Indian J. Genet. Plant Breed., (2025); 85(3): 398-406
https://doi.org/10.31742/ISGPB.85.3.5
ISSN: 0975-6906
www.isgpb.org

RESEARCH ARTICLE

Gibberellic acid and Boron application synergize enhanced seed production in a thermo-sensitive genetic male sterility (TGMS) based novel hybrid, Pusa Jawahar Rice Hybrid 56

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Abstract

The commercial adoption of hybrid rice is often constrained by low outcrossing rates in male sterile lines, necessitating agronomic interventions to improve pollination efficiency, outcrossing, and hybrid seed production. This study investigates the impact of gibberellic acid (GA_3) and boron on floral traits and yield-related parameters in the TGMS-based hybrid rice, Pusa PJRH 56. Multi-location trials were conducted across three agroclimatic zones represented by sites, Aduthurai, New Delhi, and Jabalpur, to evaluate the consistency of treatment effects under varying environmental conditions. Significant trait variations were observed across treatments and locations. Compared to the control, foliar application of GA_3 (200 ppm) and boron (1%) significantly enhanced key floral traits in both the parental lines. Stigma emergence was improved by 30.3%, glume opening angle by 51.2%, stigma receptivity by 21.3%in the female parent, and anther breadth by 13.1%and anther width by 21.3%in the male parent. These improvements facilitated increased pollen exposure and receptivity, which are critical for successful hybrid seed production. Additionally, agronomic traits such as plant height, panicle exertion, filled seeds per panicle, and seed yield per plot showed significant increases of 32.2, 87.4, 55.6, 152.6, and 103.0%, respectively. These findings demonstrate that GA_3 and boron work synergistically to improve floral development, seed formation, and overall seed yield. This study provides strong evidence for the exogenous application of GA3 and boron as an effective agronomic strategy to enhance hybrid seed production and overcome the limitations associated with TGMS-based hybrid rice cultivation.

Keywords: TGMS hybrid, gibberellic acid, boron, floral traits, seed yield.

Introduction

Rice (*Oryza sativa* L.) is the staple food for a majority of India's population, playing a vital role in national food security and the economy. India contributes over 23% to global rice production, ranks second in consumption after China, and dominates international trade by accounting for more than 40% of global rice exports to over 150 countries. However, projections from the International Global Rice Model (IGRM) baseline scenario indicate that between 2022 and 2050, rice cultivation area in India may grow by just 1.9%, while consumption and exports are expected to increase by 14.3 and 5.4%, respectively (Valerien et al. 2024). This scenario underscores the urgency of enhancing rice productivity through advanced technologies like hybrid rice and efficient water management. Although India cultivates rice across 47.83 mha and produces 206.73 mt annually, average productivity remains relatively low at 4.32 t/ha compared to China (FAOSTAT 2023). The yield gap is primarily due to limited adoption of hybrid rice, which in China covers 57% of rice area and contributes 65% of total output (Ashraf et

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How to cite this article: Pashwan V.R., Kumar M.B.A., Singh C., Krishnan S.G., Vinod K.K., Nagarajan M., Dunna V. and Gaikwad K. 2025. Gibberellic acid and Boron application synergize enhanced seed production in a thermo-sensitive genetic male sterility (TGMS) based novel hybrid, Pusa Jawahar Rice Hybrid 56. Indian J. Genet. Plant Breed., **85**(3): 398-406.

Source of support: NFST (No. 202122-NFST-TEL-00648), Ministry of Tribal Affairs, Govt. of India and ICAR-SRF (Roll No. TL01001531)

Conflict of interest: None.

Received: April 2024 **Revised:** June 2024 **Accepted:** July 2025

al. 2024). In contrast, hybrid rice cultivation in India remains minimal. Expanding hybrid rice acreage is therefore crucial to bridge the yield gap, secure national food supplies, and sustain India's competitiveness in the global rice market.

India began developing cytoplasmic male sterility (CMS) based hybrid rice in 1989, leading to the release of over 127 hybrids for commercial cultivation. However, hybrid rice adoption remains limited, with a diffusion rate of only 8% (Verma et al. 2021). Key barriers include the high cost of hybrid seeds relative to inbred varieties, inferior grain quality, and lower market prices, all of which reduce profitability. Moreover, the three-line CMS system involves a complex and labour-intensive seed production process. Limited adaptability to diverse agro-ecological conditions, increased vulnerability to pests and diseases, and inconsistent yield performance further hinder large-scale adoption. Promotional efforts have been insufficient to overcome low farmer awareness and restricted access to quality hybrid seeds (Spielman et al. 2013). To address these challenges, China adopted a two-line hybrid rice system based on environmentally sensitive genetic male sterility (EGMS), including photoperiod-sensitive (PGMS), thermosensitive (TGMS), and photo-thermosensitive (PTGMS) genic male sterility (Yuan 1987). Unlike CMS, EGMS traits are governed by nuclear genes and triggered by environmental cues such as temperature or day length, eliminating the need for a maintainer line. This simplification reduces seed production costs, enhances parental diversity, and improves adaptation across environments. As a result, two-line hybrids accounted for 37.8% of the total hybrid rice area in China in 2015, and this approach has now become the dominant method for exploiting heterosis in rice (Zhang et al. 2023).

In India, thermosensitive genic male sterility (TGMS) lines are preferred over photoperiod-sensitive lines (PGMS), due to the complex inheritance of PGMS in *indica* backgrounds and the widespread availability of rice-growing areas with temperature regimes conducive to TGMS expression (Cheng et al. 1996; Lopez and Virmani 2000). Although the research on TGMS rice began at ICAR-Indian Agricultural Research Institute (IARI), New Delhi, as early as 1995 (Ali et al. 1995), and in other institutions such as TNAU, Coimbatore (Kalaiyarasi et al. 2006), the first commercial public-bred TGMS-based stable rice hybrid in India, Pusa JRH 56 (PJRH56; IET 27333), was released in 2022 by IARI in collaboration with Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur. The female parent, PUSA 1S, is a TGMS line with a flowering duration of 110 days and sterility above 28°C, reverting to fertility below 25°C. The male parent, PRR 158, flowers 10 days earlier. PJRH56 matures in 120 days, features long, slender aromatic grains, and recorded an average yield of 61.5 q/ha across three years of trials in Madhya Pradesh. Its early maturity supports crop intensification, making it a promising option for enhancing hybrid rice cultivation in India.

The commercial success of any newly released hybrid largely depends on the efficiency and cost-effectiveness of hybrid seed production, which is primarily governed by the seed-setting percentage of the female parent. In the TGMS line Pusa 1S used as the female parent of PJRH56, limited studies have been conducted on its floral traits. Partial panicle emergence during hybrid seed production has resulted in low seed-setting rates (20–22%), posing a challenge for large-scale adoption. Enhancing seed set in Pusa 1S through agronomic interventions could reduce hybrid seed production costs, making hybrid seeds more affordable and improving their adoption. In CMS-based rice hybrids, partial panicle emergence and poor seed set have been effectively managed by applying gibberellic acid (GA_3) at the booting stage, which improves floral traits and seed setting (Mao et al. 1998; Jagadeeswari et al. 2004; Hamad et al. 2021b; ElShamey et al. 2022; Haifaa and Moses, 2022; Harshitha et al. 2024). However, such practices have not been comprehensively evaluated in PJRH56. Additionally, optimizing management practices in the male parent is critical for improving seed set in the female parent. Boron (B) application has also shown promise in enhancing seed set in CMS-based rice lines. B plays a key role in reproductive processes, including anther development, microsporogenesis, pollen viability and germination (Yang et al. 1999; Nieuwenhuis et al. 2000; Hamad et al. 2021a). During reproduction, B levels above 20 mg/kg are required for normal pollen development (Lordkaew et al. 2013). In this study, foliar application of GA3 and B was evaluated to improve seed setting in Pusa 1S during the hybrid seed production of PJRH56.

Materials and methods

Plant materials

The parental lines of the TGMS-based rice hybrid PJRH56, Pusa 1S (female) and PRR158 (male), were utilized in this study. Pusa 1S is a semi-dwarf, non-lodging genotype with a sturdy stem and erect flag leaves. It exhibits complete pollen sterility at temperatures above 28°C and achieves over 75% pollen fertility when temperatures fall below 25°C. The male parent, PRR158, shares similar traits, being a semi-dwarf, the non-lodging genotype with a sturdy stem, erect flag leaves, and aromatic, long, slender grains. Both parental lines were developed at IARI, New Delhi, and the pure seed material was obtained from the Division of Genetics, IARI, for this investigation.

Experimental sites and layout

The experiments were conducted at three distinct locations, representing three different agroclimatic zones in India: New Delhi (L1), Aduthurai, Tamil Nadu (L2) and Jabalpur, Madhya Pradesh (L3). The L1 site, situated at IARI research farm (28.4°N; 77.1°E), represents the Trans-Gangetic plain zone in northern India, which experiences an average annual rainfall of 797.3 mm over 39 rainy days, with a mean maximum temperature of 34°C during the rice-growing season. The L2 site, representing the Eastern coastal region, was located at the experimental farm of IARI-Rice Breeding and Genetics Research Centre (11°N; 79.3°E), receives an average annual rainfall of 1139 mm across 56 rainy days, with a mean maximum temperature of 31.8°C during the rice off-season (*Rabi*). The L3 site, located at the research farm of the College of Agriculture, Waraseoni (Balaghat), represented the Central Plateau and Hills region was positioned at 21°45′N and 80°50′E in Central India. This site records an annual average rainfall of 1183 mm over 43 rainy days, with a mean maximum temperature of 31.9°C during the December to May period. The experiment at L1 was conducted during the *kharif* season (June to November) of 2023, whereas those at L2 and L3 were conducted during the *Rabi* season (December–May) of 2023–24.

The experiments followed a Randomized Block Design (RCBD) with three replications, uniformly applied across all three sites. To address a 12-day difference in flowering between the parental lines observed in the initial experiment (data not shown), staggered sowing was adopted to ensure synchronized flowering. Pusa 1S was sown 10 days earlier than PRR 158, with two additional staggered sowings of PRR158on 12th and 14th day. Prior to transplanting, PRR 158 seedlings from the three sowing dates were mixed in equal proportions. Transplanting was conducted in a 2:4:2 row ratio, where two rows of PRR 158 flanked four rows of Pusa 1S on either side. Each hill was planted with a single seedling, maintaining a spacing of 20 cm × 15 cm. The individual treatment plot size was 4.8 m^2, consisting of 84 female plants and 84 male plants. To prevent cross-pollination between adjacent plots and ensure the genetic purity of F_1 hybrid seeds, a 6-ft-high polyethylene barrier surrounded each plot. Furthermore, the experimental plot was situated 150 m away from other rice fields, and isolation measures were strictly followed during flowering.

Treatments

A commercial GA_3 formulation (ProGibb®, Sumitomo Chemical India Ltd.) containing 90% active ingredient was used as the GA_3 source, while di-sodium tetraborate decahydrate ($Na_2B_4O_7.10H_2O$) of extra pure grade (Labogens Fine Chem Ind..) was used as the source of B. Eight experimental treatments were given: T_0 (Control), T_1 (GA_3 at 100 ppm), T_2 (GA_3 at 200 ppm), T_3 (Bat 0.5%), T_4 (Bat 1%), T_5 (GA_3 at 100 ppm + Bat 0.5%), T_6 (GA_3 at 200 ppm + Bat 0.5%), T_7 (GA_3 at 100 ppm + Bat 1%), and T_8 (GA_3 at 200 ppm + Bat 1%). The required quantity of GA_3, as per the treatment, was dissolved in 70% ethanol at a rate of 20 mL of ethanol per gram of GA_3, and the volume was adjusted with water to achieve the desired concentration of the GA_3 solution. Similarly, the calculated amount of disodium tetraborate decahydrate was dissolved in the appropriate volume of water to prepare the required concentration of the B solution. Treatment solutions were applied using a knapsack sprayer during the5 % heading stage. The control treatment (T_0) received only water, equivalent to the spray volume of the other treatments. To enhance pollination, supplementary pollination by rope pulling was conducted three times daily from 9:30 am to 12:00 pm for 12 consecutive days after the treatments were applied.

Methodology and data collection

At L1 and L2 sites, during flowering, observations were recorded on floral traits, including stigma emergence (StE), angle of glume opening (GlO), and stigma receptivity (StR) in the female parent, as well as anther length (AnL) and anther width (AnW) in the male parent. In each treatment and replication, five plants were selected, and from each plant, five fully opened spikelets were collected for floral trait measurements. Anther measurements were conducted using a stereomicroscope (Leica S9i, Germany) with a built-in measuring scale and analysed with the Leica Application Suite (LAS) version 4. For this, anthers were carefully excised from the spikelets without damage and mounted on a slide with a droplet of water to prevent desiccation. The anther images were captured, and their length and width were determined using the line tool in LAS. To assess stigma emergence, images of the fully opened spikelets were captured, and both the length of the stigma protruding outside the spikelet and its total length were measured using the line tool in LAS. Stigma emergence (StE) was then calculated as a percentage using the following formula:

$$\text{Stigma emergence (\%)} = \frac{\text{Length of the stigma protruding outside the spikelet (cm)}}{\text{Total length of stigma (cm)}} \times 100$$

To determine the angle of glume opening, the floret image was imported into ImageJ software (Broeke et al. 2015), and the angle was measured using the angle tool, following the guidelines provided in the software manual.

To evaluate stigma receptivity (StR), ten female plants were selected from each treatment and replication. At the onset of flowering, the main axis panicle from each of the plants was prepared by removing all opened spikelets. Among the remaining unopened spikelets, the top one-fourth of the glumes were clipped at an angle without damaging the stigma, from 100 spikelets and all anthers were removed to prevent self-pollination, despite their sterility. All the remaining unopened spikelets were removed, and the panicle was covered with a butter paper bag and labeled. Each day for the next ten days, one of the ten tagged panicles was hand-pollinated using bulk viable pollen from the male parent and then re-covered with a butter paper bag. Twenty days post-pollination, the number of filled spikelets (FSp) among the emasculated spikelets per panicle was recorded. Stigma receptivity (StR) on each plant was then calculated as a percentage using the following formula:

$$\text{Stigma receptivitypercent (StR)} = \frac{\text{Number of filled spikelets (FSp)}}{\text{Total number of emasculated spikelets}} \times 100$$

The average StR was calculated over ten plants.

During the crop maturity stage, data were collected on plant height (PlH), panicle emergence(PnE), number of productive tillers per plant (PTiP), total spikelets per panicle (TSpP), filled seeds per panicle (FSeP), and spikelet fertility rate (SpF). Measurements were taken from five randomly selected, uniform-looking plants per treatment and replication across all three locations (L1, L2, and L3). Additionally, seed yield per plot (SyP) and seed test weight (STw) were recorded for each treatment and replication after harvest, following seed drying at 36°C for 48 hours. Plant height was measured in cm from the base of the plant to the tip of the longest leaf at the milky stage of the seed. To determine panicle emergence, panicle length (PnL) was measured in centimeters from the base to the tip of the panicle on the main axis at the seed maturation stage. Panicle exertion (PnEx) was recorded as the distance (cm) from the flag leaf collar to the tip of the panicle at the same stage. Panicle emergence (PnE) was expressed as the percentage of panicle exertion length (PnEx) relative to the total panicle length (PnL), calculated using the following formula:

$$\text{Panicle emergence percent (PnE)} = \frac{\text{Panicle exsertion length (PnEx) in cm}}{\text{Total panicle length (PnL) in cm}} \times 100$$

The number of tillers that produced spikelets and/or seeds at the time of crop maturity in a plant was counted as productive tillers. The total number of spikelets in a panicle, irrespective of whether they were filled or not, including the one that had not emerged from the flag leaf, was counted and recorded as the total number of spikelets per panicle (TSpP). Similarly, the number of spikelets in a panicle, which formed the seeds, was counted and recorded as the number of filled seeds per panicle (FSeP). Spikelet fertility (%) was calculated as the proportion of filled seedsto the total number of spikelets in a panicle, using the following formula:

$$\text{Spikelet fertility percent (SpF)} = \frac{\text{Number of filled seeds (FSeP) in a panicle}}{\text{Total number of spikelets (TSpP) in a panicle}} \times 100$$

The seed test weight (g) was recorded in each treatment and replication-wise harvested seeds by following the ISTA procedure (ISTA 2024).

Statistical analysis

The pooled data analysis was conducted by combining data from all locations to assess the overall treatment effects across different locations (McIntosh 1983). Before pooling, Bartlett's K-squared test statistic was used to ensure homogeneity of error variances across the locations. As error variances across locations are homogenous, an ANOVA (Analysis of Variance) was performed using a mixed model considering the locations and treatments as random. All possible pairwise comparisons of treatment means were conducted using the Critical Difference (CD) derived at p <0.05 from the mixed-model pooled ANOVA for each respective trait. Based on the significance or non-significance of differences between treatments, they were categorized into distinct groups. All statistical analyses were carried out in the R statistical environment.

Results

Effects of GA_3, Boron, and their interactions on floral traits

ANOVA (Table 1) revealed significant ($p \leq 0.05$) effects of sites, treatment, and replication within sites on multiple floral traits in female and male parents. However, the interaction between location and treatment was not significant, suggesting that the treatment effects were consistent across locations. The treatment effects were highly significant ($p \leq 0.01$) for StE, AGO, StR, AnL and AnW, indicating that exogenous application of GA_3, B, and their combinations significantly influenced these traits. The minimal differences in mean values for floral traits between locations (Table 2) such as StE (0.73%), AGO (0.23°), StR (0.77%), AnL (0.08 mm), and AnW (0.02 mm), indicate that although the location effect was statistically significant for most of the traits except AGO ($p \leq 0.01$ or 0.05), its practical impact on treatment performance was negligible. The replication of the location effect was significant ($p \leq 0.05$ or 0.01) for stigma receptivity, anther length, and anther width, confirming some level of variability within locations.

Pooled mean performance of floral traits across locations

The pooled means for floral traits (Table 2) highlight the positive influence of GA_3, B, and their combinations on StE, AGO, and StR in female parent, AnL, and AnW in male parent. Among the nine treatments, T_8 (GA_3 at 200 ppm + B at 1%) consistently exhibited the highest values across all traits, followed by other treatments, while the untreated control (T_0) recorded the lowest values. Thehighest stigma emergence (69.8%) and glume opening angle (42.6°) were recorded in T_8, significantly exceeding the untreated control (53.6% and 28.2°, respectively), representing 30.3 and 51.2% increases, which enhanced pollen exposure and transfer [Figure 1A(T_0) and A(T_8)].GA_3 and boron applications significantly improved stigma receptivity, with T_8 recording the highest value (79.0%), a 21.3% increase over the control (65.2%), highlighting the effectiveness of the treatment in prolonging the duration of stigma receptivity to improve seed setting.In the male parent, T_8 exhibited the longest anther length (12.5 mm), 13.1% longer than the untreated

Table 1. Mixed-model pooled ANOVA for the effects of $GA_3$and B on floral traits

Source of variance	DF	Mean square				
		StE	AGO	StR	‡AnL	‡AnW
Location	1	7.15**	0.696	8.035**	0.084*	0.007*
Treatment	8	139.119**	121.421**	93.981**	1.239**	0.107**
Replication within location	4	14.911	8.476	47.420*	0.372**	0.071**
Location x Treatment	8	0.352	0.578	0.281	0.011	0.001
Error	32	12.19	16.268	18.243	0.047	0.001

StE = Stigma emergence, AGO = Angle of glume opening, StR = Stigma receptivity, AnL = Anther length, AnW = Anther width, DF = Degrees of freedom, *Significant at $p \leq 0.05$, **Significant at $p \leq 0.01$, ‡Male parent trait.

Table 2. Pooled mean performance of floral traits under GA_3 and Boron foliar application across locations

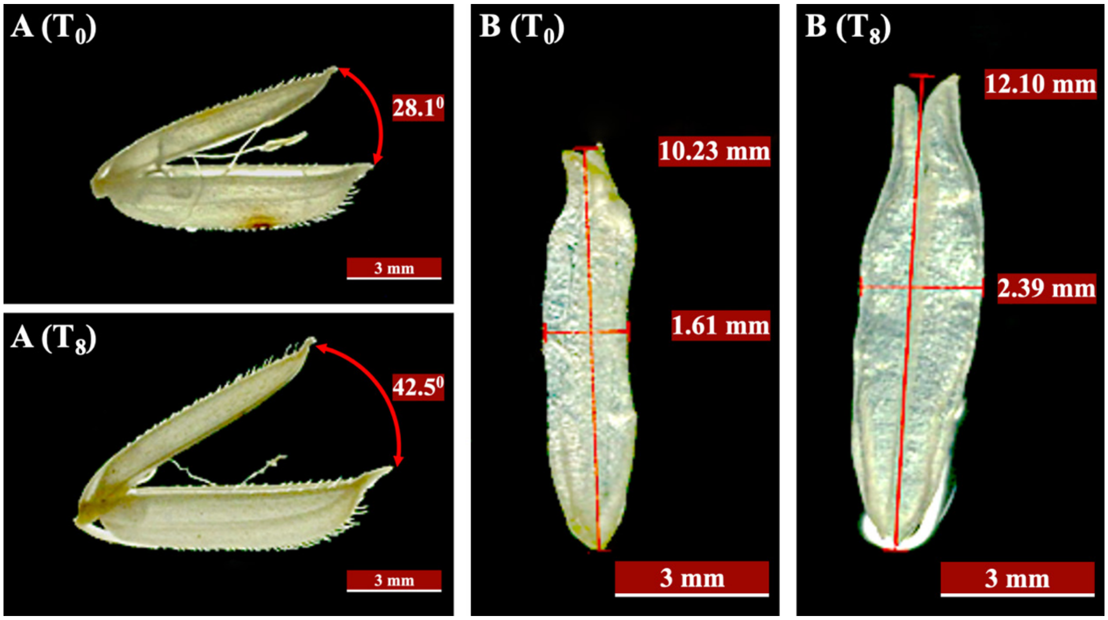
Treatments	StE (%)	AGO (°)	StR (%)	AnL (mm)	AnW (mm)
Control (T_0)	53.59^c	28.17^b	65.18^b	11.04^c	2.07^f
GA_3 @ 100 ppm (T_1)	60.18^b	37.98^a	73.31^a	11.70^b	2.21^d
GA_3 @ 200 ppm (T_2)	61.62^b	40.58^a	74.36^a	11.85^b	2.24^d
Boron @ 0.5% (T_3)	57.45^c	32.43^b	72.13^a	11.53^b	2.13^e
Boron @ 1% (T_4)	57.30^c	32.63^b	71.54^b	11.56^a	2.17^e
GA_3 @ 100 ppm + Boron @ 0.5% (T_5)	63.38^b	37.29^a	76.28^a	12.14^a	2.26^c
GA_3 @ 100 ppm + Boron @ 1% (T_6)	59.94^b	36.05^a	74.16^a	12.32^a	2.38^b
GA_3 @ 200 ppm + Boron @ 0.5% (T_7)	65.28^a	38.68^a	76.81^a	12.13^a	2.30^c
GA_3 @ 200 ppm + Boron @ 1% (T_8)	69.84^a	42.58^a	79.04^a	12.48^a	2.51^c
Mean	60.95	36.27	73.64	11.86	2.25
CD @ P=0.05	5.81	6.71	5.38	0.36	0.06
Mean at L1	61.32	36.38	74.03	11.90	2.26
Mean at L2	60.59	36.15	73.26	11.82	2.24

StE (%) = Stigma emergence in percentage, AGO (°) = Angle of glume opening, StR (%) = Stigma receptivity in percentage, AnL = Anther length in mm, AnW = Anther width in mm, CD = Critical difference, P = Probability, L1 = Delhi, L2 = Tamil Nadu, L3 = Madhya Pradesh. Means superscripted with same letters are not significantly different

control (11.0 mm), along with the widest anther (2.5 mm), a 21.3% increase over the control (2.1 mm), collectively enhancing pollen production and dispersal [Figs 1B(T_0) and B(T_8)]. These results highlight the positive influence of GA_3 and boron applications on key floral traits, which play a crucial role in enhancing pollination efficiency, seed set, and hybrid seed production success.

Effects of GA3, B, and their interactions on yield-contributing traits

The mixed-model pooled ANOVA (Table 3) revealed significant ($p \leq 0.05$) effects of location, treatment, and replication within location on various yield-contributing traits. However, the interaction between location and treatment was not significant, indicating that treatment effects remained consistent across locations. Highly significant ($p \leq 0.01$) treatment effects were observed for PlH, PnE, PTiP, FSeP, SpF, SyP, and STw, confirming the strong influence of GA3, boron, and their combinations on plant growth and reproductive traits. The treatment effect was found to be non-significant on the total number of spikelets per panicle across the locations. The small differences in mean values for yield-contributing traits across locations (L1 vs. L2, L1 vs. L3, and L2 vs. L3), such as PlH (0.2, 3.9, 4.1 cm), PnE (5.09, 6.07, 11.16%), PTiP (0.36, 0.69, 1.05), FSeP (10.27, 18.00, 7.73), SpF (4.78, 4.27, 3.49%), SyP (18.22, 51.60, 33.38 g), and STw (0.137, 0.33 g, 0.467 g) suggest that although the location effect was statistically significant ($p \leq 0.01$ or 0.05), its practical impact on treatment performance was minimal (Table 4). In contrast, TSpP remained unaffected by location, indicating stability across environments. The replication within location effect was significant ($p \leq 0.05$ or 0.01) for FSeP, SpF, and STw, suggesting some degree of variability within locations.



A(T_0) = angle of glume opening in control plant, A(T_8) = Angle of glume opening in GA_3 (200 ppm) and boron (1%) sprayed plant, B(T_0) = Anther size in control plant, B(T_8) = Anther size in GA_3 (200 ppm) and boron (1%) sprayed plant.

Fig. 1. Improved glume opening in the female parent and anther size in the male parent under GA_3 and boron foliar application compared to the control

Pooled mean performance of yield-contributing traits across locations

The pooled mean values (Table 4) confirm the positive effects of GA_3 and B on plant growth and yield traits. Among the nine treatments, T_8 (GA3 at 200 ppm + B at 1%) consistently exhibited the highest values, followed by other treatments, while the untreated control (T_0) recorded the lowest values across the traits. The highest PlH (115.62 cm) was recorded in T_8, which was 32.1% higher than the control (87.48 cm). Similarly, PnE was significantly improved, with T_8 (Fig. 2) recording 122.23%, which was 56.3% higher than the control (78.22%). These increases suggest that GA_3 and B promote vegetative growth and reproductive initiation. The PrTP was highest in T_8 (13.09), which was 24.3% greater than the control (10.53). Treatments T_7 (GA_3 at 200 ppm + B at 0.5%) and T_6 (GA_3 at 100 ppm + B at 1%) also resulted in significantly higher productive tillers per plant compared to untreated plants. While TSpP did not vary significantly among treatments, the FSeP was highest in T_8 (117.99 seeds), a 152.7% increase over the control (46.70 seeds). This suggests that GA_3 and B significantly enhanced seed filling, which is crucial for improved productivity. SpF was significantly enhanced by GA_3 and B application. T_8 recorded the highest SpF (56.91%), which was 143.5% higher than the control (23.37%). Similarly, SyP was highest in T_8 (467.41 g), representing an 85.9% increase over the control (251.42 g). This confirms that GA_3 and B significantly improve seed setting and overall productivity. Seed test weight was also significantly influenced by the foliar application of GA_3 and B. The highest STw (22.56 g) was recorded in T_8, which was 9.7% higher than the control (20.56 g).

The combination of GA_3 at 200 ppm and B at 1% (T_8) proved to be the most effective treatment, significantly enhancing growth, seed yield-associated traits, and overall seed yield performance across the location. These findings suggest that GA_3 and B application can be a valuable agronomic strategy for improving seed yield during TGMS-based rice hybrid seed production.

Discussion

Hybrid rice seed production, particularly in TGMS systems, often encounters physiological challenges due to suboptimal floral traits, inadequate panicle exertion, and inherently low natural outcrossing rates. These limitations necessitate the application of external treatments to enhance reproductive success and optimize hybrid seed yields. The present study assessed the influence of foliar-applied gibberellic acid (GA3) and boron (B) on floral characteristics, yield-determining traits, and overall seed productivity in PJRH 56, India's first public-sector TGMS hybrid rice variety.

Results from the study clearly demonstrated that the combined application of GA3 and B exerted a synergistic effect on key reproductive traits. Treatment T_8 (GA3 at 200 ppm + B at 1%) consistently outperformed all other combinations, showing significant improvements in stigma emergence, glume opening angle, anther development, and seed-setting efficiency. These enhancements are particularly

Table 3. Mixed-model pooled ANOVA for the effects of GA3 and B on yield contributing traits

Source of variance	DF	Mean Square							
		PlH	PnE	PrTP	TSpP	FSeP	SpF	SyP	STw
Location	2	146.168**	842.901**	7.689*	43.061	2201.697**	465.144**	18492.534**	1.561**
Treatment	8	1109.368**	2392.378**	5.242**	106.413	4036.505**	881.611**	32791.725**	3.015**
Replication with in location	6	19.149	4.145	2.809	19.284	358.919**	85.904**	183.622	1.995**
Location x Treatment	16	19.214	17.166	0.843	42.614	12.163	2.892	176.207	0.273
Error	48	17.982	14.356	1.94	32.005	58.957	11.156	110.85	0.204

PlH = Plant height, PnE = Panicle emergence, PrTP = Productive tillers per plant, TSpP = Total spikelets per plant, FSeP = Filled seeds per panicle, SpF = Spilet fertility, SyP = Seed yield per plot, STw = Seed test weight, DF = Degrees of Freedom, *Significant at P≤0.05, **Significant at P≤0.01

Table 4. Pooled mean performance of yield contributing traits under GA_3 and Boron foliar application across locations

Treatments	PlH (cm)	PnE (%)	PrTP	TSpP	FSeP	SpF (%)	SyP(g)	STw (g)
Control (T_0)	87.48^{c}	78.22^{e}	10.53^{b}	198.91	46.70^{e}	23.37^{e}	251.42^{h}	20.56^{c}
GA_3 @ 100 ppm (T_1)	107.09^{b}	92.07^{d}	11.73^{ab}	205.90	99.93^{bc}	48.59^{bc}	371.54^{ef}	21.68^{b}
GA_3 @ 200 ppm (T_2)	113.91^{ab}	98.81^{bc}	12.18^{ab}	208.74	103.64^{bc}	49.72^{b}	380.63^{de}	21.79^{b}
Boron @ 0.5% (T_3)	94.43^{c}	78.37^{e}	11.71^{ab}	207.39	85.37^{d}	44.14^{cd}	340.80^{g}	21.72^{b}
Boron @ 1% (T_4)	92.32^{c}	78.79^{e}	11.83^{ab}	210.51	92.80^{c}	41.10^{d}	362.84^{f}	21.92^{ab}
GA_3 @ 100 ppm + Boron @ 0.5% (T_5)	112.27^{ab}	92.94^{cd}	12.33^{ab}	208.45	106.42^{ab}	51.06^{b}	389.66^{cd}	22.20^{ab}
GA_3 @ 100 ppm + Boron @ 1% (T_6)	114.74^{a}	105.02^{b}	12.38^{ab}	210.25	112.29^{ab}	53.36^{ab}	430.34^{b}	22.29^{ab}
GA_3 @ 200 ppm + Boron @ 0.5% (T_7)	113.14^{ab}	116.04^{a}	12.96^{a}	207.39	106.45^{ab}	51.29^{b}	402.43^{c}	22.25^{ab}
GA_3 @ 200 ppm + Boron @ 1% (T_8)	115.62^{a}	122.23^{a}	13.09^{a}	207.06	117.99^{a}	56.91^{a}	467.41^{a}	22.56^{a}
Mean	105.67	95.77	12.08	207.18	96.84	46.62	377.46	21.89
CD @ P=0.05	6.96	6.22	2.29	NS	12.61	5.48	17.28	0.74
Mean at L1	108.35	101.58	12.19	208.37	106.27	50.96	400.73	21.95
Mean at L2	104.21	90.42	12.55	207.30	96.00	46.18	382.51	22.09
Mean at L3	104.44	95.51	11.50	205.86	88.27	42.70	349.13	21.62

PlH = Plant height, PnE = Panicle emergence, PrTP = Productive tillers per plant, TSpP = Total spikelets per plant, FSeP = Filled seeds per panicle, SpF = Spilet fertility, SyP = Seed yield per plot, STw = Seed test weight, CD = Critical difference, P = probability. Means superscripted with same letters are not significantly different.

important in TGMS-based systems, where seed setting relies exclusively on successful cross-pollination.

Under T_8, increases in glume opening angle (51.2%), stigma emergence (30.3%), and stigma receptivity (21.3%) were observed, indicating substantial enhancement of critical floral traits. Wider and longer-lasting floret opening, coupled with prolonged stigma emergence and higher receptivity, greatly improves the potential for cross-pollination, an essential factor in rice due to its narrow window of pollen shedding. These findings are consistent with Hamad et al. (2021a), who reported that GA3 promotes spikelet opening duration, glume angle, and stigma exsertion through its influence on cell elongation. Furthermore, GA_3's effects appear to be synergistic with boron, which enhances pollen–pistil interaction and facilitates pollen germination on the stigma surface, thereby increasing the probability of successful fertilization and seed set (Archana et al. 2022).

Similar synergistic effects were observed in the male parent. Treatment T_8 led to significant increases in anther length (13.1%) and width (21.3%), contributing to improved pollen dispersal and fertilization success. GA3 is known to promote cell division, expansion, and elongation, which likely accounted for the observed increases in anther size (Hamad et al. 2021b). Boron further reinforced GA3's effects by enhancing pollen viability, germination, and pollen tube elongation, collectively improving seed-setting efficiency under T_8 compared to the untreated control (Lordkaew et al. 2013).

Beyond reproductive traits, the integrated application of GA3 and B also led to marked improvements in vegetative growth and yield-related parameters, including plant height, panicle exertion, number of productive tillers, and the number of filled grains per panicle. The highest seed yield (467.41 g per plot) recorded under T_8 represented an 85.9% increase over the control. This significant gain can be attributed to GA3's effect on improving panicle exertion in the female parent (Thu et al. 2008) and boron's role in optimizing assimilate partitioning during grain development (Hussain et al. 2012). These findings further align with those of Lordkaew et al. (2013), who reported that boron deficiency in rice resulted in poor grain filling and reduced grain weight due to impaired pollen viability and fertilization processes.

Beneficial effects of GA3 and B remained consistent across the three agroclimatic locations included in the study, with only minor performance variations. These differences may stem from location-specific factors such as soil nutrient status and irrigation practices, which influence boron uptake and utilization. This underscores the need for site-specific nutrient management strategies to maximize the effectiveness of foliar-applied GA3 and B under varied environmental conditions.

In conclusion, this study provides compelling evidence that the integrated foliar application of GA3 and boron significantly enhances floral development, reproductive performance, and seed yield in TGMS-based hybrid rice systems. The observed consistency across environments and the improvements in both female and male reproductive traits highlight the potential of this approach to enhance the scalability and efficiency of TGMS hybrid seed production. However, further refinement of application timing, dosage, and location-specific practices is necessary to fully realize these benefits and to develop robust, environment-responsive seed production practices.

Author's contribution

Conceptualization of research (AKMB); Designing of the experiments (AKMB, GKS, KKV); Contribution of experimental material (GKS, KKV, NM); Execution of field experiments and data collection (RVP, NM, CS); Analysis of data and interpretation (RVP, AKMB, CS, VD, KG); Preparation of manuscript (AKMB, RVP, CS, KKV).

Acknowledgment

The authors extended sincere thanks to P.G. school, ICAR-Indian Agricultural Research Institute, for providing the financial support, resources and facilitation for experimentation. The first author gratefully acknowledges the financial support received in the form of a fellowship from NFST (Award No. 202122-NFST-TEL-00648), Ministry of Tribal Affairs, Government of India, and ICAR-SRF (Roll No. TL01001531) for the year 2020-21, during the Ph.D. program.

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