Optimizing *Gardenia jasminoides* Micropropagation through Cytokinin 2iP and LED Light Spectrum Modulation

Fadhil J. Kadhim [1*], Makki N. Nayyef [1], Mohammad J. Hussain [1]

Abstract

Gardenia jasminoides is an ornamental and medicinal plant of high economic value, yet its large-scale propagation is often hindered by low multiplication efficiency and limited rooting in conventional methods. This study was conducted at the Plant Tissue Culture Laboratory, Department of Horticulture and Landscape Engineering, College of Agriculture, Al-Qasim Green University, between September 2023 and April 2024, to evaluate the effects of cytokinin 2-isopentenyl adenine (2iP) concentrations and light spectra on in vitro growth performance. A factorial experiment based on a completely randomized design (CRD) with five replicates per treatment was employed. Two-node micro-cuttings derived from ex vivo multiplied branches were cultured on Murashige and Skoog (MS) medium supplemented with 0.1 mg L^{-1} NAA, 30 mg L^{-1} adenine sulfate, and four levels of 2iP (0, 0.1, 0.2, and 0.3 mg L^{-1}). Cultures were exposed to four light sources: standard white fluorescent, blue LED, red LED, and white LED, for four weeks. Growth responses were assessed based on shoot number, shoot length, leaf number, shoot fresh and dry weights, and chlorophyll content. Results revealed that increasing 2iP concentration up to 0.2 mg L^{-1} significantly enhanced all morphological traits, after which the effect plateaued. Among light treatments, blue LED illumination produced the highest means across all parameters, while white fluorescent light yielded the lowest. The combination of 0.2 mg L^{-1} 2iP and blue LED light provided the most favorable conditions for vigorous shoot proliferation and chlorophyll accumulation, offering a cost-effective and energy-efficient protocol for commercial micropropagation of *G. jasminoides*.

Keywords: *Gardenia jasminoides,* 2iP, cytokinin, LED light, in vitro propagation

Significance | This study establishes an energy-efficient micropropagation protocol enhancing Gardenia jasminoides growth and chlorophyll content using 0.2 mg L^{-1} 2iP under blue LED.

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1. Introduction

Gardenia jasminoides, a member of the Rubiaceae family (Coffee family), is an evergreen shrub native to China and Japan. The plant is highly valued for its ornamental, aromatic, and industrial applications. It is widely cultivated in gardens and as a potted ornamental due to its glossy foliage and fragrant white flowers. Beyond its aesthetic appeal, *Gardenia jasminoides* also serves as an important source of natural products. An expensive essential oil is extracted from its flowers, while its fruits yield natural dyes traditionally used to impart color to foods and textiles (Al-Dajawi, 2006).

Over the past two decades, plant tissue culture techniques have been increasingly employed to produce high-quality, disease-free, and genetically uniform planting materials. These biotechnological methods offer several advantages over conventional propagation techniques, particularly for vegetatively propagated species. Tissue culture enables mass propagation from a single explant, ensuring

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clonal fidelity to the mother plant and facilitating year-round production regardless of seasonal constraints. The controlled environment of in vitro systems allows for the regulation of growth factors, including growth regulators, light, and nutrients, thereby ensuring consistency and efficiency in plant multiplication. Cytokinins, one of the key groups of plant growth regulators, play a crucial role in tissue culture by promoting cell division, shoot initiation, and axillary bud proliferation. Numerous studies have reported that both the type and concentration of cytokinin used in the culture medium markedly influence shoot multiplication. Among the cytokinins recently gaining attention is 2-isopentenyl adenine (2iP), known for its effectiveness in inducing vegetative multiplication. The action of cytokinins, including 2iP, is closely linked to their molecular role in cell physiology. These compounds participate in cell division and organogenesis through their involvement in nucleic acid metabolism. Specifically, cytokinins form part of transfer ribonucleic acid (tRNA), facilitating the linkage of tRNA with messenger RNA (mRNA) during protein synthesis. tRNA molecules lacking the isopentenyl side chain of adenine are inactive; the addition of 2iP reactivates them, thus enhancing protein formation and cell division. Moreover, cytokinins regulate gene expression at the transcriptional level. A slight increase in endogenous cytokinin concentration has been associated with a pronounced rise in mRNA levels, highlighting their critical role in regulating genetic and physiological processes (Mohammed & Younis, 1991).

In addition to growth regulators, light quality is one of the most influential environmental factors affecting plant morphogenesis in tissue culture. Variations in light spectrum, intensity, and duration can significantly influence plant growth, physiology, and development. Different wavelengths stimulate or inhibit specific physiological responses, guiding plant scientists to investigate light quality as a means of modulating in vitro growth. Since light is essential for photosynthesis, photomorphogenesis, and chlorophyll synthesis, the spectral composition of light sources can profoundly impact shoot elongation, leaf size, pigment formation, and overall plant morphology (Batista et al., 2018).

Optimal in vitro plant growth requires precise control of environmental factors, including temperature, humidity, carbon dioxide levels, and especially light quality (Shin et al., 2008). Both insufficient and excessive light can be detrimental—low light intensity restricts photosynthetic efficiency and biomass accumulation, while excessive light may induce stress and inhibit balanced growth (Yen et al., 2011). Furthermore, light spectrum influences several morphological traits, such as stem elongation, leaf expansion, and chlorophyll concentration (Nhut & Nam, 2010). Traditionally, fluorescent lamps with a light intensity ranging from 1,000 to 10,000 lux have been the most common light sources used in plant tissue culture laboratories (Li et al., 2013). The light emitted by these lamps spans a wavelength range of 350–750 nanometers; however, not all wavelengths contribute equally to plant growth and morphogenesis (Kim et al., 2004). Moreover, fluorescent lamps have notable limitations—they consume considerable electrical energy, generate excess heat, and increase operational costs (Folta & Childers, 2008).

In recent years, Light Emitting Diodes (LEDs) have emerged as a promising alternative for plant tissue culture lighting. LEDs offer several advantages, including small size, energy efficiency, spectral specificity, long lifespan (50,000–100,000 hours), and minimal heat emission (Yeh & Chung, 2009). The ability to tailor light spectra precisely to plant requirements has made LED technology particularly attractive for controlled-environment agriculture and in vitro propagation systems.

Despite the growing global research on optimizing plant tissue culture through hormonal and light adjustments, there remains a scarcity—if not complete absence—of studies in Iraq investigating the combined effects of the cytokinin 2iP and LED-based light spectra on *Gardenia jasminoides* propagation. Given the plant's ornamental and economic importance, as well as the energy and resource efficiency offered by LED technology, systematic investigation in this area is both scientifically and practically significant.

Therefore, the present study was designed with the following objectives:

- To assess the impact of varying concentrations of the cytokinin 2iP on the development and multiplication of *Gardenia jasminoides* shoots in vitro.
- To evaluate the influence of different light sources, including standard fluorescent and LED-based lighting, on shoot growth and proliferation.
- To investigate the interactive effects between cytokinin concentration and light quality on the growth and morphological development of in vitro cultured shoots.

Through this integrated experimental approach, the research aims to establish an optimized, cost-effective, and energy-efficient protocol for the large-scale micropropagation of *Gardenia jasminoides*, contributing to both horticultural production and sustainable propagation practices in Iraq and beyond.

2. Materials and Methods

The present investigation aimed to evaluate the effects of the cytokinin growth regulator 2-isopentenyl adenine (2iP) at three concentrations (0.1, 0.2, and 0.3 mg L^{-1}) in addition to a control (0 mg L^{-1}), combined with two light sources (LED plant growth lamps and standard fluorescent lamps), on the growth and multiplication of *Gardenia jasminoides* shoots. The study was conducted from September 2023 to April 2024 at the Plant Tissue Culture

Laboratory, Department of Horticulture and Landscape Engineering, College of Agriculture, Al-Qasim Green University.

2.1 Preparation of Nutrient Medium

Murashige and Skoog (MS) basal medium (Himedia, India), supplemented with 100 mg L^{-1} myo-inositol and vitamins including pyridoxine (0.1 mg L^{-1}), thiamine (10 mg L^{-1}), and nicotinic acid (1 mg L^{-1}), was used for all culture procedures (Murashige & Skoog, 1962). To prepare 1 liter of medium, 4.43 g of MS powder was dissolved in 700 mL of distilled water, and 30 g of sucrose was added as a carbohydrate source. The medium was solidified with 7 g L^{-1} agar, and the pH was adjusted to approximately 5.7 using 0.1 N NaOH or HCl. The final volume was made up to 1 liter with distilled water. The medium was heated using a hot plate magnetic stirrer to ensure complete dissolution and homogeneity. Subsequently, 50 mL portions of the medium were dispensed into glass culture jars (14 cm height × 6 cm diameter) and sterilized in an autoclave at 121°C and 1.4 kg/cm² for 20 minutes. After sterilization, the jars were stored in a sterile environment until use.

2.2 Sterilization of Culture Cabinet and Tools

All culture tools, including scalpels, forceps, and Petri dishes, were sterilized in a drying oven at 160–180°C for 1–3 hours. The culture cabinet was sanitized by wiping surfaces with 70% ethanol, followed by exposure to UV light for 30 minutes prior to use.

2.3 Source of Plant Material

Explant material was obtained from a cultivated two-year-old *Gardenia jasminoides* plant grown in a private nursery in Babil Governorate.

2.4 Establishment of Tissue Culture

Tissue culture initiation involved selecting tender shoots from new growth (5–10 cm in length) of the mother plant. Leaves were removed, and shoots were washed with tap water and liquid soap before cutting into 2–2.5 cm segments containing a single node. The segments were rinsed under running tap water for 30 minutes, then immersed in a solution of 100 mg L^{-1} ascorbic acid and 150 mg L^{-1} citric acid to prevent oxidation.

Sterilization was conducted in a laminar airflow cabinet. Explants were dipped in 70% ethanol for 10 seconds, then immersed in 1% sodium hypochlorite (commercial bleach, 5.25% chlorine) for 15 minutes with gentle shaking to remove air bubbles. Following sterilization, explants were rinsed three times with sterile distilled water for 3–5 minutes each to eliminate residual sterilizing agents. The ends of explants in contact with sterilant were trimmed to approximately 1 cm using fine forceps and a surgical blade, rendering them ready for cultivation.

2.5 Experimental Design and Statistical Analysis

The experiment followed a factorial design based on a Completely Randomized Design (CRD), with two factors: four light sources × four 2iP concentrations, including a control, with five replicates per treatment. Statistical analyses were performed using the GenStat software package on a Windows platform. Treatment means were compared using the Least Significant Difference (LSD) test at a 0.05 probability level to identify statistically significant differences among treatments (Al-Rawi & Khalaf Allah, 2000).

Studied Traits

The study focused on two categories of traits:

a) Vegetative Growth Indicators

- Average number of shoots formed (shoot multiplication rate)
- Average shoot length
- Average number of leaves per shoot
- Average fresh weight of the vegetative mass (mg)
- Average dry weight of the vegetative mass (mg)

b) Biochemical Characteristics

- Leaf chlorophyll content (mg/100 g fresh weight)
- Total carotene content (mg/100 g fresh weight) according to the method described by Goodwin et al. (1976)

This approach allowed for comprehensive evaluation of both morphological and biochemical responses of *Gardenia jasminoides* shoots to varying concentrations of 2iP and light conditions, facilitating the identification of optimal in vitro propagation protocols.

3.Results

3.1 Average Dry Weight

Significant differences were observed among treatments in terms of average dry weight (Table 1). The light source exhibited a notable influence, with the Blue LED light producing the highest average dry weight (0.3368 g), whereas the Red light recorded the lowest average (0.2196 g). The effect of 2iP concentration was also significant, as the 0.2 mg L^{-1} treatment yielded the highest mean dry weight (0.4636 g), compared to the lowest value (0.1874 g) obtained at 0.3 mg L^{-1}. Moreover, a significant interaction effect was evident between light source and cytokinin concentration. The combination of Blue LED light with 0.2 mg L^{-1} 2iP produced the greatest average dry weight (0.6240 g), while the lowest interaction mean (0.1160 g) was recorded under Red light at 0.1 mg L^{-1}. These results confirm that Blue LED light in conjunction with an optimal 2iP concentration of 0.2 mg L^{-1} effectively enhances biomass accumulation in *Gardenia jasminoides* shoots (Table 1).

3.2 Average Fresh Weight

The average fresh weight also showed significant differences among treatments (Table 2). Among the light sources, Blue LED light again produced the highest mean fresh weight (3.240 g), whereas White light yielded the lowest (2.105 g). Regarding 2iP concentrations, plants cultured with 0.2 mg L^{-1} exhibited the highest mean fresh weight (3.922 g), while the control treatment (0 mg L^{-1}) showed the

Table 1. The effect of 2iP concentrations and light source on the dry weight of *Gardenia jasminoides* grown in vitro.

Light source	2iP concentrations (mg/L)				Mean of light source
	0	0.1	0.2	0.3	
White fluorescent	0.1967	0.2713	0.4283	0.1560	**0.2631**
Red LED	0.1990	0.1160	0.3877	0.1757	**0.2196**
Blue LED	0.2267	0.3430	0.6240	0.1533	**0.3368**
Red + Blue + White LED	0.1410	0.1507	0.4143	0.2647	**0.2427**
Mean of 2iP concentration	**0.1909**	**0.2203**	**0.4636**	**0.1874**	
LSD 0.05	2iP concentration		Light source		Interaction
	0.055		0.0553		0.110

Table 2. The effect of 2iP concentrations and light source on the average fresh weight (gm) of *Gardenia jasminoides* grown in vitro.

Light source	2iP concentrations (mg/L)				Mean of light source
	0	0.1	0.2	0.3	
White fluorescent	1.494	2.187	2.883	1.854	**2.105**
Red LED	2.807	0.795	3.224	1.997	**2.206**
Blue LED	2.054	2.748	5.171	2.987	**3.240**
Red + Blue + White LED	1.427	2.302	4.410	2.743	**2.721**
Mean of 2iP concentration	**1.946**	**2.008**	**3.922**	**2.395**	
LSD 0.05	2iP concentration		Light source		Interaction
	0.483		0.483		0.967

Table 3. The effect of 2iP concentrations and light source on average number of leaves of *Gardenia jasminoides* grown in vitro.

Light source	2iP concentrations (mg/L)				Mean of light source
	0	0.1	0.2	0.3	
White fluorescent	13.00	19.33	27.67	15.33	**18.83**
Red LED	11.67	9.00	23.00	11.00	**13.67**
Blue LED	18.67	24.00	40.67	17.33	**25.17**
Red + Blue + White LED	10.33	16.33	35.00	20.00	**20.42**
Mean of 2iP concentration	**13.42**	**17.17**	**31.58**	**15.92**	
LSD 0.05	2iP concentration		Light source		Interaction
	3.47		3.47		6.95

lowest (1.946 g). The interaction between light source and cytokinin concentration was significant, with the Blue LED at 0.2 mg L^{-1} resulting in the highest fresh weight (5.171 g). Conversely, the lowest interaction mean was found under Red light at 0.1 mg L^{-1}, with a value of 0.795 g. This trend emphasizes the combined influence of optimal cytokinin levels and blue spectrum light in stimulating higher tissue hydration and metabolic activity, leading to improved shoot vigor (Table 2).

3.3 Average Number of Leaves

The number of leaves per shoot varied significantly across treatments (Table 3). Among light treatments, Blue LED produced the highest average number of leaves (25.17), while the Red light yielded the lowest (13.67). Similarly, the 0.2 mg L^{-1} 2iP concentration led to the highest mean leaf number (31.58), whereas the control group (0 mg L^{-1}) had the lowest (13.42). The interaction between Blue LED and 0.2 mg L^{-1} 2iP resulted in the maximum mean number of leaves (40.67), while Red light combined with 0.1 mg L^{-1} 2iP recorded the lowest (9.00). These findings demonstrate that Blue LED illumination in conjunction with moderate cytokinin supplementation promotes enhanced shoot morphogenesis and leaf initiation in *G. jasminoides* (Table 3).

3.4 Average Number of Branches

As shown in Table (4), there were significant differences in the number of branches among treatments. The Blue LED light recorded the highest mean branch number (3.71), while the Red fluorescent light resulted in the lowest (2.59). The 2iP concentration effect followed a similar pattern, with the highest mean (4.29) at 0.2 mg L^{-1} and the lowest (2.59) at 0 mg L^{-1}. The interaction effect revealed that Blue LED light at 0.2 mg L^{-1} produced the greatest branching (5.67), whereas Red light at 0.1 mg L^{-1} yielded the lowest (1.50). This suggests that Blue LED lighting enhances cytokinin responsiveness, facilitating axillary bud development and branching (Table 4).

3.5 Average Branch Length

Significant differences were also evident in branch length (Table 5). Blue LED light yielded the longest branches, averaging 4.59 cm, whereas Red fluorescent light produced the shortest (2.33 cm). The influence of 2iP concentration was similarly pronounced, with the

Table 4. The effect of 2iP concentrations and light source on average number of branches of *Gardenia jasminoides* grown in vitro.

Light source	2iP concentrations (mg/L)				Mean of light source
	0	0.1	0.2	0.3	
White fluorescent	2.17	3.17	3.83	2.50	2.92
Red LED	3.17	1.50	3.50	2.17	2.59
Blue LED	3.00	3.33	5.67	2.83	3.71
Red + Blue + White LED	2.00	2.67	4.17	3.33	3.04
Mean of 2iP concentration	2.59	2.67	4.29	2.71	
LSD 0.05	2iP concentration		Light source		Interaction
	0.62		0.62		1.25

Table 5. The effect of 2iP concentrations and light source on average branch length of *Gardenia jasminoides* grown in vitro.

Light source	2iP concentrations (mg/L)				Mean of light source
	0	**0.1**	**0.2**	**0.3**	
White fluorescent	1.67	3.00	4.00	2.67	**2.84**
Red LED	2.33	1.00	4.33	1.67	**2.33**
Blue LED	3.00	3.67	8.00	3.67	**4.59**
Red + Blue + White LED	1.33	2.67	5.00	3.67	**3.17**
Mean of 2iP concentration	**2.08**	**2.59**	**5.33**	**2.92**	
LSD 0.05	2iP concentration		Light source		Interaction
	0.66		0.66		1.33

Table 6. The effect of 2iP concentrations and light source on chlorophyll content of the branches of *Gardenia jasminoides* grown in vitro.

Light source	2iP concentrations (mg/L)				Mean of light source
	0	**0.1**	**0.2**	**0.3**	
White fluorescent	7.320	6.143	10.320	10.112	**8.474**
Red LED	9.520	7.650	12.576	11.763	**10.377**
Blue LED	11.862	19.653	13.543	13.320	**14.595**
Red + Blue + White LED	10.740	7.591	12.630	10.986	**10.487**
Mean of 2iP concentration	**9.861**	**10.259**	**12.267**	**11.545**	
LSD 0.05	2iP concentration		Light source		Interaction
	0.44		0.44		0.88

highest mean length (5.33 cm) at 0.2 mg L^{-1} and the lowest (2.08 cm) at 0 mg L^{-1}. The interaction between Blue LED and 0.2 mg L^{-1} 2iP generated the longest mean branch length (8.00 cm), while the Red light at 0.1 mg L^{-1} produced the shortest (1.00 cm). These results ($p \leq 0.05$) affirm that Blue LED lighting, in combination with an optimal cytokinin concentration, significantly enhances shoot elongation and vegetative growth performance in G. jasminoides (Table 5).

4. Discussion

The results on chlorophyll content and associated growth parameters of *Gardenia jasminoides* revealed significant variations among treatments involving different light sources and cytokinin (2iP) concentrations (Table 6). The highest mean chlorophyll content was recorded under Blue LED light (14.595), while the lowest was observed with white fluorescent light (8.474). Similarly, among the cytokinin concentrations, 0.2 mg L^{-1} produced the highest chlorophyll mean (12.267), whereas the control (0 mg L^{-1}) yielded the lowest (9.861). The interaction effect showed that Blue LED light combined with 0.1 mg L^{-1} 2iP achieved the maximum chlorophyll value (19.653), while the white fluorescent light with the same 2iP concentration resulted in the lowest (6.143). These findings highlight the strong synergistic impact of light spectrum and cytokinin concentration on chlorophyll biosynthesis and overall shoot vigor in *G. jasminoides*.

The data across Tables (1–6) collectively indicate that increasing 2iP concentration significantly enhanced all major growth indicators, including shoot number, shoot length, fresh and dry weights, leaf count, and chlorophyll content. This positive response aligns with the fundamental physiological role of cytokinins, such as 2iP, in stimulating cell division, shoot initiation, and organogenesis. Cytokinins function as essential components of transfer RNA (tRNA), where the isopentenyl side chain attached to adenine is critical for maintaining biological activity. In the absence of this group, tRNA becomes functionally inactive, but the incorporation of 2iP restores its efficiency in protein synthesis. Furthermore, cytokinins influence gene expression by enhancing RNA transcription. As reported by Mohammed and Younis (1991), a

modest increase in endogenous cytokinin concentration correlates with a significant elevation in messenger RNA (mRNA) levels, thereby promoting active cellular growth.

These results are in agreement with several previous studies that emphasized the necessity of cytokinins, particularly 2iP and benzyl adenine (BA), for shoot proliferation in plant tissue cultures. Al-Marri and Al-Ghamdi (1997), Loutfi and Chlyah (1998), Bekheet and Saker (1998), Hameed (2001), Al-Khateeb et al. (2014), and Bader and Khierallah (2009) all reported that cytokinins play a crucial role in the initiation and multiplication of shoots in various plant species.

The superior performance of cultures grown under Blue LED light can be attributed to its optimal wavelength range (610–700 nm), which coincides with the absorption peak of chlorophyll pigments, thus maximizing photosynthetic efficiency. Blue light is known to stimulate photosystem II activity, increase chloroplast development, and enhance overall vegetative growth. Additionally, it prolongs the duration of the cell division phase, resulting in higher rates of cell elongation and biomass accumulation. Sprinchanu and Butenko (1991) observed similar effects, noting that blue light enhances both morphogenesis and chlorophyll synthesis.

The elevated chlorophyll content in branches under Blue LED conditions (Table 6) may also stem from the stimulation of enzyme systems responsible for pigment biosynthesis. Blue light likely promotes nutrient absorption—particularly nitrogen, a vital element in chlorophyll structure—thereby improving pigment concentration and photosynthetic potential. Collectively, these findings demonstrate that combining an optimal cytokinin concentration (0.2 mg L^{-1} 2iP) with Blue LED illumination constitutes an effective strategy for maximizing growth, photosynthetic capacity, and overall tissue culture efficiency in *G. jasminoides*.

5. Conclusion and Recommendations

The study demonstrated that varying concentrations of the cytokinin plant growth regulator 2iP significantly affected culture growth and vegetative indicators during the multiplication stage. Among the tested concentrations, 0.2 mg L^{-1} 2iP produced superior results across most growth parameters. Furthermore, the use of LED lamps during the multiplication stage proved highly effective, yielding better outcomes in shoot multiplication and growth indicators compared to conventional white fluorescent lamps.

Based on these findings, it is recommended that the nutrient medium in the multiplication stage be supplemented with 0.2 mg L^{-1} 2iP and that LED light sources be adopted in tissue culture laboratories to enhance plant growth and development. Future research should focus on exploring the physiological and practical effects of different LED light spectra during the rooting and acclimatization stages. Given the enhanced shoot growth and higher multiplication rates observed under LED lighting during both multiplication and elongation stages, the continued use of LED lights in these phases is strongly encouraged for optimal tissue culture performance.

Author contributions

M.J. H. was responsible for fieldwork, data collection, and literature review. F.J. K. and M.N. N. contributed to manuscript writing, conducted statistical analyses, discussed the results, and jointly finalized the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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