

Impact of plant growth regulators on shortening maturity duration and enhancing grain yield in *Oryza sativa* cv. OM5451

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ABSTRACT

In rice, the maturity duration is an important stage that determines grain yield. This study examines the relationship between phytohormones and rice development, providing a foundation for plant growth regulators (PGRs) application to shorten maturity time and improve rice yield. High-performance liquid chromatography with diode-array detection analysis revealed that indole-3-acetic acid (IAA) and gibberellic acid (GA₃) levels decline from tillering to panicle initiation, gibberellin A4 appears during tillering, and zeatin emerges during stem elongation and panicle initiation. PGRs treatment was done at the phases of tillering, panicle initiation (pre-booting), or booting stages. The experiment followed a Randomized Block Design. Compared to the control, the flowering was reduced by 2.19 days, and the 1000-grain weight increased by 1.37 g when 50 mg/L IAA was applied at the tillering stage. Treatment with 50 mg/L GA₃ at the booting stage improved grain filling and rice production by reducing flowering time by 5.88 days and maturing time by 9.06 days. In addition, it significantly increased panicle length, flag leaf size, and 1,000-grain weight. Treatments with GA₃ at panicle initiation have shown significant effects, promoting earlier flowering and maturity during pre-booting. These results suggest the applicability of PGRs in the future of agriculture.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a vital global crop that feeds over 3.5 billion people, especially in Asia, Africa, and Latin America. Therefore, increasing rice production is critical to tackle the growing food shortages [1,2]. The global demand for rice continues to rise due to population growth, urbanization, putting immense pressure on agricultural systems to enhance productivity while tackling challenges such as climate change, water scarcity, and land degradation [3,4]. A sudden change in the environment, such as a change in temperature or water level (due to floods and droughts), affects the development of plants, yield, and grain quality, thereby affecting the world's food security. Rice plants undergo three distinct stages of development in their life cycle.

The vegetative phase includes germination, seedling establishment, tillering (the formation of lateral shoots), and stem elongation. The reproductive stage begins with panicle initiation (pre-booting), continues through booting (when the panicle is enclosed in the leaf sheath), heading, and ultimately, grain filling and maturity [5,6]. According to Yoshida [7] and Ashikari *et al.*, [8] the heading is vital because it influences flowering time and pollination, as well as grain formation and filling, all of which directly affect the quantity, size, and

quality of harvested grains. The yield of rice is influenced by three primary factors: The number of panicles, the number of spikelets per panicle, and the grain-filling rate. Rice cultivars characterized by larger panicles demonstrate greater yield potential due to a higher spikelet count per panicle. Therefore, shortening flowering time should also be considered one of the options to finish the harvest before bad weather arrives. However, scientists have found that when shortening the maturity period, rice yield and grain quality are also affected [9].

In rice, phytohormones are essential for growth and yield regulation, particularly during the heading period, affecting grain filling, floret development, and panicle exertion [10]. Phytohormones coordinate important cellular functions such as division, elongation, differentiation, and senescence, acting as crucial regulators of the development stage [11]. According to Zhang *et al.*, maturation is sensitive to environmental factors such as temperature and water availability [12,13]. Imbalances in hormone levels at this stage can lead to poor yield outcomes, including incomplete panicle emergence, poor fertility, and poor grain production [14]. During the heading stage, hormonal changes redirect the plant from vegetative growth to reproduction. Gibberellins play a key role in stem elongation and panicle exertion, which are important for pollination and self-pollination in rice [15]. Mutants that lack gibberellin biosynthesis, such as the *semi-dwarf1* (*sd1*) genotype, show reduced height and incomplete panicle emergence, highlighting the hormone's necessity during heading [16]. Studies using high-performance liquid chromatography (HPLC) have shown that gibberellin levels peak during stem elongation and decline

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as the plant approaches panicle initiation, suggesting a temporal regulation that aligns with reproductive priorities [17]. Auxins regulate apical meristem activity and vascular development, influencing heading, panicle formation, and nutrient transport [18,19]. The level of indole-3-acetic acid (IAA) decreases, signaling the cessation of vegetative growth and the onset of reproductive differentiation at the transition from tillering to panicle initiation [20]. According to Kieber and Schaller, cytokinins play a crucial role in heading and grain filling by promoting cell proliferation and maintaining sink activity in the panicle [10]. Zeatin concentration rises during stem elongation and peaks around heading, which facilitates floret development and prevents premature senescence of cells in the reproductive stage [21]. This surge in cytokinins is vital for determining grain number, a key component of yield, as it ensures the viability of spikelets [8]. However, insufficient research has been conducted on the precise timing of plant growth regulator (PGR) treatment.

Therefore, we investigated the effects of PGRs applied at different developmental stages to shorten the maturation period of rice. In addition, the analysis of correlations between morphology, agronomy, physiology, and biochemistry in this study contributes to sustainable agriculture and global food security in other crops, especially in climate change conditions.

2. MATERIALS AND METHODS

2.1. Plant Material

The seeds of *Oryza sativa* L. cv. *OM5451* was sourced from the Mekong Delta Rice Research Institute in Vietnam.

2.2. Cultivation Conditions

Seeds were treated by soaking in warm water (50°C) for 2 h, followed by 24 h at 35°C [22]. Then, seeds were placed in wet paper and incubated in the dark for approximately 48 h until seedling roots reached 1–2 mm in length. After germination, seedlings were transplanted into boxes measuring 40 cm in width, 60 cm in length, and 40 cm in height. Each box was filled with 50 dm³ of soil (pH 6.0–6.5), supplied by Saigon Green Biotechnology Company (Ho Chi Minh City). The soil texture comprised 63.4% sand, 28.5% silt, and 8.1% clay. The soil had an organic matter content of 24.91 g/kg, available phosphorus of 0.062%, and potassium of 0.93%. Each box accommodated 24 plants, spaced 10 cm apart.

Plants were fertilized with a total of 100 kg/ha nitrogen (N), 40 kg/ha phosphorus (P₂O₅), and 30 kg/ha potassium (K₂O). Fertilizer application was split across growth stages: all P₂O₅ was applied pre-sowing; 50% of N and 50% of K₂O were applied 7 days after sowing (seedling stage); 25% of N and the remaining 50% of K₂O were applied 22 days after sowing (tillering stage); and the final 25% of N was applied 38 days after sowing (panicle initiation stage). Water levels were maintained at 6–7 cm above the soil surface during vegetative growth, reduced to 4–5 cm during the reproductive phase, and discontinued 15 days before harvest.

The experiment was conducted in conditions with a 12-h photoperiod (light intensity 150 ± 20 klux at noon), daytime temperatures of 33 ± 3°C (noon), nighttime temperatures of 24 ± 3°C (20:00), and relative humidity of 65 ± 5% (noon).

2.3. PGR Treatments

Exogenous PGRs, including IAA, GA₃, and benzyladenine (BA) (Merck, Germany) at 25 or 50 mg/L, were applied on three different

developmental stages: Tillering (26 days after sowing), panicle initiation (36 days after sowing), or booting (46 days after sowing). Treatments involved spraying 50 mL of PGR solution, containing 0.01% Tween-20 as a surfactant, onto the apical shoots daily for 3 consecutive days at a time between 16:00 and 17:00. Distilled water served as the control. Each treatment was replicated 4 times, each replicate included three containers, each container comprised 24 plants. The spikelets were tagged for subsequent monitoring. The following parameters were assessed:

- At the flowering stage: Days to 50% flowering (from sowing), flag leaf length (tip to blade base), and flag leaf area (calculated using LeafByte software, Getman-Pickering).
- At the ripening stage: Days to 85% seed yellowing on panicles, plant height (base to panicle tip), panicle length (main stem), percentage of filled grains per panicle, and 1000-grain weight (dried to 13% moisture).

2.4. Determination of Phytohormone Content in the Shoot Tip at Different Development Stages

Endogenous levels of IAA, GA₃, GA₄, and cis-zeatin riboside (cZR) in the shoot apex were measured on days 23 (tillering), 30 (internode elongation), and 37 (panicle initiation) after sowing, corresponding to the tillering, stem elongation, and panicle initiation stages, respectively. The apical shoots (0.5 cm in length) with mature leaves removed were ground, and extracted in either a methanol: water:formic acid mixture (15:4:1 by volume) for IAA and cZR or pure methanol for GA₃ and GA₄. Extracts were filtered and analyzed using HPLC-diode-array detection (DAD) (Agilent Series 1200, USA) on a Zorbax Extend C18 column, following the method of Nakagawa *et al.* [23]. Hormone concentrations were expressed as µg/g fresh weight (FW).

2.5. Experimental Design and Statistical Analysis

The experiment followed a randomized block design with four replicates per treatment, each replicate consisting of three containers (72 plants total per treatment). The data were tested for homogeneity of group variances (Levene test). Then, data were analyzed using one-way analysis of variance in Statistical Package for the Social Sciences 26.0 (IBM Corp., Armonk, NY, USA), with significant differences determined at *P* < 0.05 using Duncan’s test. Results are reported as means ± standard deviation.

3. RESULTS

3.1. Changes in Phytohormone Content in the Shoot Apex at Different Development Stages

The level of phytohormones in the shoot apex of *O. sativa* cv. *OM5451* exhibited distinct patterns across the tillering, internode elongation, and panicle initiation stages, as determined by HPLC-DAD analysis according to Table 1. Content of IAA decreased progressively from

Table 1: Changes in phytohormone content in the shoot apex at different development stages.

Development stages	Content of phytohormone (µg/g FW)			
	Zeatin	IAA	GA ₄	GA ₃
Tillering	-	1.45±0.07 ^a	8.15±0.66	19.33±0.37 ^a
Internode elongation	0.45±0.08 ^{ns}	0.91±0.13 ^b	-	1.04±0.05 ^b
Panicle initiation	0.55±0.04 ^{ns}	0.40±0.08 ^c	-	0.48±0.33 ^c

Different letters in each column indicate significant differences according to the Duncan test (*P*≤0.05). (–) Not present in the sample; (ns) no significant difference at *P*≤0.05. IAA: Indole-3-acetic acid, FW: Fresh weight, GA: Gibberellic acid.

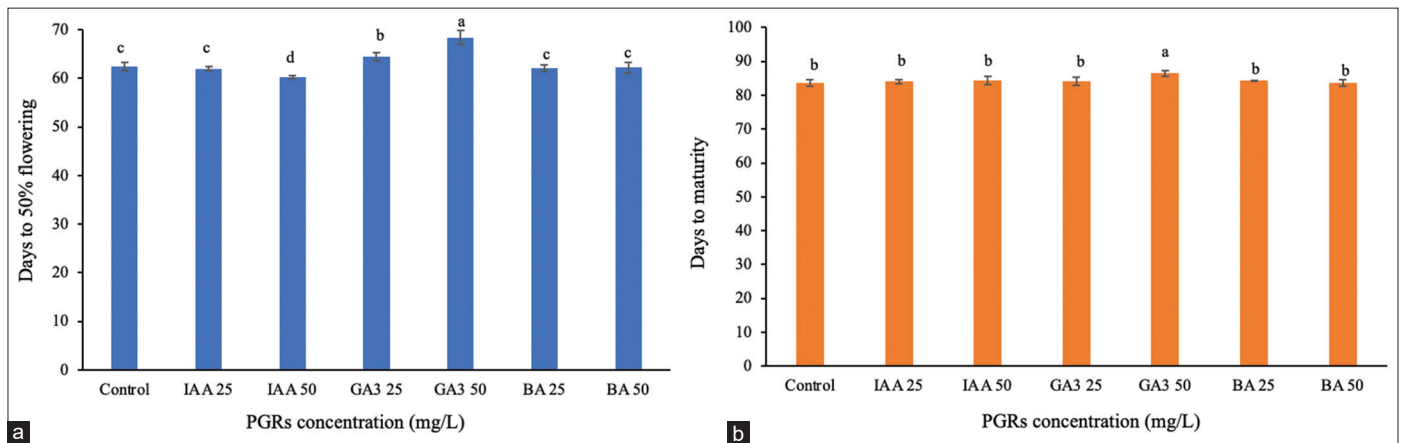


Figure 1: Effect of plant growth regulators treatment at the tillering stage on flowering and maturity time. (a) Days to 50% flowering, (b) days to maturity.

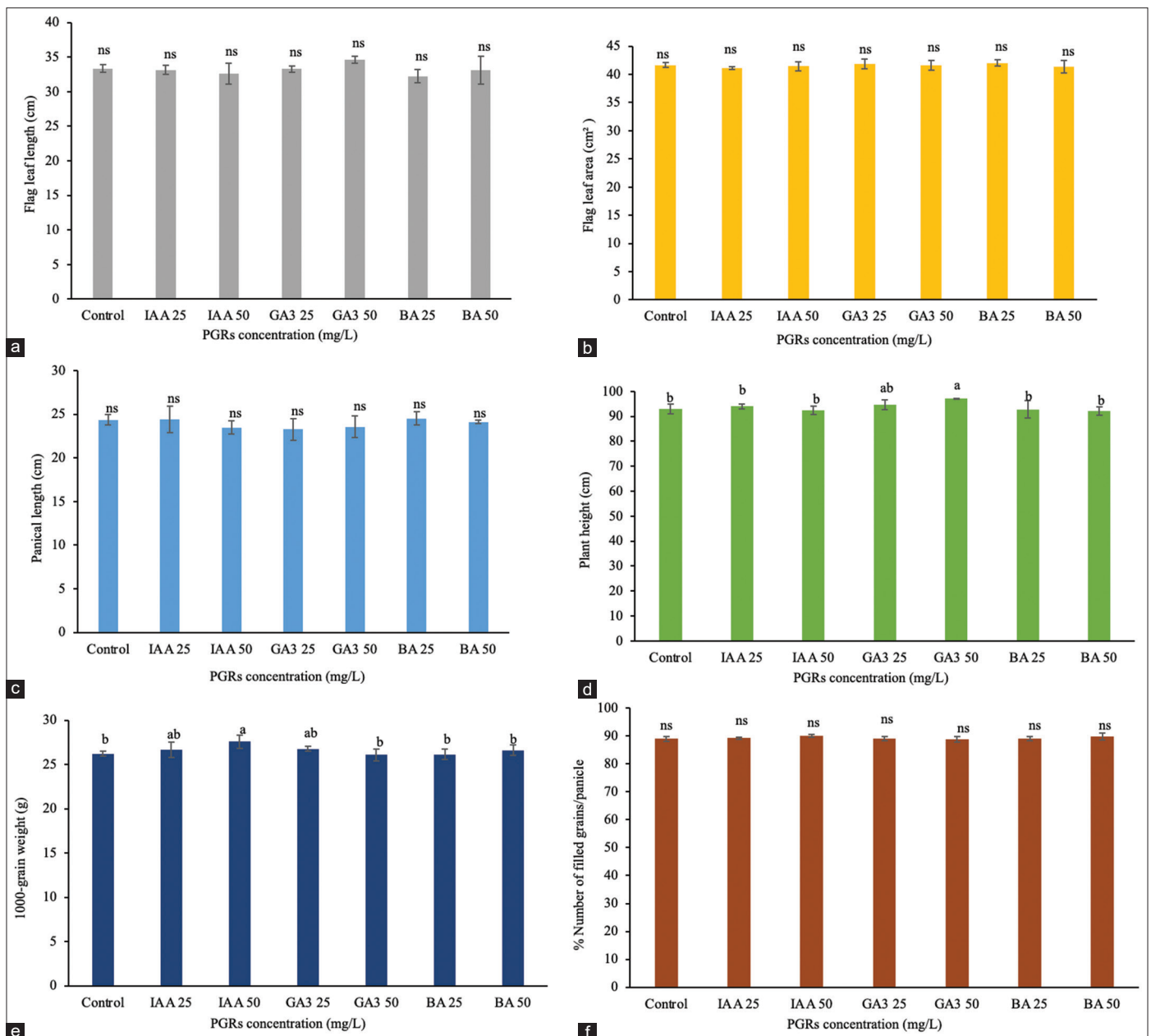


Figure 2: Effect of plant growth regulators on the development and grain yield during tillering stages. (a) Flag leaf length, (b) flag leaf area, (c) panicle length, (d) plant height, (e) 1000-grain weight, (f) the number of filled grains per panicle.

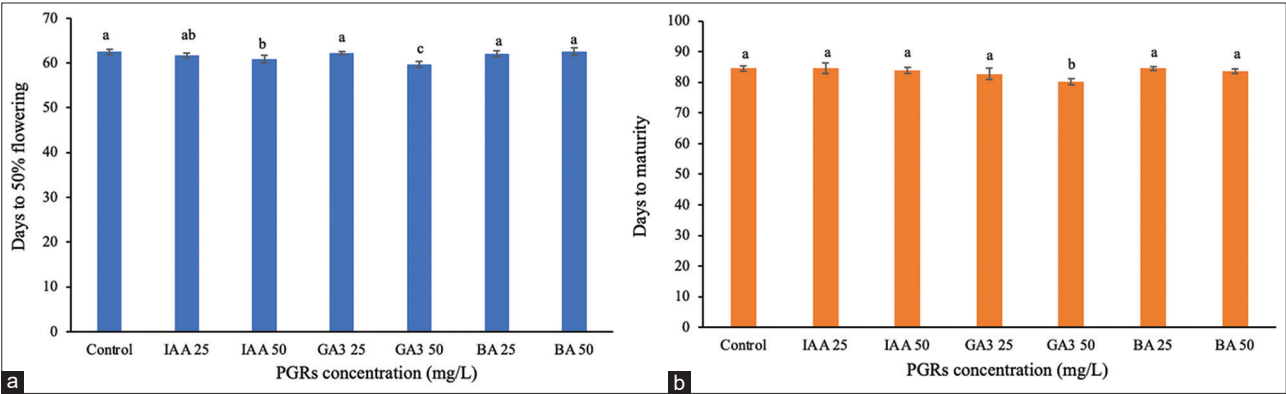


Figure 3: Effect of plant growth regulators treatment at pre-booting stage on flowering and maturity time. (a) Days to 50% flowering, (b) days to maturity. Data are presented as the mean $\pm$ standard deviation, according to Duncan's multiple range test.

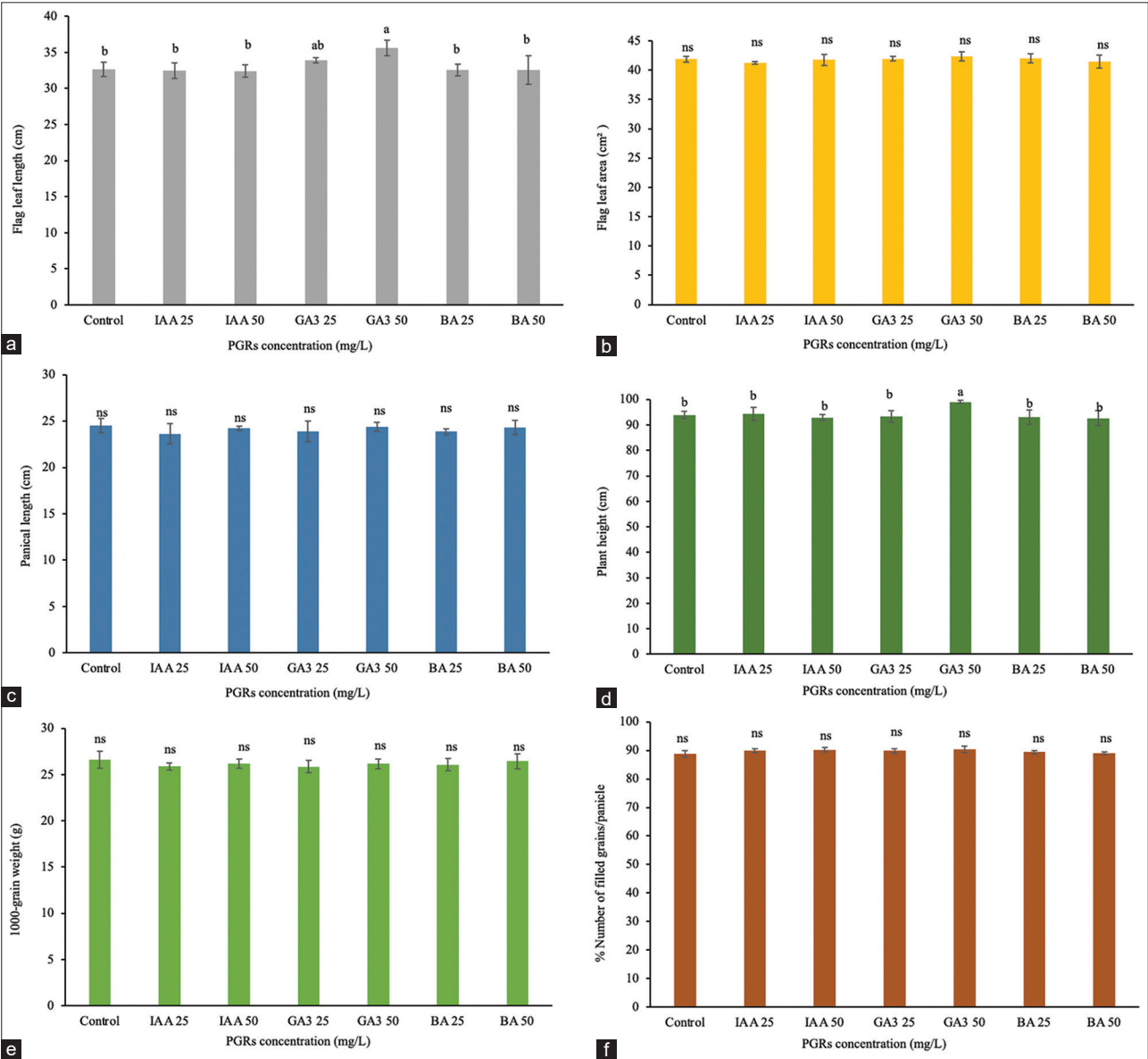


Figure 4: Effect of plant growth regulators treatment at pre-booting stage on the development and grain yield. (a) Flag leaf length, (b) Flag leaf area, (c) panicle length, (d) plant height, (e) 1000-grain weight, (f) the number of filled grains per panicle. Data are presented as the mean $\pm$ standard deviation, according to Duncan's multiple range test.

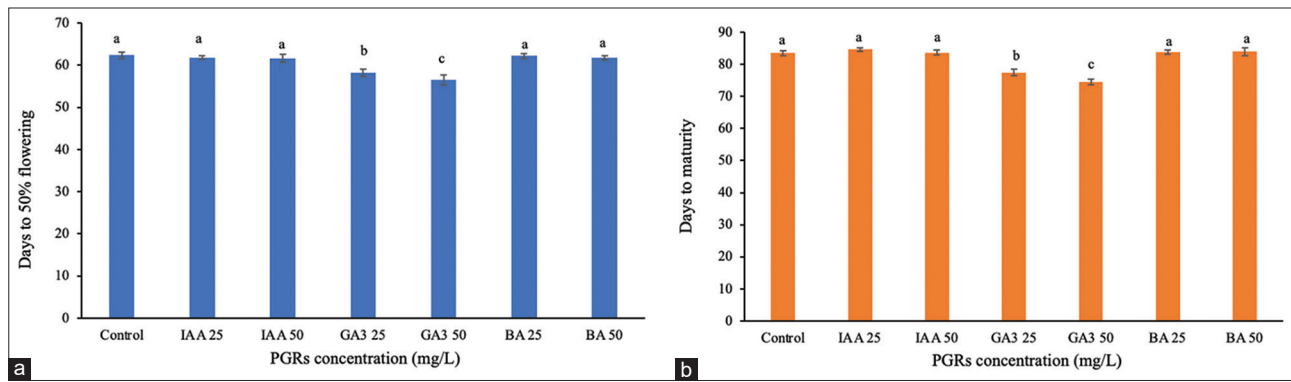


Figure 5: Effect of plant growth regulators treatment at the booting stage on flowering and maturity time. (a) Days to 50% flowering, (b) days to maturity. Data are presented as the mean $\pm$ standard deviation, according to Duncan's multiple range test.

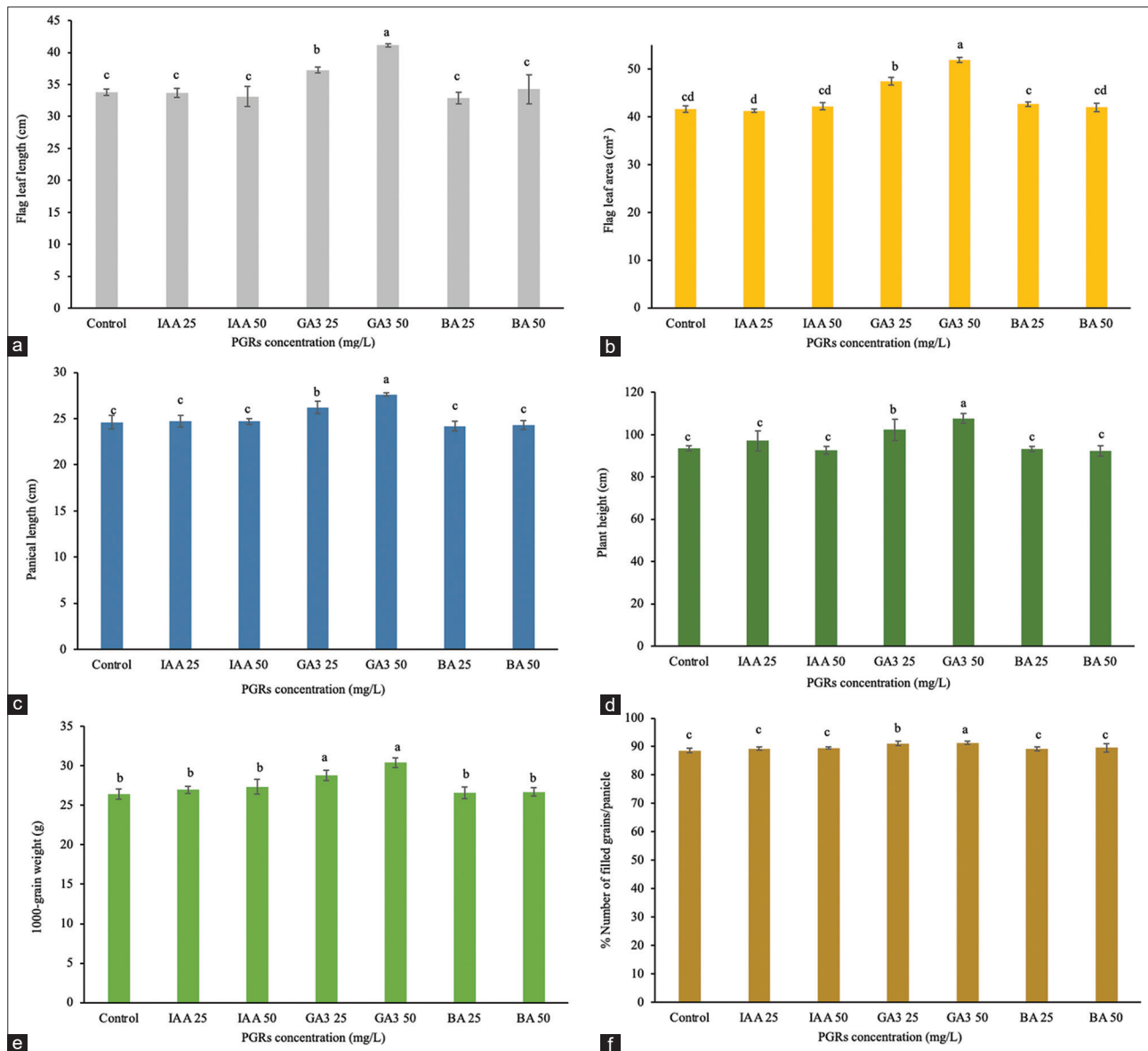


Figure 6: Effect of plant growth regulators treatment at the booting stage on the development and grain yield. (a) Flag leaf length, (b) flag leaf area, (c) panicle length, (d) plant height, (e) 1000-grain weight, (f) the number of filled grains per panicle. Data are presented as the mean $\pm$ standard deviation, according to Duncan's multiple range test.

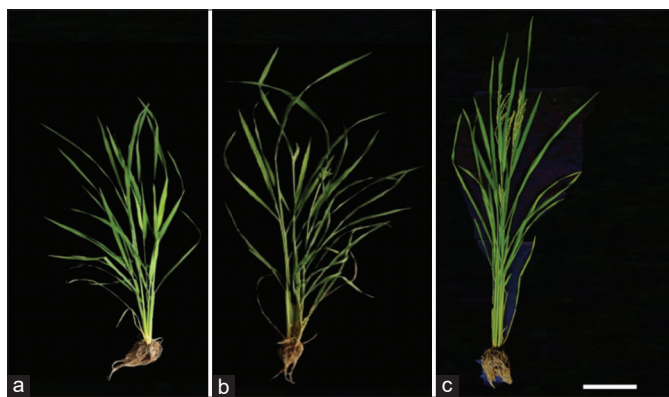


Figure 7: Effect of gibberellic acid (GA) treatment at the booting stage on the development, 58 days after sowing. (a) Rice plants without flowers are in control, (b) rice plants with newly emerged spikelets when treated with 25 mg/L GA_3 , (c) rice plants with fully opened spikelets when treated with 50 mg/L GA_3 . (Bar: 20 cm).

1.45 $\mu\text{g/g}$ FW at tillering to 0.91 $\mu\text{g/g}$ at internode elongation, and further to 0.40 $\mu\text{g/g}$ at panicle initiation, with statistically significant ($P < 0.05$) differences across all stages. GA_3 experienced a decline of 18.29 $\mu\text{g/g}$ from tillering to internode elongation, followed by a decrease of 0.56 $\mu\text{g/g}$ from internode elongation to panicle initiation, with both changes differing significantly ($P < 0.05$). GA_4 was detected exclusively at tillering (8.15 $\mu\text{g/g}$) and was absent in subsequent stages. In contrast, cZR was undetectable at tillering. Still, it emerged at internode elongation and panicle initiation, with no significant difference between these latter stages. These shifts indicate a transition from vegetative to reproductive priorities, with auxins and gibberellins dominating early growth and cytokinins supporting later reproductive development.

3.2. Effect of PGRs Applied at the Tillering Stage

Treatments with PGRs at the tillering stage (day 26) affected reproductive and yield traits [Figures 1 and 2]. Treatment with IAA at 50 mg/L significantly reduced ($P < 0.05$) the time to 50% flowering by 2.19 days compared to controls, increasing the 1000-grain weight to 27.58 g compared to the control 26.21 g. The flag leaf length (32.59 cm), area (41.47 cm^2), and panicle length (23.47 cm) remained unchanged [Figure 2]. In contrast, the application of GA_3 at 25 mg/L extended flowering by 2 days, whereas GA_3 at 50 mg/L delayed flowering by 5.94 days. In addition, GA_3 at 50 mg/L increased plant height to 97.16 cm, compared to 93.05 cm in the control, and prolonged maturity by 2.81 days, resulting in a total of 86.44 days compared to 83.63 days in the controls. However, there was no significant effect on grain weight, which remained at 26.08 g. BA at 25 mg/L (62.06 days) and 50 mg/L (62.19 days) had no significant impact on flowering time, maturity, or yield parameters. These results suggest that IAA promotes early reproductive onset and grain filling, whereas GA_3 at this stage prioritizes vegetative growth.

3.3. Effect of PGRs Applied at the Pre-booting (Panicle Initiation) Stage

During the panicle initiation stage (day 36), the treatments had an impact on reproductive development [Figures 3 and 4]. IAA at 50 mg/L reduced the time to 50% flowering by 1.68 days (60.88 days compared to the control at 62.56 days), with no significant changes in flag leaf traits, maturity (83.81 days), or 1000-grain weight (26.20 g). GA_3 at

50 mg/L decreased flowering time by 2.87 days, lowered maturity by 4.37 days (84.50 days in the control), increased flag leaf length to 35.58 cm, and raised plant height to 99.02 cm, whereas 1000-grain weight (26.16 g) remained unchanged. GA_3 at 25 mg/L and BA at both 25 and 50 mg/L concentrations showed no significant differences from the control group in most traits. These findings suggest that GA_3 enhances both reproductive development and vegetative growth traits such as flag leaf length and plant height at this stage, whereas IAA primarily accelerates the onset of flowering without affecting yield-related traits.

3.4. Effect of PGR Treated at the Booting Stage

The most significant effects were observed with treatments at the booting stage (day 46) [Figures 5 and 6]. The GA_3 at 25 mg/L treatment showed significantly impacted plant development, reducing the time to 50% flowering by 4.25 days (58.13 days for treated plants and 62.38 days for controls) and shortening maturity by 6 days (77.50 days for treated and 83.50 days for controls) [Figure 7]. The treatment resulted in increased flag leaf length (37.27 cm), flag leaf area (47.41 cm^2), panicle length (26.21 cm), and 1000-grain weight (28.82 g). GA_3 at 50 mg/L reduced flowering time by 5.88 days and maturity by 9.06 days, while also enhancing growth parameters, such as flag leaf length (increased by 7.35 cm) and flag leaf area (increased by 10.27 cm^2). The plant height rose by 14.08 cm, and panicle length grew by 3.01 cm. The percentage of filled grains increased to 91.33% from 88.59% in the control group ($P \leq 0.05$). In addition, the weight of 1000-grain grew from 26.43 g to 30.40 g. Treatment with IAA at 25 mg/L and 50 mg/L, as well as BA at both concentrations, showed no significant effects on any measured traits.

4. DISCUSSION

In *O. sativa* cv. OM5451, IAA concentration gradually decreased through the stages from 1.45 $\mu\text{g/g}$ FW at tillering to 0.91 $\mu\text{g/g}$ FW at internode elongation and continued to decline at panicle initiation (0.40 $\mu\text{g/g}$ FW) [Table 1]. IAA levels decreased significantly from tillering to flowering initiation, suggesting a key role for auxin in growth processes such as shoot formation and stem elongation. This decrease may facilitate the diversion of essential nutrients to reproductive structures, supporting the growth of the cotton shoot meristem, as observed in previous studies [24,25]. Similarly, GA_3 exhibited the highest concentration at tillering (19.33 $\mu\text{g/g}$ FW), followed by a marked decline to 1.04 $\mu\text{g/g}$ FW at internode elongation and 0.48 $\mu\text{g/g}$ FW at panicle initiation. GA_3 showed a significant decrease during these stages, whereas GA_4 was present only at tillering (8.15 $\mu\text{g/g}$ FW) and was absent in later stages [Table 1]. High levels of gibberellins during tillering promote stem elongation and vegetative growth, consistent with their established functions in rice [15]. The decrease in gibberellin levels indicates the transition from the vegetative to reproductive growth phase, consistent with the timing regulation of gibberellins [21]. GA_4 at tillering suggests a role in early vegetative growth as previously studied [26]. In contrast, zeatin was undetectable during the tillering stage but emerged at 0.45 $\mu\text{g/g}$ FW during internode elongation, increasing slightly to 0.55 $\mu\text{g/g}$ FW at panicle initiation, with no significant difference between these stages. Zeatin was not detected during tillering but increased during internode elongation and panicle initiation, suggesting an important involvement of cytokinin in reproductive development, especially in maintaining panicle formation. This increase supported flower development, as shown in a previous study [10,14]. Decreased IAA and gibberellin, and increased cZR highlight a coordinated hormonal transition from

a vegetative to a reproductive priority, with implications for yield-enhancing.

Treatments with PGRs provide deeper insight into their hormonal functions and their ability to boost crop productivity. When 50 mg/L of IAA is applied during the tillering phase, flowering is hastened by 2.19 days, and the 1000-grain weight increases to 27.58 g from 26.21 g observed in untreated plants [Figures 1 and 2]. Auxin likely increases meristematic activity, triggering panicle formation, which accelerates flowering and promotes grain growth [18,20]. According to Yu *et al.* [27], IAA increases grain filling in young rice panicles by improving sugar transport. Auxins play a significant role in the development of vascular tissues, which function as essential conduits for the long-distance transport of assimilates [28,29]. The absence of notable changes in flag leaf dimensions or panicle length indicates that IAA mainly influences developmental timing and grain filling. Conversely, applying 50 mg/L of GA₃ at the tillering stage postpones flowering by 5.94 days and maturity by 2.81 days, whereas elevating plant height to 97.16 cm [Figure 1]. This postponement stems from GA₃ prioritizing vegetative growth over the start of reproduction, as elevated gibberellin levels during tillering encourage stem lengthening. Thus, our results corroborate those of Nagai *et al.* [30]. Treatments possibly interfering with the processes that initiate flowering, such as the results of Sun and Gubler [31].

The results of treatment at the initial stage of rice panicle formation showed the effect of PGRs at this stage [Figures 3 and 4]. When 50 mg/L IAA is administered, flowering occurs 1.68 days earlier, whereas the same concentration of GA₃ cuts flowering duration by 2.87 days and maturity by 4.37 days. In addition, GA₃ induces slight improvements in flag leaf length (35.58 cm) and plant height (99.02 cm). These observations imply that both phytohormones can advance reproductive timing when introduced at panicle formation. Although their effects on grain yield were not as pronounced as those of GA₃ treatments applied earlier at the heading stage, these results were also reported by Yang *et al.* [21]. In contrast, BA at 25–50 mg/L concentrations – tested at multiple growth phases – failed to produce measurable changes in flowering, maturation, or grain weight. This lack of response may stem from inadequate dosage, suboptimal application timing, or divergent activity between synthetic cytokinins and naturally occurring zeatin, which aids panicle development without hastening flowering in rice systems [32].

The greatest improvements in crop yield are observed when GA₃ is applied during the booting phase. A 50 mg/L application of GA₃ reduces flowering time and maturity while increasing flag leaf length and area, plant height, panicle length, filled grain percentage, and 1000-grain weight [Figures 5 and 6]. These results indicate that GA₃ enhances panicle exertion, photosynthetic capacity through larger flag leaves, and grain filling efficiency, consistent with its documented role in promoting stem elongation and spikelet development during reproductive stages. Comparable results were also identified in a research study focused on *Indica* hybrid rice [33]. According to previous research, GA₃ and IAA treatment at flowering reduced sterility and improved yield by elongating leaf length [34]. The accelerated maturity and higher grain weight suggest improved assimilate partitioning, likely mediated by gibberellin-induced sink strength [35]. In addition, GA₃ treatment at 25 mg/L during the heading stage reduced flowering time by 4.25 days and maturity by 6 days, and increased grain weight by 28.82 g, highlighting a clear correlation between concentration and plant response to treatment. The effects of PGRs vary depending on the growth phase of the plants, highlighting their applications in

agriculture. In other studies, IAA applied during tillering may enable earlier flowering, whereas GA₃ treatment at late tillering stage and 10–30% flowering stage (early reproductive) increased yield by more than 27%, thus GA₃ treatment at heading stage increased plant yield [36]. While IAA promotes cell division and elongation, GA₃ not only stimulates these processes but also enhances the translocation of photosynthetic assimilates to developing seeds and plays a key role in inflorescence formation. These combined actions accelerate floral initiation and development, resulting in earlier flowering. Therefore, the timely application of GA₃ at specific developmental stages serves as an effective strategy to regulate flowering time and improve crop yield. These outcomes corroborate existing evidence identifying auxins and gibberellins as hormones of rice reproduction [37]. This suggests that the PGR treatment was more effective at the pre-flowering stage than previously reported. These findings highlight the intricate correlations among morphological, physiological, and biochemical changes during rice grain development, providing valuable insights for optimizing growth and yield. The application of PGRs at specific developmental stages demonstrates potential in reducing the maturation period without compromising grain quality, which is particularly important in the context of climate variability and changing growing seasons.

5. CONCLUSION

In *O. sativa* cv. OM5451, the level of endogenous IAA and GA₃ decreased from tillering to panicle initiation stage, marking the transition from vegetative to reproductive growth. GA₄ levels were only detected during the tillering stage, whereas zeatin appeared during the stem elongation and panicle initiation, supporting reproductive processes. Foliar application of 50 mg/L IAA at the tillering stage advanced flowering by 2.19 days and increased the 1000-grain weight by 1.37 g, hastening reproductive onset. Treatment with 50 mg/L GA₃ at booting reduced flowering and maturity durations by 5.88 and 9.06 days, respectively, and enhanced flag leaf size, panicle length, and grain weight, resulting in improved grain filling and yield. These findings demonstrate a strong correlation between the known roles of PGRs and their practical applications in agriculture. Targeted PGR application, based on agronomic analysis, significantly enhanced the development and yield. Nevertheless, further molecular analysis and large-scale field trials are necessary to validate and optimize these effects under changing environmental conditions.

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7. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data are available to the authors and shall be provided upon request.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI) tools for writing and editing the manuscript, and no images were manipulated using AI.

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