisms underlying these effects are still not fully understood, particularly in relation to the interplay between bio-stimulants, chlorophyll biosynthesis, and hormone signaling pathways. This study aims to explore the regulatory role of plant-based bio-stimulants in chlorophyll development and the concentration of endogenous hormones in plants. By examining the effects of different plant extracts on chlorophyll biosynthesis and hormone regulation under varying growth conditions, this research seeks to contribute to a deeper understanding of how bio-stimulants can be used to optimize plant growth and productivity, particularly under stress conditions such as drought or nutrient deficiency [12].

2. Materials and Methods

2.1. Sample Collection

The plant extracts were prepared from the leaves of three species: J. gendarussa, O. regalis, and S. occidentalis, all collected from various locations within Jhenaidah District, Bangladesh. J. gendarussa was sourced from Garagang, near the local market and surrounding agricultural fields. O. regalis was gathered from Vatoi Bazar, situated near riverbanks and wetland areas. S. occidentalis was collected from Sheikhpara, on the outskirts of the village, in open fields. After collection, the leaves were thoroughly washed,

air-dried, and ground into fine powder. The extract was prepared by boiling the powdered leaves in double-distilled water, followed by filtration, and storage at 4°C until further use.

2.2. Plant Extract Preparation

Aqueous plant extracts were prepared using a standard extraction procedure. To prepare the extract, 10 grams of dried leaves from each species were boiled in 100 mL of distilled water for 15-20 minutes. The resulting solution was filtered using Whatman No. 1 filter paper to remove any solid residues. The filtrate was stored at 4°C for later use. To create different concentrations of the plant extracts, the following dilution series was prepared: for a 10% concentration, 10 mL of extract was mixed with 90 mL of distilled water; for a 20% concentration, 20 mL of extract was mixed with 80 mL of distilled water; and for a 50% concentration, 50 mL of extract was combined with 50 mL of distilled water.

2.3. Seed Preparation

Prior to experimentation, the seeds underwent surface sterilization to eliminate potential contaminants. Initially, the seeds were soaked in 70% ethanol for 1 minute to remove surface impurities. The ethanol was drained, and the seeds were then treated with 3% hydrogen peroxide for 10 minutes to sterilize any remaining microbial contaminants. After this, the seeds were rinsed five times with sterile distilled water to remove any residual chemicals. The sterilized seeds were used immediately to ensure sterility and prevent contamination.

2.4. Plant Extract Effect on Chlorophyll Content

To assess the impact of plant extracts on chlorophyll content, fully expanded, mature leaves (5 g) from treated rice plants were harvested after 4 weeks. The leaves were ground in a mortar and pestle with 30 mL of 80% acetone, filtered, and diluted to a final volume of 50 mL with additional 80% acetone. Chlorophyll content was determined by measuring absorbance at 663 nm and 645 nm using a spectrophotometer for chlorophyll a and b, respectively. The concentrations of chlorophyll a and b were calculated using the following equations based on the absorbance values:

Chlorophyll a (mg/L) = (12.7 × A663) − (2.69 × A645)

Chlorophyll b (mg/L) = (22.9 × A645) − (4.68 × A663)

Where:

A663 = Absorbance at 663 nm (for chlorophyll a)

A645 = Absorbance at 645 nm (for chlorophyll b)

2.5. Plant Extract on Plant Hormonal Concentration

To analyze the effect of plant extracts on plant hormones, 5 g of shoot tissue from the 4-week treated rice plants was ground with 10 mL of 80% acetone, then filtered and diluted to a final volume of 25 mL. The hormonal concentrations were measured by spectrophotometry at A280 nm for auxins, A254 nm for gibberellins, and A270 nm for cytokinin. One-way ANOVA was used to analyze the hormone levels across the treatments. The concentrations were converted to ng/g using the following formula:

$$Concentration\ (ng/g) = \frac{Concentration\ (ng/mL)\ x\ (\ Volume\ (mL\ of\ extract)}{Weight\ of\ the\ shoot\ sample\ (g)}$$

3. Results

3.1. Measurement of Chlorophyll Content on Plant Extract Treatment

The effect of different plant extracts and their concentrations on chlorophyll content was evaluated using the quantified levels of chlorophyll a and b, as presented in Table 1. The findings revealed a notable increase in chlorophyll concentrations with increasing doses of *J. gendarussa*, O. *regalis*, and S. *occidentalis* extracts, indicating a dose-dependent stimulatory effect on chlorophyll biosynthesis. Among all treatments, *J. gendarussa* extract at 100% concentration recorded the highest chlorophyll accumulation, with 15.49 mg/L of chlorophyll a and 20.14 mg/L of chlorophyll b. Similarly, O. *regalis* at 100% concentration showed elevated chlorophyll levels, registering 14.01 mg/L (chlorophyll a) and 19.38 mg/L (chlorophyll b). The extract from S. *occidentalis* also enhanced chlorophyll content, albeit to a slightly lower extent than the other two, with 12.75 mg/L of chlorophyll a and 17.65 mg/L of chlorophyll b at full concentration. In contrast, the control group exhibited the lowest chlorophyll content (1.84 mg/L for chlorophyll a and 1.95 mg/L for chlorophyll b). ANOVA analysis confirmed significant effects of the plant extract type ($p < 0.01$) and concentration ($p < 0.001$) on chlorophyll synthesis, with an F-value of 50.70 for the plant factor and 183.07 for concentration (Table S9). The interaction between plant extract and concentration also had a significant impact ($p < 0.01$), with an F-value of 20.95. However, the residual variation was relatively low, confirming that the model's explanatory power was high ($p > 0.05$). This analysis supports the conclusion that both the type of plant extract and its concentration significantly influenced chlorophyll content, and the interaction between these factors was also statistically significant (Figure 1).

Plant Extract	Concentration (%)	Chlorophyll a (mg/L)	Chlorophyll b (mg/L)
J. gendarussa	20	2.84	3.77
J. gendarussa	50	8.09	9.73
J. gendarussa	100	15.49	20.14
O. regalis	20	3.44	4.87
O. regalis	50	9.24	12.37
O. regalis	100	14.01	19.38
S. occidentalis	20	3.06	3.91
S. occidentalis	50	7.45	11.06
S. occidentalis	100	12.75	17.65
Control	100	1.84	1.95

Table 1: Overall Data of Chlorophyll Content

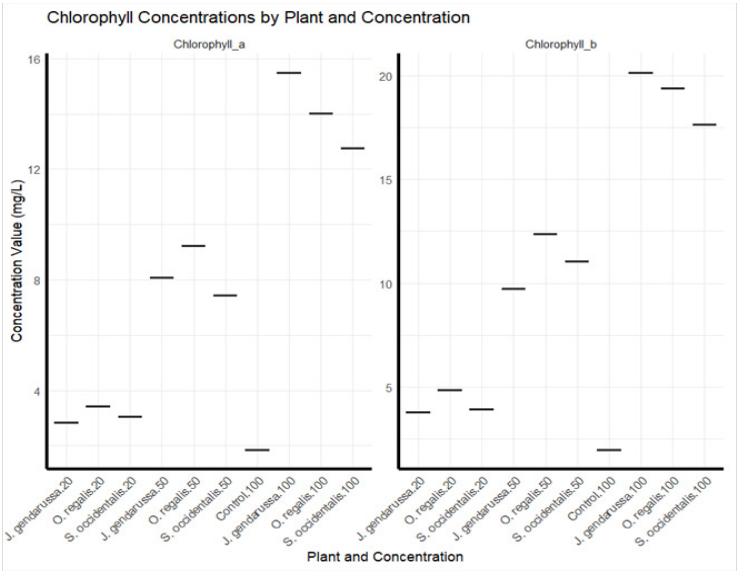

Figure 1: Chlorophyll Concentrations in Different Plant Species at Varying Concentration Levels. Boxplot showing the concentration of Chlorophyll a and Chlorophyll b in different plant species (*J. gendarussa, O. regalis, S. occidentalis*, and Control) at concentrations of 20%, 50%, and 100%. The data illustrate the significant differences in chlorophyll content across plants and concentrations. Statistical significance was determined using ANOVA, with p-values indicating differences between treatment groups

3.2. Exploration of Plant Extract on Plant Hormonal Concentration

The hormonal concentrations, including auxins, gibberellins, and cytokinin, were measured for various plant extracts at different concentrations, as summarized in Table 2. The results indicated that both the plant type and concentration significantly influenced the concentrations of auxins, gibberellins, and cytokinin, though the interaction between these two factors was less pronounced. For auxins, *J. gendarussa* at 100% concentration showed the highest levels (46.5 ng/g), followed by *O. regalis* (52.1 ng/g) at the same concentration (Figure 2). On the other hand, *S. occidentalis* demonstrated the lowest auxin concentration at all concentrations, with 26.3 ng/g at 100% concentration. The control group exhibited 14.8 ng/g of auxins. ANOVA results for auxins indicated that both the plant extract type ($p < 0.0255$) and concentration ($p < 0.0119$) had statistically significant effects, with the interaction between plant extract and concentration not being significant ($p < 0.4374$). Similarly, for gibberellins, *J. gendarussa* at 100% concentration showed the highest value (36.7 ng/g), followed by *O. regalis* at 100% concentration (41.3 ng/g). The lowest gibberellin levels were observed in *S. occidentalis*, particularly at 20% concentration (9.2 ng/g). The control had 12.4 ng/g of gibberellins. The ANOVA results for gibberellins indicated significant effects of both plant type ($p < 0.0355$) and concentration ($p < 0.0205$), with no significant interaction between the two factors ($p < 0.5712$). As for cytokinin, J. gendarussa at 100% concentration showed the highest value (15.8 ng/g), with *O. regalis* and *S. occidentalis* also showing increasing cytokinin concentrations at higher concentrations of their extracts. The control group exhibited a cytokinin concentration of 6.9 ng/g. ANOVA analysis revealed that both the plant type ($p < 0.00934$) and concentration ($p < 0.01609$) significantly affected cytokinin concentrations, while the interaction between plant extract and concentration was not significant ($p < 0.66029$).

Plant Extract	Concentration (%)	Auxins (ng/g)	Gibberellins (ng/g)	Cytokinin (ng/g)
J. gendarussa	20%	17.2	13.5	8.6
J. gendarussa	50%	36.8	30.2	13.4
J. gendarussa	100%	46.5	36.7	15.8
O. regalis	20%	31.5	26.2	16.4
O. regalis	50%	41.7	36.5	18.9
O. regalis	100%	52.1	41.3	20.7
S. occidentalis	20%	11.4	9.2	5.6
S. occidentalis	50%	21.5	16.1	9.8
S. occidentalis	100%	26.3	21.7	11.6
Control	100%	14.8	12.4	6.9

Table 2: Overall Data of Plant Hormonal Concentration Indicating the Effect of Plant Extract

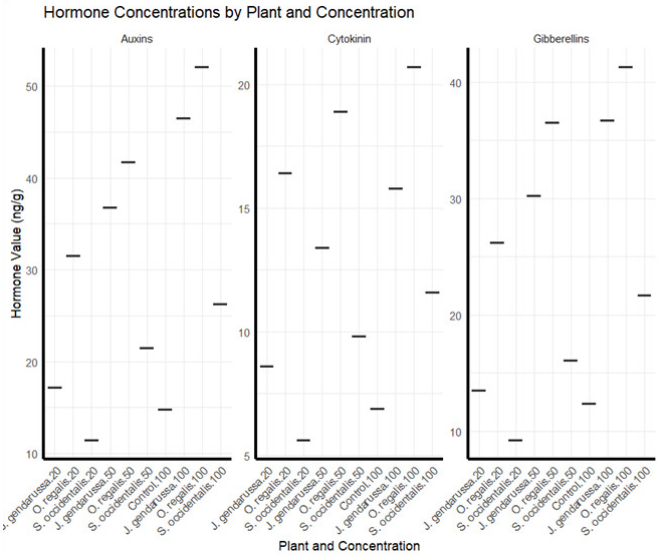

Figure 2: Hormone Concentrations in Different Plant Species at Varying Concentration Levels. Boxplot showing the concentration of Auxins, Gibberellins, and Cytokinin in different plant species (*J. gendarussa, O. regalis, S. occidentalis*, and Control) at concentrations of 20%, 50%, and 100%

4. Discussion

4.1. Plant Extracts Effects on Chlorophyll Content

The significant increase in chlorophyll a and b levels observed in this study suggests that the applied plant extracts positively influenced photosynthetic pigment synthesis, likely by enhancing nutrient uptake and reducing oxidative stress. Notably, the presence of phenolic compounds in *J. gendarussa* may have contributed to this increase by upregulating genes involved in tetrapyrrole metabolism, a key pathway in chlorophyll biosynthesis. These findings are in agreement with earlier research, such as the study by which demonstrated that various plant extracts applied to *Callistemon viminalis* cuttings improved chlorophyll levels [12]. The bioactive compounds present in these extracts are believed to either stimulate chlorophyll biosynthesis or protect existing chlorophyll molecules from degradation due to oxidative stress. Among the three plant species tested, *J. gendarussa* consistently showed the highest chlorophyll content, followed by *O. regalis* and *S. occidentalis,* with all showing dose-dependent effects. This suggests that higher concentrations of plant-based bio-stimulants may more effectively enhance photosynthetic efficiency and overall plant vigor. Further research, including transcriptomic and proteomic analyses, is recommended to identify the specific genes and chlorophyll-binding proteins involved in this enhancement and to better understand the underlying molecular mechanisms.

4.2 Plant Extracts Effects on Hormonal Concentration

The application of plant extracts also led to notable increases in the concentrations of key endogenous hormones, including auxins, gibberellins, and cytokinins. This hormonal modulation likely plays a role in promoting plant growth and enhancing stress resilience. Among the treatments, *J. gendarussa* and *O. regalis* showed the strongest stimulatory effects on auxin and gibberellin levels, while *S. occidentalis* had relatively moderate effects. The elevated auxin concentrations may promote root and shoot elongation, while increased gibberellins could enhance stem elongation and seed germination. Cytokinins, which influence cell division and delay

leaf senescence, were also significantly elevated, particularly in treatments with higher extract concentrations. These hormonal shifts suggest that bioactive compounds in the extracts may interact with hormonal biosynthesis or signaling pathways, amplifying growth responses. Although the interaction between plant type and extract concentration was not statistically significant for hormones, both factors independently showed strong effects, emphasizing their importance in optimizing plant growth regulators. Further studies should investigate the molecular pathways triggered by these plant-based bio-stimulants, including potential cross-talk among hormone signaling networks. Insights into these mechanisms will be essential for developing sustainable bio-stimulant formulations for use in stress-prone agricultural environments.

5. Conclusions

This study demonstrates that plant-based bio-stimulants derived from *Justicia gendarussa, Osmunda regalis, and Senna occidentalis* significantly enhance chlorophyll content and regulate endogenous hormone levels in rice plants. The effect was concentration-dependent, with 100% extracts showing the highest improvement in both chlorophyll a and b levels, as well as increased concentrations of auxins, gibberellins, and cytokinin. *J. gendarussa* was particularly effective in promoting chlorophyll biosynthesis and hormonal modulation. These findings suggest that such natural plant extracts hold great potential as eco-friendly alternatives to chemical enhancers, contributing to sustainable agriculture by improving plant vigor and stress tolerance. Further molecular studies are needed to identify the precise pathways involved and to validate their use across different crop species.

Acknowledgment

The authors wish to thank the department of Biotechnology and Genetic Engineering for supporting this research.

Ethics Declarations

This article does not include any studies by any of the authors that used human or animal participants. All authors are conscious and accept responsibility for the manuscript. No part of the manuscript content has been published or accepted for publication elsewhere.

Funding

There was no fund available.

Conflict of Interest

The authors declare no competing interests.

Data Availability

The corresponding author Abdullah Al Mamun is responsible for all data and materials.

Code Availability

There was no code available.

Authors Contributions

AAM comprehended and planned the study, carried out the analysis, wrote the manuscript; and prepared the graphs and illustrations; STR and SK contributed to the critical revision of the manuscript and wrote the manuscript; AAM supervised the whole work, and all authors approved the final manuscript.

References

1. Balliu A, Zheng Y, Sallaku G, Fernández JA, Gruda NS, Tuzel Y (2021) Environmental and cultivation factors affect the morphology, architecture and performance of root systems in soilless grown plants. *Horticul 7*(8):243.
2. Pant P, Pandey S, Dall'Acqua S (2021) The influence of environmental conditions on secondary metabolites in medicinal plants: A literature review. *Chem Biod* (11):e2100345.
3. Ahsan SM, Injamum-Ul-Hoque M, Shaffique S, Ayoobi A, Rahman MA, Rahman MM, Choi HW (2024) Illuminating Cannabis sativa L.: the power of light in enhancing C. sativa growth and secondary metabolite production. *Plants 13*(19):2774.
4. Hudeček M, Nožková V, Plíhalová L, Plíhal O (2023) *Plant hormone cytokinin at the crossroads of stress priming and control of photosynthesis*. F Plant Sci 13:1103088.
5. EL Sabagh A, Islam MS, Hossain A, Iqbal MA, Mubeen M, Waleed M, Reginato M, Battaglia M, Ahmed S, Rehman A, Arif M (2022) Phytohormones as growth regulators during abiotic stress tolerance in plants. *F in Agro 4*:765068.
6. Singh AL, Singh S, Kurella A, Verma A, Mahatama MK, Venkatesh I (2022) Plant bio-stimulants, their functions and use in enhancing stress tolerance in oilseeds. In *New and future developments microbial biotech bioengpp*. 239-259. Elsevier.
7. Ghosh P, Pal H (2023) Biotechnological Attributes of Bio-stimulants for Relieving Abiotic Stress. In *Climate-Resilient Agriculture, Vol 2: Agro-Biotechnological Advancement for Crop Production pp*. 677-688. Cham: Springer International Publishing.
8. Baltazar M, Correia S, Guinan KJ, Sujeeth N, Bragança R, Gonçalves B (2021) Recent advances in the molecular effects of biostimulants in plants: An overview. *Biomol 11*(8):1096.
9. Shahrajabian MH, Chaski C, Polyzos N, Petropoulos SA (2021) Biostimulants application: A low input cropping management tool for sustainable farming of vegetables. *Biomol 11*(5):698.
10. Bhupenchandra I, Chongtham SK, Devi EL, Choudhary AK, Salam MD, Sahoo MR, Bhutia TL, Devi SH, Thounaojam AS, Behera C, MN H (2022) Role of biostimulants in mitigating the effects of climate change on crop performance. *F Plant Sci 13*:967665.
11. Rai N, Rai SP, Sarma BK (2021) Prospects for abiotic stress tolerance in crops utilizing phyto-and bio-stimulants. *F in Sustain Food Syste 5*:754853.
12. Vashishth DS, Bachheti A, Bachheti RK, Husen A (2023) Allelopathic effect of Callistemon viminalis's leaves extract on weeds, soil features, and growth performance of wheat and chickpea plants. *J Plant Inter 18*(1):2248172.