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An Efficient Micropropagation Protocol and Optimization of Phytohormones for *Gladiolus* Species for Commercial Cultivation

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Abstract

Conventional propagation of *Gladiolus* through corms and cormels is slow, season-dependent, and insufficient to meet the increasing demand for quality planting materials, highlighting the need for an efficient micropropagation system for commercial uses. This study aimed to develop a reproducible *in vitro* protocol and optimization of various concentration of phytohormones using five *Gladiolus* spp. Healthy cormel explants of five gladiolus varieties, e.g. (add names of the five varieties) were cultured on Murashige and Skoog medium supplemented with different concentrations of 6-benzylaminopurine (BAP). Significant variation was observed among treatments and varieties for shoot proliferation, shoot length, and leaf number. The highest shoot multiplication (4.80 shoots explant^{-1}), shoot length (6.45 cm), and leaf number (5.28 leaves explant^{-1}) were obtained on MS medium with 2.0 mg/l BAP. Among the varieties, Red Sindoor showed better performance, producing up to 5.60 shoots explant^{-1} under the same treatment. Rooting was most effective on MS medium supplemented with 1.5 mg/l indole-3-butyric acid (IBA), where Red Sindoor also exhibited the highest rooting percentage (94.5%), root number (6.0), and root length (6.35 cm). The regenerated plantlets were successfully acclimatized and recorded the survival rates from 78.0 to 90.0%. These findings indicated that 2.0 mg/l BAP and 1.5 mg/l IBA were optimal for shoot multiplication and rooting, respectively. The developed protocol offers a practical and scalable approach for rapid production of disease-free, genetically uniform planting materials, contributing to the advancement of commercial application in floriculture.

Introduction

Gladiolus (*Gladiolus grandiflorus* L.), a prominent member of the family Iridaceae, is one of the most valued bulbous ornamental crops in global floriculture. Native to South Africa, where numerous wild species still exist, gladiolus has been cultivated for over

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two millennia (Kumari et al. 2025). The genus name is derived from the Latin word *Gladius*, meaning sword, referring to its characteristic sword-shaped leaves, hence the common name 'Sword lily' (Cantor and Tolety 2011). Owing to its striking spikes, wide range of lively colors, and prolonged vase life, gladiolus occupies a distinguished position among cut flowers and ranks fifth in the international floriculture trade (Darras 2025).

In Bangladesh, Gladiolus was introduced in the early 1990s and has since gained considerable commercial importance, particularly in Jashore, Kushtia, Chuadanga, Satkhira, Khulna, Gazipur, and Rangpur regions (Roy et al. 2023). The expansion of urbanization, increasing demand for ornamental plants, and export opportunities have collectively contributed to the rapid growth of gladiolus cultivation. As a high-value crop, it offers substantial economic returns to small and marginal farmers, especially during the winter season.

Despite its economic significance, large-scale production of Gladiolus planting materials is severely constrained by the limitations of conventional propagation methods. Traditionally, gladiolus is propagated vegetatively through corms and cormels (Kumar et al. 2024a). However, the multiplication rate is inherently low, as a single mother corm typically produces only 1-2 daughter corms and 20-30 cormels in a season (Memon et al. 2009), which require 2-4 growing cycles to reach flowering size. Furthermore, smaller cormels exhibit poor survivability and delayed flowering, reducing propagation efficiency (Memon et al. 2016). The system is also highly susceptible to diseases, particularly Fusarium corm rot, and postharvest storage losses, leading to significant depletion of viable planting materials. Additionally, prolonged dormancy of corms and cormels restricts timely planting and continuous production cycles (Li et al. 2023). Although seed propagation is possible, it is not commercially viable due to genetic variability and the extended juvenile phase required for flowering (Misra et al. 1994).

To overcome these limitations, plant tissue culture techniques, particularly *in vitro* propagation, offer a viable alternative for rapid and large-scale multiplication of Gladiolus. Micropropagation enables the production of genetically uniform, disease-free planting materials under controlled aseptic conditions, independent of seasonal constraints (Bhardwaj et al. 2025). It also ensures high multiplication rates, efficient use of space, and the conservation of elite germplasm. The success of *in vitro* regeneration largely depends on the composition of the culture medium, especially the type and concentration of plant growth regulators (Pasternak and Steinmacher 2024).

Among PGRs, cytokinins play a critical role in shoot induction and proliferation by promoting cell division and axillary bud development (Sosnowski et al. 2023). BAP is one of the most widely used cytokinins in gladiolus tissue culture due to its effectiveness in enhancing shoot multiplication over a wide range of explants (Kumar et al. 2024b). Previous studies have demonstrated that optimal concentrations of BAP significantly improve shoot proliferation, shoot elongation, and leaf formation, though the response varies depending on genotype and culture conditions (Singh et al. 2025).

Similarly, auxins such as IBA and NAA are essential for root induction and successful acclimatization of regenerated plantlets (Justamante et al. 2022).

Although considerable progress has been made in the micropropagation of Gladiolus using different explants and growth regulator combinations, still there have some limitations. Most studies have focused on a limited number of genotypes, often without systematically evaluating varietal responses to PGRs. The regeneration efficiency of Gladiolus is highly genotype-dependent, and protocols optimized for one variety may not be applicable to others (Naik et al. 2025). In Bangladesh, there is a notable lack of standardized and reproducible micropropagation protocols for commercially important Gladiolus cultivars. Furthermore, limited research has been conducted on optimizing various concentrations of cytokinin as plant growth regulators for maximizing shoot multiplication while ensuring efficient rooting and acclimatization under local conditions.

Therefore, to develop genotype-specific and optimized micropropagation protocols for gladiolus varieties cultivated in Bangladesh, the present study was undertaken to establish an efficient and reproducible *in vitro* regeneration and multiplication system. The study aimed to optimize the concentration of BAP for enhanced shoot proliferation in different varieties, to evaluate varietal responses to *in vitro* culture conditions and growth regulators, and to develop an effective rooting and acclimatization protocol for successful field establishment of regenerated plantlets.

Materials and Methods

Healthy, non-sprouted cormels (Fig. 1A) of five Gladiolus varieties *viz*., Pink, Saffron, Red Sindoor, Violet, and White were used as explants. The cormels were collected from a commercial source in Jhikorgacha, and Jashore, Bangladesh. Outer tunics were removed, and cormels were washed under running tap water for 30 min, followed by treatment with Tween-20 for 10 min. Surface sterilization was carried out using 0.1% carbendazim + sodium dichloroisocyanurate for 2 hrs, followed by rinsing with sterile distilled water. The explants were then treated with 70% ethanol for 30 sec followed by treating with 0.2% $HgCl_2$ for 7 min under laminar air flow cabinet, and finally rinsed thoroughly with sterile distilled water.

The MS medium supplemented with 30 g/l sucrose and 8 g/l agar was used. The pH was adjusted to 5.6 before autoclaving at 121°C for 20 min. For shoot multiplication (Fig. 1B-D), MS medium was fortified with different concentrations of BAP (0.0, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) along with 0.5 mg/l Kn and 0.5 mg/l thidiazuron (TDZ).

Cultures were maintained at 25 ± 1°C under a 16 hrs photoperiod with approximately 3000 lux (light intensity) and 50-70% relative humidity. Sub-culturing was performed at 4-week intervals. Shoot multiplication was evaluated in a factorial Completely Randomized Design (CRD) with two factors: five varieties and six BAP concentrations. Each treatment was replicated five times.

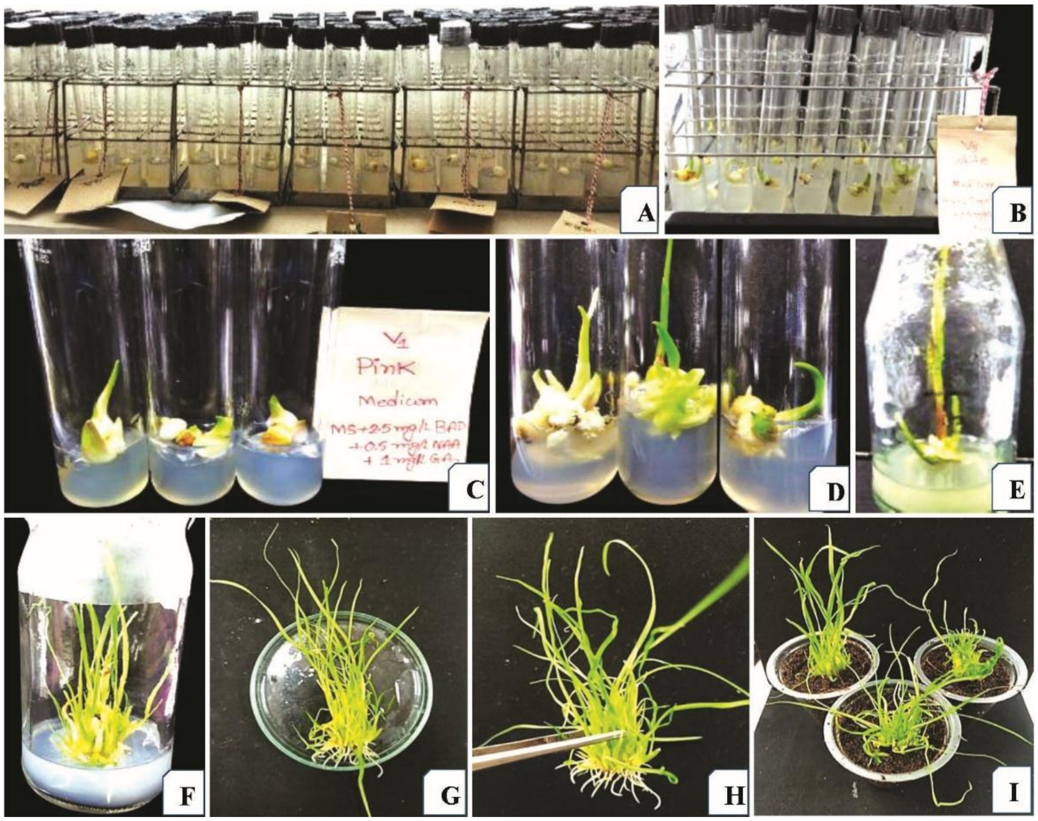

Fig. 1(A-I). The sequential steps of *in vitro* propagation of Gladiolus: (A) culture initiation of gladiolus cormels in MS medium, (B) first seedling stage of the germinated cormels, (C) established culture of gladiolus, (D) initiation of shoot multiplication, (E) multiple shoots ready for subculture, (F) *in vitro* shoots cultured on rooting media, (G-H) well rooted plantlets ready for *ex vitro* transfer, and (I) acclimatized and *ex vitro* established plants of gladiolus.

In vitro regenerated shoots of around 4-6 cm long (Fig. 1E)) were excised and transferred to MS medium supplemented with 1.5 mg/l indole-3-butyric acid (IBA) for root induction (Fig. 1F). The medium was solidified with 0.7% agar. Cultures were incubated at 25 ± 2°C under a 16 hrs photoperiod for 4 weeks. Rooted plantlets (Fig. 1G-H) were removed from culture vessels, washed gently, and transferred to pots containing suitable substrate (Fig. 1I) for acclimatization under greenhouse conditions.

Data were recorded after each sub-culture cycle for number of shoots explant^{-1}, shoot length (cm), number of leaves explant^{-1}, number of roots plantlet^{-1}, root length (cm), survival percentage (%) during acclimatization, and plant height (cm) of acclimatized plantlets. The collected data were subjected to analysis of variance (ANOVA) using R statistical software (R Core Team, 2025). Treatment means were compared using Duncan's Multiple Range Test (DMRT) at the 5% level of significance (Gomez and Gomez 1984).

Results and Discussion

Significant variation was observed among the five gladiolus varieties in terms of shoot multiplication, shoot length, and leaf number under *in vitro* conditions (Fig. 2). The highest number of shoots per explant was recorded in Red Sindoor (4.68), followed by Violet (4.12) and Saffron (3.95). Lower shoot proliferation was observed in Pink (3.24), while the minimum was found in White (2.85).

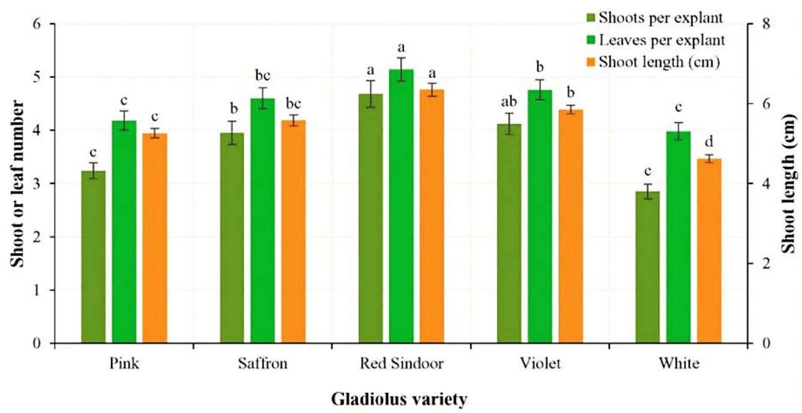

Fig. 2. Varietal response of five *Gladiolus* spp. under *in vitro* conditions. Values with same letters of each bar type are not significantly different at 5% level by DMRT.

Shoot length also varied significantly among the varieties. The longest shoots were obtained in Red Sindoor (6.35 cm), followed by Violet (5.85 cm) and Saffron (5.58 cm). Pink produced moderately long shoots (5.26 cm), whereas the shortest shoots were observed in White (4.62 cm).

In terms of leaf production, Red Sindoor produced the highest number of leaves explant^{-1} (5.14), followed by Violet (4.76) and Saffron (4.60). The variety Pink showed moderate leaf number (4.18), while the lowest leaf number was recorded in White (3.98).

The effects of different concentrations of BAP on *in vitro* growth attributes of *Gladiolus* spp. was found to be highly significant ($p \leq 0.01$) for shoot multiplication, shoot length, and leaf number (Table 1). The number of shoots explant^{-1} increased progressively with increasing concentrations of BAP up to 2.0 mg/l. The highest shoot proliferation (4.80 shoots explant^{-1}) was recorded at 2.0 mg/l BAP, which was significantly higher than all other treatments. This was followed by 2.5 mg/l (4.12) and 1.5 mg/l (4.05), which were statistically similar. Lower shoot numbers were observed at 1.0 mg/l (3.25) and 0.5 mg/l (2.84), while the control (without BAP) produced the lowest number of shoots (1.60).

A similar trend was observed for shoot length. The longest shoots (6.45 cm) were obtained at 2.0 mg/l BAP, followed by 1.5 mg/l (5.88 cm) and 2.5 mg/l (5.75 cm), which were statistically comparable. Moderate shoot elongation was recorded at 1.0 mg/l (5.28 cm) and 0.5 mg/l (4.62 cm), whereas the shortest shoots (3.45 cm) were observed in the control.

Table 1. Effects of different concentrations of BAP on *in vitro* growth attributes of *Gladiolus* spp.

BAP (mg/l)	Shoots explant^{-1}	Shoot length (cm)	Leaves explant^{-1}
0.0	1.60 ± 0.10^{e}	3.45 ± 0.12^{e}	2.92 ± 0.08^{e}
0.5	2.84 ± 0.13^{d}	4.62 ± 0.15^{d}	3.75 ± 0.10^{d}
1.0	3.25 ± 0.18^{c}	5.28 ± 0.17^{c}	4.28 ± 0.12^{c}
1.5	4.05 ± 0.22^{b}	5.88 ± 0.20^{b}	4.65 ± 0.14^{b}
2.0	4.80 ± 0.25^{a}	6.45 ± 0.23^{a}	5.28 ± 0.18^{a}
2.5	4.12 ± 0.20^{b}	5.75 ± 0.19^{b}	4.70 ± 0.13^{b}
Level of significance	**	**	**

Values with same letters are not significantly different at 5% level by DMRT. ** = significant at 1% level of significance.

Leaf number explant^{-1} also increased with increasing BAP concentration up to 2.0 mg/l. The maximum number of leaves (5.28 leaves explant^{-1}) was recorded at 2.0 mg/l BAP, followed by 2.5 mg/l (4.70) and 1.5 mg/l (4.65), which were statistically similar. The lowest leaf number (2.92) was observed in the control, followed by 0.5 mg/l (3.75) and 1.0 mg/l (4.28).

The interaction between five Gladiolus varieties and BAP concentrations had a highly significant ($p \leq 0.01$) effect on shoot multiplication, shoot length, and leaf number (Table 2). Shoot proliferation varied markedly across treatment combinations. The highest number of shoots explant^{-1} (5.60) was recorded in Red Sindoor at 2.0 mg/l BAP, followed by Saffron (5.28) and Violet (4.90) at the same concentration. The variety Pink also showed a high response (4.80) at 2.0 mg/l BAP in addition with MS medium. In contrast, the lowest shoot number (1.60) was observed in Pink under the control. In general, shoot multiplication increased with increasing BAP concentration up to 2.0 mg/l in all varieties, followed by a slight decline at 2.5 mg/l.

Shoot length showed a similar pattern of variation among the treatment combinations. The longest shoots (6.45 cm) were obtained in Pink at 2.0 mg/l BAP, followed by Red Sindoor (6.25 cm) and Saffron (6.15 cm) at the same concentration. The variety Violet and White also produced comparatively longer shoots at 2.0 mg/l BAP, measuring 6.00 cm and 5.80 cm, respectively. The shortest shoot length (3.45 cm) was recorded in Pink under control condition.

Leaf production explant^{-1} was also significantly influenced by the interaction effect of BAP concentrations and gladiolus varieties. The highest number of leaves (5.28) was observed in Pink at 2.0 mg/l BAP, followed by Saffron and Red Sindoor (5.10 each) at the same concentration. Variety Violet and White also showed maximum leaf numbers (5.00) at 2.0 mg/l BAP. The lowest leaf number (2.92) was recorded in Pink in the absence of BAP.

Root induction of *Gladiolus* spp. at 1.5 mg/l IBA showed significant variation among the varieties for rooting percentage, number of roots plantlet^{-1}, and root length (Table 3).

Table 2. Interaction effect of gladiolus variety and BAP concentrations on shoot multiplication, shoot length, and leaf number.

Gladiolus variety	BAP (mg/l)	Shoots explant^{-1}	Shoot length (cm)	Leaves explant^{-1}
Pink	0.0 (Cont.)	1.60 ± 0.10^{e}	3.45 ± 0.12^{e}	2.92 ± 0.08^{e}
	0.5	2.84 ± 0.13^{d}	4.62 ± 0.15^{d}	3.75 ± 0.10^{d}
	1.0	3.25 ± 0.18^{c}	5.28 ± 0.17^{c}	4.28 ± 0.12^{c}
	1.5	4.05 ± 0.22^{b}	5.88 ± 0.20^{b}	4.65 ± 0.14^{b}
	2.0	4.80 ± 0.25^{a}	6.45 ± 0.23^{a}	5.28 ± 0.18^{a}
	2.5	4.12 ± 0.20^{b}	5.75 ± 0.19^{b}	4.70 ± 0.13^{b}
Saffron	0.0 (Cont.)	2.84 ± 0.13^{d}	4.62 ± 0.15^{d}	3.75 ± 0.10^{d}
	0.5	3.95 ± 0.22^{c}	5.58 ± 0.20^{c}	4.60 ± 0.14^{c}
	1.0	4.05 ± 0.20^{b}	5.75 ± 0.19^{b}	4.70 ± 0.13^{b}
	1.5	4.28 ± 0.18^{b}	5.88 ± 0.20^{b}	4.85 ± 0.12^{b}
	2.0	5.28 ± 0.23^{a}	6.15 ± 0.22^{a}	5.10 ± 0.14^{a}
	2.5	4.60 ± 0.14^{b}	5.60 ± 0.19^{b}	4.70 ± 0.12^{b}
Red Sindoor	0.0 (Cont.)	3.24 ± 0.15^{d}	4.65 ± 0.17^{d}	4.18 ± 0.11^{d}
	0.5	4.12 ± 0.22^{c}	5.28 ± 0.18^{c}	4.48 ± 0.13^{c}
	1.0	4.25 ± 0.18^{b}	5.60 ± 0.19^{b}	4.72 ± 0.14^{b}
	1.5	4.50 ± 0.20^{a}	5.85 ± 0.20^{a}	4.85 ± 0.15^{a}
	2.0	5.60 ± 0.26^{a}	6.25 ± 0.23^{a}	5.10 ± 0.18^{a}
	2.5	5.02 ± 0.18^{b}	5.90 ± 0.21^{b}	4.95 ± 0.16^{b}
Violet	0.0 (Cont.)	2.85 ± 0.14^{e}	4.62 ± 0.16^{e}	3.98 ± 0.10^{e}
	0.5	3.28 ± 0.19^{d}	4.85 ± 0.17^{d}	4.30 ± 0.11^{d}
	1.0	3.75 ± 0.20^{c}	5.18 ± 0.19^{c}	4.50 ± 0.13^{c}
	1.5	4.12 ± 0.22^{b}	5.60 ± 0.20^{b}	4.60 ± 0.15^{b}
	2.0	4.90 ± 0.24^{a}	6.00 ± 0.22^{a}	5.00 ± 0.16^{a}
	2.5	4.20 ± 0.21^{b}	5.55 ± 0.20^{b}	4.60 ± 0.13^{b}
White	0.0 (Cont.)	2.30 ± 0.12^{e}	4.50 ± 0.15^{e}	3.90 ± 0.11^{e}
	0.5	3.10 ± 0.18^{d}	4.85 ± 0.17^{d}	4.30 ± 0.12^{d}
	1.0	3.50 ± 0.20^{c}	5.15 ± 0.19^{c}	4.50 ± 0.13^{c}
	1.5	3.80 ± 0.22^{b}	5.40 ± 0.18^{b}	4.60 ± 0.14^{b}
	2.0	4.20 ± 0.23^{a}	5.80 ± 0.21^{a}	5.00 ± 0.15^{a}
	2.5	3.90 ± 0.19^{b}	5.20 ± 0.18^{b}	4.40 ± 0.12^{b}
Level of significance		**	**	**

Values with same letters are not significantly different at 5% level by DMRT. ** = significant at 1% level of significance.

The highest rooting percentage (94.5%) was recorded in Red Sindoor, which was significantly higher than the other varieties. This was followed by Violet (92.3%) and Saffron (91.2%), which were statistically similar. Lower rooting percentages were observed in Pink (88.0%) and White (87.5%), with White showing the minimum response.

The number of roots plantlet^{-1} also differed significantly among the varieties. The variety Red Sindoor produced the highest number of roots (6.0), followed by Violet (5.6) and Saffron (5.2). The lowest number of roots was recorded in White (4.4), which was statistically similar to Pink (4.6).

Table 3. Root induction efficiency of gladiolus varieties under *in vitro* conditions.

Five Gladiolus variety	Rooting (%)	Number of roots plantlet^{-1}	Root length (cm)
Pink	88.0 ± 1.8^{bc}	4.6 ± 0.15^{c}	5.28 ± 0.22^{c}
Saffron	91.2 ± 1.5^{ab}	5.2 ± 0.18^{b}	5.72 ± 0.20^{b}
Red Sindoor	94.5 ± 1.3^{a}	6.0 ± 0.20^{a}	6.35 ± 0.25^{a}
Violet	92.3 ± 1.6^{ab}	5.6 ± 0.17^{ab}	5.98 ± 0.23^{ab}
White	87.5 ± 1.9^{c}	4.4 ± 0.14^{c}	5.20 ± 0.18^{c}
Level of significance	**	**	**

Values with same letters are not significantly different at 5% level by DMRT. ** = significant at 1% level of significance.

Similarly, root length varied significantly across the varieties. The longest roots (6.35 cm) were observed in Red Sindoor, followed by Violet (5.98 cm) and Saffron (5.72 cm). The shortest roots were recorded in White (5.20 cm), which was statistically similar to Pink (5.28 cm).

The *ex vitro* performance of regenerated gladiolus plantlets varied significantly among the varieties in terms of survival rate and plant height at the 5% level of significance (Fig. 3). The highest survival rate (90.0%) was recorded in Red Sindoor, which was statistically superior to the other varieties. The variety Pink also showed a relatively high survival rate (85.5%), which was statistically similar to Red Sindoor. Moderate survival rates were observed in Saffron (82.0%) and Violet (80.5%), which were statistically similar. The lowest survival rate (78.0%) was recorded in White.

Plant height also showed significant variation among the varieties. The tallest plants (13.5 cm) were obtained from Red Sindoor, which was significantly higher than the other varieties. The variety Pink produced moderately tall plants (12.2 cm), which was statistically similar to Red Sindoor. The remaining varieties *viz*., Saffron (11.8 cm), Violet (11.4 cm), and White (10.8 cm) showed comparatively shorter plant height and were statistically similar among themselves.

The present study demonstrates a systematic and genotype-responsive optimization of *in vitro* micropropagation in *Gladiolus* spp., with particular emphasis on cytokinin-mediated shoot proliferation and auxin-induced rooting. The findings not only confirm the central role of Plant Growth Regulators (PGRs) in organogenesis but also provide refined, genotype-specific insights that contribute to improve commercial micropropagation efficiency.

A pronounced varietal effect was observed for all *in vitro* parameters, including shoot multiplication, shoot elongation, leaf production, rooting, and acclimatization. Among the tested cultivars, Red Sindoor consistently outperformed others, indicating superior morphogenetic competence. This genotype-dependent response aligns with earlier observations that regeneration efficiency in gladiolus is strongly influenced by genetic background (Mousavimatin et al. 2025), which governs endogenous hormonal balance and tissue responsiveness (Singh et al. 2025). However, the present study advances beyond previous reports by demonstrating that a single optimized hormonal regime (particularly BAP 2.0 mg/l) can produce consistently superior results across multiple varieties, though with differential magnitudes of response. This suggests that while genotype plays a critical role, a partially universal protocol with predictable varietal scaling is achievable, which is particularly valuable for commercial applications where multiple cultivars are propagated simultaneously.

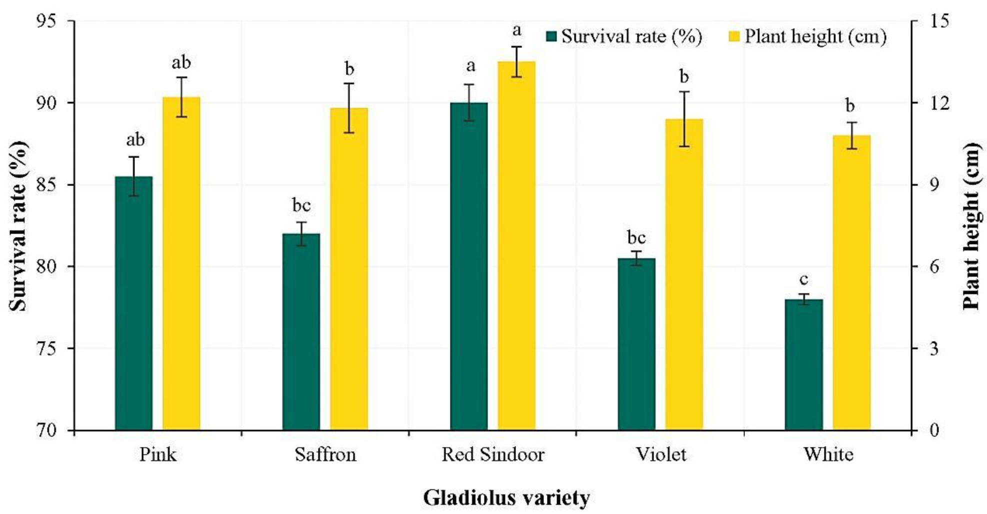

Fig. 3. The *ex vitro* survival rate, and plant height of five Gladiolus varieties. Values with same letters of each bar type are not significantly different at 5% level by DMRT.

Cytokinin concentration, particularly BAP, significantly influenced shoot proliferation, shoot length, and leaf number. The results clearly indicate that 2.0 mg/l BAP is the optimal concentration for maximizing all three growth parameters. This is consistent with previous studies reporting effective shoot induction within the range of 2-3 mg/l BAP. However, the present study provides a more precise refinement of this range, demonstrating that increasing BAP up to 2.0 mg/l enhances shoot regeneration, but further increase leads to a decline in growth attributes. This decline at higher concentrations may be attributed to cytokinin-induced physiological imbalance, leading to reduced shoot quality or inhibition of elongation (Hussain et al. 2025). The identification of this threshold is particularly important for commercial laboratories, as it prevents unnecessary use of higher hormone concentrations, thereby reducing cost and improving plantlet quality. An additional advancement of this study lies in the simultaneous optimization of multiple traits (shoot number, length, and leaf number) rather than focusing on a single parameter. This integrated optimization is critical for producing robust plantlets suitable for successful acclimatization, which is often overlooked in earlier protocols.

The significant interaction between variety and BAP concentration highlights the importance of genotype × hormone interaction in determining *in vitro* response. While 2.0 mg/l BAP was optimal across all varieties, the magnitude of response varied, with Red Sindoor showing the highest shoot proliferation (5.60 shoots explant^{-1}). This finding contributes a novel perspective by demonstrating that optimal hormone concentration can be standardized, but maximum efficiency depends on genotype-specific responsiveness (Torun et al. 2020, Padyab et al. 2026). Such interaction-based optimization is particularly relevant for commercial micropropagation, where maximizing output per explant is crucial. Compared to earlier protocols that often focus on single cultivars, this study provides a comparative framework for selecting high-performing varieties (e.g., Red Sindoor) for large-scale multiplication, thereby enhancing production efficiency.

Root induction was successfully achieved using 1.5 mg/l IBA, with significant varietal differences. The variety Red Sindoor again showed superior performance in terms of rooting percentage, root number, and root length. Previous studies have reported effective rooting in gladiolus using IBA concentrations ranging from 0.75 to 1.0 mg/l (Malviya et al. 2018, Kumar et al. 2024). The findings suggest that a slightly higher concentration (1.5 mg/l) is more effective under the tested conditions, indicating that rooting response may depend on prior shoot proliferation conditions and genotype. This highlights an important advancement: rooting optimization should not be considered independently but rather as a continuation of the shoot multiplication phase. Moreover, the production of well-developed root systems (in terms of both number and length) is critical for successful acclimatization. The results demonstrate that IBA not only enhances rooting frequency but also improves root quality, which directly contributes to higher survival rates.

Successful acclimatization is a critical step in commercial micropropagation, often limiting the practical applicability of *in vitro* protocols. In this study, high survival rates (up to 90%) were achieved, particularly in Red Sindoor, indicating the production of physiologically competent plantlets. These survival rates are comparable to or higher than those reported in previous studies (typically 80-100%) under controlled conditions. However, the present study adds value by linking acclimatization success directly to optimized *in vitro* conditions, particularly balanced shoot and root development. The variation in plant height among varieties further reflects the carry-over effect of *in vitro* vigor on *ex vitro* growth, highlighting the importance of selecting both optimal hormone concentrations and responsive genotypes for commercial success.

The study demonstrates an efficient and reproducible micropropagation protocol for *Gladiolus* spp. with MS medium supplemented with 2.0 mg/l BAP proved to be the most effective for shoot multiplication, shoot elongation, and leaf production across all varieties, while 1.5 mg/l IBA was optimal for root induction. Significant genotypic variation was observed, with Red Sindoor consistently exhibiting superior performance in terms of shoot proliferation, rooting ability, and *ex vitro* survival. The integration of high multiplication efficiency and successful acclimatization highlights the potential of this protocol for large-scale production of uniform, disease-free planting materials.

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