



Growth regulator mediated mitigation of moisture stress in *Gladiolus grandiflorus* L.

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ABSTRACT

Drought stress is a key constraint that hampers growth, flowering, and postharvest quality in gladiolus. This study examined the effect of growth regulators in mitigating moisture stress in two gladiolus cultivars, Arka Amar and Arka Darshan, under a rain shelter during 2024–2025. The study was set up in a factorial Completely Randomized Design consisting of two moisture regimes. (100 and 75% field capacity) and three growth regulator treatments comprising melatonin (100 µM), brassinolide (0.3 mM), and an untreated control. Moisture stress at 75% field capacity significantly reduced spike length, rachis length, number of florets per spike, vase life, and photosynthetic rate. Arka Amar consistently outperformed Arka Darshan under both moisture regimes. Among the treatments, melatonin was most effective in improving floral, physiological, and biochemical traits, followed by brassinolide. The best performance was recorded in Arka Amar under 100% field capacity with melatonin, which showed maximum spike length (97.05 cm), vase life (10.50 days), and enhanced photosynthetic and antioxidant responses. The results indicate that melatonin can effectively mitigate moisture stress in gladiolus by improving floral quality, photosynthetic efficiency, and biochemical responses, with Arka Amar exhibiting greater tolerance than Arka Darshan.

Key words: Melatonin, brassinolide, Arka Amar, Arka Darshan, drought stress, growth regulators.

INTRODUCTION

Gladiolus (*Gladiolus grandiflorus* L.) is a popular ornamental plant grown mainly for use as a cut flower. However, its productivity and ornamental value are highly influenced by environmental conditions, particularly abiotic stresses. Among these, drought is a major constraint affecting growth and flower quality, and its impact is increasing under changing climatic conditions (Das *et al.*, 5). Adequate soil moisture is essential throughout the growth cycle of gladiolus for optimal development, and water limitation, particularly during vegetative growth and spike elongation stages, reduces biomass and floral attributes. Earlier studies have shown that optimal irrigation improves spike yield and quality, whereas moisture deficit adversely affects flowering and prolongs crop duration (Begum *et al.*, 3; Santos *et al.*, 13).

Under drought conditions, plants undergo various physiological and biochemical adjustments regulated by phytohormones. In this context, the use of growth regulators as stress-mitigating agents has gained considerable attention (Manghwar *et al.*, 10). Melatonin is known to enhance drought tolerance by improving antioxidant defence and protecting the photosynthetic system (Sharma and Zheng, 14), and its exogenous application has been shown to improve

stress tolerance in several crops (Kamatchi *et al.*, 7; Luo *et al.*, 9). Brassinosteroids are also important regulators of plant growth and stress response, with reported benefits under moisture stress conditions (Omidian *et al.*, 11; Yang *et al.*, 15).

Despite these findings, comparative information on the effectiveness of melatonin and brassinolide in mitigating moisture stress in gladiolus under controlled conditions remains limited, and varietal differences in response are not well understood. Hence, the present investigation was undertaken to assess the effects of these growth regulators on floral, physiological, and biochemical traits of gladiolus under different moisture regimes, and to compare the performance of two cultivars, Arka Amar and Arka Darshan.

MATERIALS AND METHODS

The study was carried out at the Department of Floriculture and Landscaping, College of Agriculture, Vellayani, Thiruvananthapuram during 2024–2025. Uniform-sized corms of gladiolus cultivars Arka Amar and Arka Darshan were planted in growbags under a rain shelter using a potting mix prepared using equal quantities of sand, soil, and cow dung. The study was set up in a factorial Completely Randomised Design (CRD) consisting of two varieties, two moisture regimes (100% and 75% field capacity), and two growth regulator treatments [melatonin (100

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μM), brassinolide (0.3 mM)] and control, resulting in 12 treatment combinations. Each treatment was replicated three times with 15 plants per experimental unit. Corms were treated with fungicide (SAAF) and planted at a depth of 7 cm, and all plants were irrigated uniformly during establishment. A gravimetric method was adopted to maintain soil moisture, where container weight was monitored regularly and water was added to achieve the desired moisture level. Growth regulators were applied as foliar sprays at spike initiation and one week later, while control plants received water spray.

Floral parameters were recorded at flowering, including number of florets per spike, spike length (base to tip), rachis length (first to last floret-bearing portion), and vase life (duration from harvest to complete senescence in water at room temperature). Photosynthetic rate was recorded using a portable photosynthesis system under ambient conditions (IRGA) between 09:00 and 11:00 h under clear conditions. Proline content in leaf tissue was quantified following the standard ninhydrin-based procedure (Sadasivam and Manickam, 12), and Superoxide dismutase activity was determined based on its ability to inhibit photochemical reduction of NBT (Kakkar *et al.*, 6). Proline was determined from leaf tissue (0.5 g) extracted in sulphosalicylic acid and measured at 520 nm using an L-proline standard curve. SOD activity was measured from leaf extract prepared in

phosphate buffer, with absorbance recorded at 560 nm, and expressed as activity $\text{g}^{-1} \text{min}^{-1}$ based on inhibition of NBT photoreduction.

The collected data were statistically evaluated with the help of RAISINS software developed by Kerala Agricultural University. Analysis of variance (ANOVA) was applied following a factorial Completely Randomized Design, and differences among treatment means were tested using the critical difference (CD) at the 5 per cent level of significance.

RESULTS AND DISCUSSION

Spike length was significantly affected by variety, moisture regime, and growth regulator treatments (Table 1). 'Arka Amar' produced significantly longer spikes than 'Arka Darshan'. Plants maintained at 100% field capacity recorded greater spike length compared to those at 75% field capacity, indicating the adverse effect of moisture stress on spike development. This reduction under water-deficit conditions may be attributed to limited cell expansion, reduced photosynthetic activity, and restricted assimilate translocation (Becker *et al.*, 2). Among growth regulators, the highest values were observed under melatonin treatment, followed by brassinolide, while the control recorded the lowest value. The interaction effect revealed that 'Arka Amar' under 100% field capacity with melatonin produced the maximum spike length (97.05 cm), which was on

Table 1: Effect of varieties, stress levels and growth regulators on spike length, number of florets per spike and vase life of gladiolus.

Treatment	Spike length (cm)	Rachis length (cm)	Number of florets per spike	Vase life (days)
Varieties (Factor A)				
Arka Amar	87.09 $\pm$ 10.34 ^a	47.25 $\pm$ 5.64 ^a	12.16 $\pm$ 1.64 ^a	7.50 $\pm$ 1.83 ^a
Arka Darshan	65.36 $\pm$ 5.74 ^b	34.04 $\pm$ 2.95 ^b	10.70 $\pm$ 0.91 ^b	6.13 $\pm$ 1.08 ^b
SE(m)	0.51	0.35	0.06	0.08
CD	1.48	1.01	0.17	0.24
Stress levels (Factor B)				
100% FC	80.21 $\pm$ 12.78 ^a	42.75 $\pm$ 7.77 ^a	12.23 $\pm$ 1.27 ^a	7.67 $\pm$ 1.67 ^a
75% FC	72.25 $\pm$ 13.90 ^b	38.54 $\pm$ 7.96 ^b	10.63 $\pm$ 1.30 ^b	5.96 $\pm$ 1.08 ^b
SE(m)	0.51	0.35	0.06	0.08
CD	1.48	1.01	0.17	0.24
Growth regulators (Factor C)				
Control	68.50 $\pm$ 11.68 ^c	36.64 $\pm$ 6.83 ^c	10.28 $\pm$ 1.49 ^c	5.58 $\pm$ 1.03 ^c
Melatonin	83.32 $\pm$ 13.69 ^a	44.32 $\pm$ 8.36 ^a	12.27 $\pm$ 1.12 ^a	7.98 $\pm$ 1.78 ^a
Brassinolide	76.86 $\pm$ 12.60 ^b	40.98 $\pm$ 7.53 ^b	11.74 $\pm$ 1.17 ^b	6.88 $\pm$ 1.06 ^b
SE(m)	0.62	0.42	0.07	0.1
CD	1.81	1.24	0.21	0.3

par with its performance under 75% field capacity with melatonin, whereas the minimum spike length was observed in 'Arka Darshan' under 75% field capacity without growth regulator (Table 2). Similar reductions in floral traits under moisture stress have been reported in gladiolus and other ornamental crops (Begum *et al.*, 3; Chyliński *et al.*, 4), whereas improvement under melatonin application may be attributed to enhanced physiological efficiency and stress tolerance (Ahsan *et al.*, 1).

Rachis length was significantly influenced by variety, moisture regime, and growth regulator treatments (Table 1). 'Arka Amar' recorded longer rachis length than 'Arka Darshan', indicating varietal differences in growth potential. Plants at 100% field capacity showed greater rachis length compared to those at 75% field capacity, confirming the negative impact of moisture stress on floral development. Melatonin treatment resulted in the highest rachis length, followed by brassinolide, while the control recorded the lowest value. The interaction effect revealed that 'Arka Amar' under 100% field capacity with melatonin recorded the maximum rachis length (52.57 cm), whereas the minimum was observed in 'Arka Darshan' under 75% field capacity without growth regulator. The improvement in rachis length under melatonin treatment may be due to enhanced metabolic activity and better assimilate partitioning under stress conditions.

The number of florets per spike was affected by variety, moisture regime, and growth regulator treatments (Table 1). 'Arka Amar' produced more florets than 'Arka Darshan'. Plants at 100% field capacity recorded a higher number of florets compared to those at 75% field capacity, reflecting the sensitivity of floret development to moisture availability. Among growth regulators, melatonin produced the maximum number of florets, followed by brassinolide, while the control recorded the lowest. The interaction effect showed that 'Arka Amar' under 100% field capacity with melatonin recorded the highest number of florets (13.97), whereas the lowest value was observed under moisture stress without growth regulator. These findings indicate that moisture stress adversely affects floral initiation and development, while melatonin improves floret production possibly through enhanced photosynthetic efficiency and hormonal regulation.

Vase life was significantly influenced by variety, moisture regime, and growth regulator treatments (Table 1). 'Arka Amar' exhibited longer vase life than 'Arka Darshan'. Plants maintained at 100% field capacity recorded longer vase life compared to those at 75% field capacity, indicating that moisture stress negatively affects postharvest longevity. The

Table 2: Effect of variety x stress level x growth regulator interaction on spike length, number of florets per spike and vase life.

	Spike length (cm)						Rachis length (cm)						Number of florets per spike						Vase life (days)					
	Arka Amar			Arka Darshan			Arka Amar			Arka Darshan			Arka Amar			Arka Darshan			Arka Amar			Arka Darshan		
	100%	75%	FC	100%	75%	FC	100%	75%	FC	100%	75%	FC	100%	75%	FC	100%	75%	FC	100%	75%	FC	100%	75%	FC
Control	86.01 ± 1.41 ^c	67.71 ± 2.91 ^e	64.77 ± 1.37 ^{ef}	55.52 ± 1.83 ^g	46.81 ± 1.24 ^b	36.73 ± 1.16 ^{cd}	33.80 ± 1.61 ^e	29.22 ± 1.04 ^f	12.47 ± 0.32 ^c	12.47 ± 0.32 ^c	8.97 ± 0.06 ^g	10.57 ± 0.40 ^f	9.10 ± 0.10 ^g	7.17 ± 0.15 ^d	5.10 ± 0.36 ^{hi}	5.37 ± 0.25 ^{gh}	4.67 ± 0.45 ⁱ							
	97.05 ± 1.07 ^a	95.37 ± 0.81 ^{ab}	73.11 ± 2.74 ^d	67.74 ± 0.85 ^e	52.57 ± 2.70 ^a	51.66 ± 1.06 ^a	37.84 ± 1.85 ^c	35.21 ± 1.41 ^{de}	13.97 ± 0.11 ^a	13.97 ± 0.11 ^a	12.10 ± 0.09 ^{cd}	11.93 ± 0.43 ^d	11.09 ± 0.33 ^e	10.50 ± 0.44 ^a	7.80 ± 0.40 ^c	7.87 ± 0.15 ^c	5.77 ± 0.42 ^g							
Brassinolide	92.42 ± 3.46 ^b	84.00 ± 3.41 ^c	67.87 ± 1.59 ^e	63.13 ± 1.83 ^f	50.24 ± 1.32 ^a	45.51 ± 1.23 ^b	35.23 ± 1.31 ^{de}	32.92 ± 0.77 ^e	13.40 ± 0.38 ^b	13.40 ± 0.38 ^b	12.03 ± 0.18 ^d	11.04 ± 0.08 ^e	10.47 ± 0.08 ^f	8.50 ± 0.60 ^b	5.93 ± 0.31 ^{fg}	6.63 ± 0.15 ^{de}	6.47 ± 0.21 ^{ef}							
SE(m)	1.24						0.85						0.15						0.2					
CD	3.61						2.48						0.43						0.59					

highest values were observed under melatonin treatment, followed by brassinolide, while the control recorded the shortest. The interaction effect indicated that 'Arka Amar' under 100% field capacity with melatonin recorded the maximum vase life (10.50 days), whereas the minimum was observed in 'Arka Darshan' under 75% field capacity without growth regulator. Improved vase life under melatonin treatment may be attributed to reduced oxidative damage and delayed senescence.

Photosynthesis rate was significantly influenced by variety, moisture regime, and growth regulator treatments (Table 3). 'Arka Amar' recorded higher photosynthetic rate than 'Arka Darshan', indicating better physiological adaptability. Plants maintained at 100% field capacity showed higher photosynthesis compared to those at 75% field capacity, likely due to stomatal limitation, reduced CO₂ availability, and impairment of photosynthetic machinery under moisture stress (Zahra *et al.*, 16). Among growth regulators, melatonin resulted in the highest photosynthetic rate, followed by brassinolide, while the control recorded the lowest. The interaction effect showed that 'Arka Amar' under 100% field capacity with melatonin recorded the maximum photosynthetic rate (10.92 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), whereas the minimum was observed under moisture stress without growth regulator (Table 4). Enhancement in photosynthesis under melatonin treatment may be due to protection of the photosynthetic apparatus and improved antioxidant activity.

Proline content was significantly affected by variety, moisture regime, and growth regulator treatments (Table 3). 'Arka Amar' recorded higher proline content than 'Arka Darshan'. Plants at 75% field capacity showed higher proline accumulation

compared to those at 100% field capacity, indicating osmotic adjustment under drought stress. Melatonin treatment resulted in the highest proline content, followed by brassinolide, while the control recorded the lowest. The interaction effect revealed that 'Arka Amar' under 75% field capacity with melatonin recorded the maximum proline content (8.62 $\mu\text{mol g}^{-1} \text{ FW}$), whereas

Table 3: Effect of varieties, stress levels and growth regulators on photosynthesis rate, proline content and superoxide dismutase activity.

Treatment	Photosynthesis rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Proline content ($\mu\text{mol g}^{-1} \text{ FW}$)	Superoxide dismutase (activity $\text{g}^{-1} \text{ min}^{-1}$)
Varieties (Factor A)			
Arka Amar	8.37 $\pm$ 1.99 ^a	7.28 $\pm$ 1.04 ^a	3.62 $\pm$ 0.61 ^a
Arka Darshan	7.24 $\pm$ 1.62 ^b	6.89 $\pm$ 1.36 ^b	3.21 $\pm$ 0.70 ^b
SE(m)	0.06	0.05	0.03
CD	0.18	0.15	0.09
Stress levels (Factor B)			
100% FC	8.61 $\pm$ 1.53 ^a	6.16 $\pm$ 0.96 ^b	2.89 $\pm$ 0.54 ^b
75% FC	7.00 $\pm$ 1.88 ^b	8.01 $\pm$ 0.53 ^a	3.94 $\pm$ 0.29 ^a
SE(m)	0.06	0.05	0.03
CD	0.18	0.15	0.09
Growth regulators (Factor C)			
Control	6.09 $\pm$ 0.91 ^c	6.26 $\pm$ 1.25 ^c	3.09 $\pm$ 0.64 ^c
Melatonin	9.73 $\pm$ 1.24 ^a	7.86 $\pm$ 0.75 ^a	3.77 $\pm$ 0.70 ^a
Brassinolide	7.58 $\pm$ 1.23 ^b	7.15 $\pm$ 1.07 ^b	3.39 $\pm$ 0.57 ^b
SE(m)	0.08	0.06	0.04
CD	0.22	0.18	0.11

Table 4: Effect of variety x stress level x growth regulator interaction on photosynthesis rate, proline content and superoxide dismutase activity.

	Photosynthesis rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)				Proline content ($\mu\text{mol g}^{-1} \text{ FW}$)				Superoxide dismutase (activity $\text{g}^{-1} \text{ min}^{-1}$)			
	Arka Amar		Arka Darshan		Arka Amar		Arka Darshan		Arka Amar		Arka Darshan	
	100% FC	75% FC	100% FC	75% FC	100% FC	75% FC	100% FC	75% FC	100% FC	75% FC	100% FC	75% FC
Control	7.02 $\pm$ 0.09 ^g	5.39 $\pm$ 0.07 ^{hi}	6.87 $\pm$ 0.15 ^g	5.10 $\pm$ 0.35 ⁱ	5.56 $\pm$ 0.10 ^f	7.46 $\pm$ 0.32 ^d	4.69 $\pm$ 0.40 ^g	7.33 $\pm$ 0.10 ^d	2.60 $\pm$ 0.17 ^{gh}	3.73 $\pm$ 0.06 ^{de}	2.38 $\pm$ 0.03 ^h	3.63 $\pm$ 0.21 ^e
Melatonin	10.92 $\pm$ 0.17 ^a	10.34 $\pm$ 0.24 ^b	9.83 $\pm$ 0.54 ^c	7.84 $\pm$ 0.23 ^{ef}	7.14 $\pm$ 0.13 ^d	8.62 $\pm$ 0.12 ^a	7.17 $\pm$ 0.04 ^d	8.51 $\pm$ 0.29 ^{ab}	3.92 $\pm$ 0.11 ^{bcd}	4.36 $\pm$ 0.20 ^a	2.65 $\pm$ 0.11 ^g	4.13 $\pm$ 0.06 ^b
Brassinolide	9.05 $\pm$ 0.24 ^d	7.50 $\pm$ 0.02 ^f	7.95 $\pm$ 0.24 ^e	5.81 $\pm$ 0.36 ^h	6.73 $\pm$ 0.27 ^e	8.21 $\pm$ 0.05 ^{bc}	5.70 $\pm$ 0.08 ^f	7.96 $\pm$ 0.27 ^c	3.15 $\pm$ 0.17 ^f	3.97 $\pm$ 0.12 ^{bc}	2.64 $\pm$ 0.04 ^g	3.80 $\pm$ 0.17 ^{cde}
SE(m)	0.15				0.12				0.08			
CD	0.45				0.36				0.23			

the minimum was observed in 'Arka Darshan' under 100% field capacity without growth regulator (Table 4). Increased proline accumulation under melatonin treatment suggests enhanced osmotic regulation and stress tolerance (Langaroudi *et al.*, 8).

Superoxide dismutase (SOD) activity was significantly influenced by variety, moisture regime, and growth regulator treatments (Table 3). 'Arka Amar' recorded higher SOD activity than 'Arka Darshan', indicating better antioxidant capacity. Plants at 75% field capacity exhibited higher SOD activity compared to those at 100% field capacity, reflecting activation of antioxidant defense mechanisms under stress conditions. Among growth regulators, melatonin resulted in the highest SOD activity, followed by brassinolide, while the control recorded the lowest. The interaction effect showed that 'Arka Amar' under 75% field capacity with melatonin recorded the maximum SOD activity ($4.36 \text{ activity g}^{-1} \text{ min}^{-1}$), whereas the minimum was observed in 'Arka Darshan' under 100% field capacity without growth regulator. Increased SOD activity under melatonin treatment indicates enhanced detoxification of reactive oxygen species and improved stress tolerance (Luo *et al.*, 9).

The findings of the present investigation demonstrate that moisture stress led to a reduction in floral quality, photosynthetic performance, and postharvest longevity in gladiolus, whereas growth regulator application mitigated these adverse effects. Among the treatments, melatonin was more effective than brassinolide in improving floral, physiological, and biochemical traits, likely due to its role in enhancing antioxidant defense and maintaining cellular homeostasis under stress conditions. 'Arka Amar' consistently outperformed 'Arka Darshan' across all parameters, indicating greater tolerance to moisture stress, possibly due to inherent genotypic differences.

AUTHOR'S CONTRIBUTION

Conceptualization of research (SK, RCR, RM, PI, BR); Designing of the experiments (SK, RCR, RM, PI, BR); Contribution of experimental materials (SK, RCR); Execution of field/lab experiments and data collection (SK); Analysis of data and interpretation (S.K.); Preparation of the manuscript (S.K.); Editing of manuscript (S.K., R.C.R.).

DECLARATION

The authors declare no conflict of interest

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