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Original research article

Effects of Gibberellic Acid Applied at Different Flowering Stages on Agronomic Traits and Yields of Hybrid Rice Parental Lines

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ABSTRACT

Gibberellic acid (GA_3) application in hybrid rice seed production plays a vital role in increasing seed yield; however, improper use of GA_3 may affect diversely the growth of hybrid parental lines. This study aimed to investigate the effects of GA_3 application at different flowering stages on agronomic traits and seed yield of hybrid parental lines. A micro-crossing plot experiment was employed for A line multiplication (HCS^A/HCS^B) under five treatments: applying GA_3 at 0%, 10%, 30% and 50% panicle heading stages and not applying GA_3. The results indicated flag leaf length, number of internodes, length of base internode, spikelets per panicle, total dry biomass of both lines, panicle length of HCS^A line and seed setting rate of HCS^B line were not significantly different among the treatments. However, applying GA_3 at 10% panicle heading to 50% flowering stage significantly increased plant height, length of 1st, 2nd, and 3rd upper internodes, total length of these three upper internodes and panicle exsertion rate of these lines. Interestingly, applying GA_3 at 30% panicle heading stage enhanced A line to produce significantly higher panicle exsertion, stigma exsertion, and seed setting rates, ultimately leading to the highest seed yield of A line, while applying GA_3 at 0% panicle heading stage slightly reduced panicle exsertion rate, and produced lower seed yield of both parental lines. These results suggest that applying GA_3 at 30% panicle heading stage can be an effective method for increasing seed yield of A line. A verification test is necessary to confirm the present results.

Keywords: Hybrid rice; Gibberellic acid application; Flowering stage; Seed yield.

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1. Introduction

Rice (Oryza *sativa L.*) is a staple food, providing a major source of calories and nutrients for more than half of the world's population. With a future increasing population, the rice demand is projected to rise by 26% in 2035 while the production is faced with decreasing availability of arable land, water, labor, chemical inputs and climate change issues [1-3].

Hybrid rice technology is an alternatively miraculous method to increase rice production per unit area of land as it gains at least 15% above high-yielding varieties [4]. The vitally important step towards cultivation of hybrid rice is its hybrid seed production. However, hybrid seed production has been facing many major problems which limited the seed production and adoption of the technology because of complex crop management, genetic background of parental lines and environmental factors [5-6]. Gibberellic acid (GA_3) is a plant growth regulator for cell elongation. In hybrid rice seed production, GA_3 has been used to increase seed yields as it enhanced plant height, stigma exsertion, panicle exsertion, duration of floret opening, panicle exsertion from the flag leaf sheath and seed setting rate of A lines[7-8] .

In Thailand, hybrid rice programs have existed since 1980. Many hybrid rice parental lines have been studied and successfully developed [9-10]. Kasetsart University is one institute which has researched and developed new hybrid rice lines from Thai rice varieties. Cytoplasmic genetic male sterility (A), maintainer (B) and restorer (R) lines were developed successfully for a three-line hybrid system. However, the hybrid seed productivity is still low when compared to other countries. In addition, the GA_3 application has been limited as its cost is expensive. In a new environmental growing condition and with different hybrid parental lines, an appropriate time for and amount of the GA_3 application must be determined. Therefore, this study aimed to investigate the effects of the GA_3 application at different flowering stages on agronomic traits and seed yield of hybrid parental lines.

2. Materials and Methods

2.1 Experimental design

A micro-crossing plot experiment was employed in a nursery at the department of agronomy of the faculty of agriculture, Kasetsart University. A crossing plot with a dimension of 1.6 m x 4 m long was arranged, and two hybrid parental lines, HCS^A and HCS^B, were used in the experiment. HCS^A, which is a cytoplasmic genetic male sterility (CGMS), has stable sterility of pollen, and it is a non-selfing plant. Its maintainer is Homchonsith variety (HCS^B) which is a fertile pollen and a selfing plant. Both HCS^A and HCS^B lines were developed by Kasetsart University. These varieties have low amylose contents and are aromatic, which are desirable characteristics of a good quality of hybrid rice. HCS^A (A) and HCS^B (B) lines were germinated on 4 July 2018, and 30-day old seedlings were transplanted with a row ratio of A: B (1: 1) in the micro-crossing plots. The planting space was 20 cm from hill to hill and A line to B line. At the booting stage of rice development phase, the crossing plot was divided into five subplots. Each subplot consisted of two rows of A line and two rows of B line for GA_3 application. The application of 90 ppm/ha of GA_3 was applied to each subplot at different flowering stages using a knapsack sprayer. The arranged treatments consisted of five treatments (Table 1). The GA_3 application rate was divided over two periods of spraying (60ppm/ha for the first spraying and 30ppm/ ha for the second spraying). The second spraying was applied on the second day after the first spraying [11]. In addition, temperature, rainfall and relative humidity are reported in Table 2.

Table 1. Treatment description of the experiment.

Treatment	Description
T_0	Control (no GA_3 application)
T_1	Applied GA_3 at 0% heading stage
T_2	Applied GA_3 at 10% heading stage
T_3	Applied GA_3 at 30% heading stage
T_4	Applied GA_3 at 50% flowering stage

Table 2. Temperature, rainfall and relative humidity during the trial from June to November 2018.

Month	Temperature (°C)			Rain (mm)	RH (%)
	Mean	Max.	Min.		
Jun.	29.9	33.6	26.1	157.1	74
Jul.	29.5	33.2	25.7	175.1	75
Aug.	29.2	32.9	25.5	219.3	76
Sep.	28.9	32.8	25.0	334.3	79
Oct.	28.7	32.6	24.8	292.1	78
Nov.	28.2	32.4	23.9	49.5	70

2.2 Measurement of traits

The recorded data were plant height (cm), flag leaf length (cm), panicle length (cm), stigma exsertion rate (%), number of internodes, length of based internode (cm), 1^{st}, 2^{nd}, 3^{rd} upper internode (cm), total length of these three uppermost internodes (cm), seed setting rate (SSR) (%) (Eq.1), seed yield (g/plant) and total dry biomass (g/plant). Measuring these traits was carried out following a standard evaluation system for rice [12]. Stigma exsertion rate (SER) was calculated using Eq. 2 and panicle exsertion rate (PER) was calculated using Eq. 3 [13-14]. Five replicates of independent plant materials of the treatments were observed, and harvested individually.

$$SSR\ (\%) = \frac{\text{No. of filled grain}}{\text{Total spikelets}} \times 100 \quad (1)$$

$$SER\ (\%) = \frac{SSE + DSE}{(DSE + SSE + NSE)} \times 100 \quad (2)$$

Where: NSE, spikelets with no stigma exsertion; SSE, single stigma exsertion and DSE, double stigma exsertion

$$PER\ (\%) = b/(a + b) \times 100 \quad (3)$$

Where: a, length of panicle enclosed to the sheath and b, length of exserted panicle from the flag leaf sheath.

2.3 Statistical analysis

Statistical differences between treatments of parental lines were analyzed separately by One-way ANOVA using STAR 2.0.1 software. A probability of $P > 0.05$ was considered to be non-significant difference. Also, Duncan's multiple range test at 5% level of significance was used to compare the means.

3. Results and Discussion

3.1 The effect of GA_3 on plant height, flag leaf length, panicle length, panicle exsertion rate, stigma exsertion rate of hybrid parental lines

The results of statistical analysis revealed that applying GA_3 at 0% heading to 50% flowering stage had significant effect on plant height and panicle exsertion rate of both hybrid parental lines and panicle length of B line ($P < 0.01$). But the different treatments did not affect significantly flag leaf length of both either parental line or panicle length of A lines (Table.3). Plant height and panicle exsertion rate of both lines showed the same trend when the plants were treated with GA_3 from 0% heading to 50% flowering stage. On average, the treated plants increased height significantly by 29.92%, 17.46%, 11.89% and 10.74% for the treatments of GA_3 applied at 0%, 10%, 30% and 50% flowering stage, respectively when compared to the control. When the HCS^A line had GA_3 applied at 10% to 50% flowering stage, its panicle exsertion rate was increased significantly from 73.66% to

80.80% when compared to the control without effect on the panicle exsetion of the HCS^B line. Besides, the panicle exsertion rate of the HCS^B line had no significant difference between the control and applying GA_3 at 10% to 50% flowering stage. In contrast, the panicle exsertion rates of both parents were reduced slightly when the plants had GA_3 applied at 0% heading stage compared to others due to significant reduction of the length of the 1st uppermost internode (Table 3 and Table 4). Applying GA_3 at 30% and 50% flowering stage gave higher panicle exsertion rate of A line when compared to the control due to higher growth of the 1st upper internode which was stimulated by GA_3 [15]. Genetically, the B line has good panicle exsertion, and almost no spikelets are enclosed in the flag leaf sheath, and the A line has poor panicle exsertion due to poor elongation of the uppermost internode. Increasing plant height and panicle exsertion rate is a result of cell elongation in the three uppermost internodes enhanced by GA_3 application [16]

Table 3. The effect of GA_3 application at different flowering stages on traits of HCS^A and HCS^B line.

Line	Treatment	PH	FLL	PL	PER
HCS^A	T_0	121.00 ± 1.34d	43.40 ± 1.86	29.20 ± 0.58	63.10 ± 2.50bc
	T_1	159.60 ± 3.14a	46.20 ± 1.96	31.20 ± 0.97	52.66 ± 3.12c
	T_2	141.40 ± 2.16b	42.40 ± 1.29	30.60 ± 0.51	73.66 ± 7.38ab
	T_3	134.40 ± 3.43bc	45.80 ± 1.07	30.80 ± 0.49	78.93 ± 5.15a
	T_4	131.40 ± 2.84c	45.00 ± 2.07	30.20 ± 0.20	80.79 ± 5.52a
	F-test	**	ns	ns	**
HCS^B	T_0	122.80 ± 1.53c	43.60 ± 1.69	28.60 ± 0.68b	92.37 ± 1.21a
	T_1	157.40 ± 2.56a	44.60 ± 1.21	31.40 ± 0.51a	66.71 ± 5.31b
	T_2	145.20 ± 3.80b	43.00 ± 1.64	30.80 ± 0.66a	87.21 ± 3.02a
	T_3	138.60 ± 1.63b	40.40 ± 1.86	28.40 ± 0.60b	91.36 ± 4.65a
	T_4	136.80 ± 4.25b	41.80 ± 1.98	28.20 ± 0.73b	93.48 ± 4.14a
	F-test	**	ns	**	**

Note: T_0, control (no GA_3 application); T_1, applied GA_3 at 0% heading; T_2, applied GA_3 at 10% heading; T_3, applied GA_3 at 30% heading; T_4, applied GA_3 at 50% heading stage; PH, plant height (cm); FLL, flag leaf length (cm) and PL, panicle length (cm); PER, panicle exsertion rate (%). *, ** indicates significant at 5% and 1% level of probability; ns, indicates non-significant difference. Mean ± SE (n=5) in the same column with the different letter indicates significantly different at the 5% level according to DMRT

Stigma exsertion is one of main traits which enhanced the seed set of the A line. The result showed that GA_3 application at different flowering stages affected significantly the stigma exsertion of the A line ($P < 0.01$). The plants treated with GA_3 at 30% and 50% flowering stage gave significantly higher stigma exsertion when compared to the treatment of 0% , 10% panicle heading stage and the control (Fig. 1). Virmani and Sharma [11] mentioned that GA_3 application was used to adjust the plant height of both parents, and enhanced panicle exsertion rate and stigma exsertion rate of the A line.

3.2 The effect of GA_3 on number of internode and internode length of main culm of hybrid parental lines

The results revealed that the number of internodes and length of based internode of both lines were not significantly different among the treatments. However, there was a significantly different effect on the length 1st, 2nd, 3rd of upper internode and the length of these three uppermost internodes of both lines due to the treatments ($P < 0.01$) (Table 4). Both lines responded with the same trend when the plants had GA_3 applied at 0% to 50% flowering stage. Applying GA_3 at 0% and 10% heading stage gave the highest length of these three upper internode because of increases in the growth of the 3rd and 2nd upper internodes, while the length of the 1st internode reduced. However, applying GA_3 at 30% and 50% flowering stage caused all treated plants to increase significantly the length of 2nd, 1st and total length of these three uppermost internodes (Fig 2). It clearly indicated that the GA_3 application has significant effect on the uppermost internodes, not the number of internodes of plant species, e.g. rice [16-17] and soybean [18].

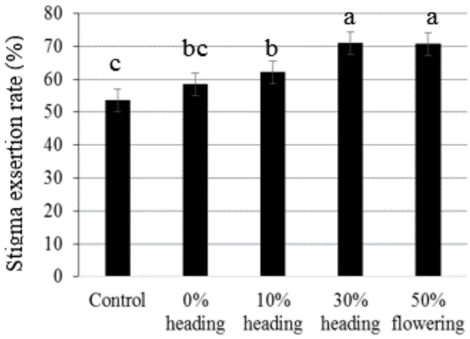

Fig. 1. The effect of GA_3 application at different flowering stage on stigma exsertion of HCS^A line. There was a statistically significant difference among treatments ($P < 0.01$) according to one-way ANOVA test. Mean ± SE (n= 5) with the different letter indicating a significant difference at the 5% level.

Table 4. The effect of GA_3 application at different flowering stages on the number of internodes and internode length of main culm of HCS^A and HCS^B line.

Line	Treatment	NI	BI	UI3	UI2	UI1	TUI
HCS^A	T_0	5.80±0.20	3.60±0.24	10.20±0.80c	16.80±0.73b	25.80±1.59ab	52.80±3.13d
	T_1	5.80±0.20	3.80±0.58	28.10±1.40a	27.20±2.75a	22.20±1.53b	77.50±5.68a
	T_2	6.00±0.00	3.40±0.24	20.20±2.03b	27.20±0.97a	27.80±1.24a	75.20±4.24ba
	T_3	5.80±0.20	3.00±0.00	13.60±1.60c	25.60±1.50a	31.20±1.88a	70.40±4.98bc
	T_4	5.60±0.25	3.20±0.20	10.80±0.64c	23.80±1.93a	31.60±1.21a	66.20±3.79c
	F-test	ns	ns	**	**	**	**
HCS^B	T_0	5.60±0.24	3.00±0.55	10.50±0.89d	15.40±0.40b	34.80±0.80a	60.70±0.44c
	T_1	5.60±0.24	3.40±0.51	28.20±1.76a	31.20±2.58a	29.80±1.32b	89.20±5.66a
	T_2	5.80±0.20	3.00±0.45	21.80±1.16b	28.80±1.59a	33.80±1.07a	84.40±1.81a
	T_3	5.80±0.20	3.20±0.20	17.70±1.46c	26.80±0.86a	32.40±1.17ba	76.90±1.21b
	T_4	5.80±0.20	3.40±0.24	14.20±1.31c	26.80±1.36a	34.80±0.58a	75.80±3.25b
	F-test	ns	ns	**	**	**	**

Note: T_0, control (no GA_3 application); T_1, applied GA_3 at 0% heading; T_2, applied GA_3 at 10% heading; T_3, applied GA_3 at 30% heading; T_4, applied GA_3 at 50% heading stage;NI, number of internode; BI, length of based internode from top soil (cm); UI1, UI2 and UI3 are the length of upper internode 1, 2, 3 from the top (cm), respectively; TUI, total length of these three uppermost internode (cm). *, ** indicate significant at the 5% and 1% level; ns, indicates non-significant difference. Mean ± SE (n=5) in the same column with the different letter indicates significantly different at the 5% level according to DMRT.

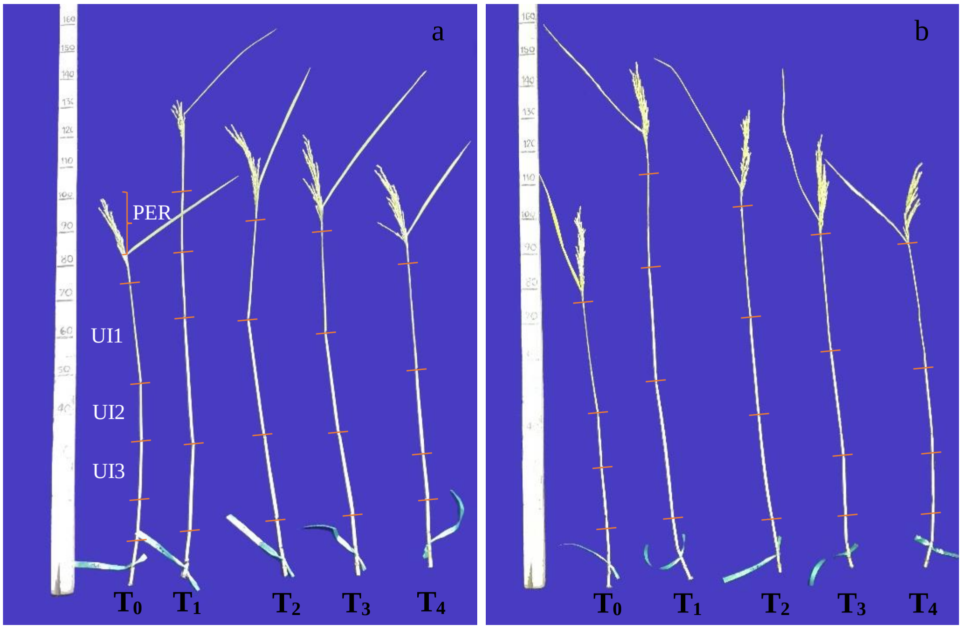

Fig. 2. The effect of GA_3 applied at different flowering stages on internode length and panicle exsertion of HCS^A line (a) and HCS^B line (b). UI1, UI2, and UI3 is 1st, 2nd and 3rd upper internode from the top, respectively. PER, panicle exsertion rate. T_o, control; T_1, GA_3 applied at 0% heading; T_2, GA_3 applied at 10% heading; T_3, GA_3 applied at 30% heading and T_4, GA_3 applied 50% flowering stage.

3.3 The effect of GA_3 on spikelets per panicle, seed setting rate, seed yield and dry biomass of hybrid parental lines

The results showed that applying GA_3 when plants were at 0% heading to 50% flowering had significant effect on seed setting rate and seed yield of the HCS^A line ($P < 0.05$), while it was not significantly different in spikelets per panicle and dry biomass of either parental line. Also, the application of GA_3 at different flowering stages did not affect significantly the seed setting rate and seed yield of the HCS^B line (Table 5). Mu and Yamagishi [19] reported that the application of GA_3 at the panicle initiation stage did not affect the spikelets per panicle of Nipponbare variety, while Akenohoshi variety increased its spikelets per panicle. As HCS^A is an isogenic line of the HCS^B line, their number of spikelets per panicle did not differ significantly, but seed yield of the HCS^A (sterile pollen) which depended on degree of pollen supply of HCS^B (fertile pollen) and evironment factors was lower than HCS^B's (Table 5). However, Yamagishi et al. [20] revealed that the application of GA_3 had a significantly different effect on the number of spikelets among the varieties. Besides, seed yield of HCS^B was not substantially different between the application of GA_3 at 0% heading to 50% flowering stage and the control. This result is in accordance with the finding of Umezaki et al. [18] who reported that seed weight per plant of soybean was not tremendously increased by the GA_3 application when compared to the control.

It clearly indicated that applying GA_3 at 30% heading stage gave significantly higher seed yield per plant of the A line as the seed setting rate of the A line was increased when compared to the application at 0%, 10%, 50% flowering stage and the control. These results are in accordance with the findings of Neik et al. [21] who mentioned that the application of GA_3 at the pre-flowering stage was the most effective time as it significantly affected the seed setting rate and seed yield of hybrid parental lines. In our results, high temperature and heavy rain in October (Table 2) during heading to flowering stage may have influenced seed setting rate and seed yield of HCS^A line. Seetharamaiah et al. [22] reported that more floral traits and oucrossing of A lines were better expressed in the dry season than in the wet season. Mao and Virmani [23] also revealed that the seed yield of hybrid rice seed production in the dry season was higher than the wet season because of favorable weather conditions (low temperature, high humidity and no heavy rain) during the heading to flowering period.

GA_3 application is a key to success of hybrid rice seed production. As the cost of GA_3 is very expensive outside China, its application rate varies based on hybrid production condition and avalability of GA_3 [24-25]. Virmani and Sharma [11] revealed that 90 ppm of GA_3 was a suitable application in hybrid seed production. However, Li and Yuan [16] mentioned that the dosage of GA_3 application was increased in China because of its potential to increase hybrid seed yield, and they also reported that GA_3 application could be sprayed three times, two times and one time if started at 1-5%, 10%, and 30% of panicle heading, respectively; however, the application of GA_3 at 50% panicle heading stage was found to have no benefit. Susilawati et al [26] reported that the optimun rate of 200 ppm of GA_3 application with two splits at the 5-10% panicle heading stage increased stigma exsertion, panicle length, panicle exsertion, seed setting rate and seed yield of A lines. Suralta [27] aslo found that the optimum rate of 300 ppm of GA_3 with three splits at a ratio of 25:50:25, beginning at the 5-10% panicle heading stage, increased panicle exsertion, stigma exsertion, seed setting rate and seed yield of the A line.

Table 5. The effect of GA_3 application at different flowering stages on spikelets per panicle, seed setting rate, seed yield and dry biomass of HCS^A and HCS^B line.

Line	Treatment	SP	SSR	SY	DBM
HCS^A	T_0	203.80 ± 18.83	4.84 ± 0.97b	1.76 ± 0.43b	33.03 ± 4.63
	T_1	187.20 ± 18.91	3.68 ± 0.49b	1.15 ± 0.43b	28.51 ± 2.69
	T_2	232.80 ± 8.97	5.52 ± 0.82b	1.37 ± 0.19b	41.60 ± 5.58
	T_3	221.40 ± 9.71	12.32 ± 1.21a	3.47 ± 0.93a	31.41 ± 4.36
	T_4	197.80 ± 8.35	10.13 ± 1.01ab	2.78 ± 0.49ab	31.36 ± 2.32
	F- test	ns	*	*	ns
HCS^B	T_0	186.20 ± 13.78	60.35 ± 1.77	14.34 ± 1.80	50.20 ± 5.58
	T_1	174.60 ± 13.19	64.77 ± 1.54	13.05 ± 1.92	50.99 ± 6.77
	T_2	185.20 ± 16.48	65.68 ± 2.19	14.14 ± 1.68	51.72 ± 4.09
	T_3	183.20 ± 14.77	64.01 ± 1.41	15.42 ± 1.47	53.80 ± 5.65
	T_4	180.00 ± 9.95	63.80 ± 0.79	15.63 ± 1.58	52.67 ± 3.98
	F- test	ns	ns	ns	ns

Note: SP, spikelets per panicle; SY, seed yield (g/plant); DBM, total dry biomass (g/plant); *, ** indicate significant at the 5% and 1% level; ns, non-significant difference. Mean values in the same column with the different letter indicates significantly different at the 5% level according to DMRT.

4. Conclusion

GA_3 is an effective plant hormone, used to increase seed yield in hybrid rice seed production. The results show that applying GA_3 at 0% heading to 50% flowering stage did not significantly affect flag leaf length, panicle length, number of internode, length of based internode, spikelets per panicle and dry biomass of hybrid parental lines. Interestingly, applying GA_3 at 10% to 50% flowering stage had positive effect on plant height, length of 1st and 2nd upper internodes, and panicle exsertion rate of hybrid parental lines, while applying GA_3 at 0% reduced slightly panicle exsertion rate, seed setting rate and seed yield of A line, and decreased significantly panicle exsertion rate of B line which may reduce the seed yield of hybrid parental lines. The present result confirmed that applying GA_3 at 30% panicle heading stage improved significantly main traits of the A line including panicle exsertion rate, stigma exsertion rate and seed setting rate, ultimately leading to the highest seed yield. Thus, applying GA_3 at 30% panicle heading stage can be an effective method for increasing seed yield of the A line. Verification tests that include other application rates should be investigated in multiple locations and seasons.

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