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Effect of supplementary pollination along with GA_3 combination on yield attributing characters and hybrid seed yield in hybrid rice (PRH-10)

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Abstract
The present experiments were conducted during Kharif 2017 & 2018 for rice hybrid seed production of PRH-10 at Sabour condition. The successful development and use of hybrid rice production technology is prerequisite to exploit its benefit across the rice growing agro climatic regions. It was reported that the production technology of hybrid seed is very much influenced by the provenance. The parental line i.e. seed parent Pusa 6A and pollen parent PRR78 significantly differ in growth duration difference based on 50 percent flowering. Application of GA_3 was having significantly and positive effect on enhancing the panicle exertion alone or in combination with flag leaf clipping. For achieving maximum hybrid seed yield application of GA_3 in combination with flag leaf clipping and supplementary pollination (rope pulling) is best in sabour (Bihar) condition.

Keywords: Pollen parent, seed parent, supplementary pollination & hybrid

Introduction
Rice *(Oryza sativa* L.), (2n=24) is one of the major staple food crops in the world which are cultivated in India around 43 mha, out of which 2.6 mha area is covered under hybrid rice. In Bihar, 15 percent of total acreage under rice is covered by hybrid rice (Directorate of Economics and Statistics, 2018).But due to limited studies on flowering behavior and seed setting pattern in parental makes seed production of hybrid rice a challenging task. Hybrid rice have the potential to increase productivity 15-20 percent over inbreed varieties. The successful development and use of hybrid rice production technology is prerequisite to exploit its benefit across the rice growing agro climatic regions. It was reported that the production technology of hybrid seed is very much influenced by the provenance. That why this technology is need to be redefined in environments for better synchronization of seed parent and pollen parent of each hybrid. Rice is basically a self-pollinated crop and hence it is essential to provide supplementary pollination to enhance the extent of out crossing. Also, among all the available sources of cytoplasm only wild abortive (CMS-WA) type is stable. This system is widely used and all the released hybrids in India are based on this system. A major drawback in the system is partial opening of spikelet of seed parent which leads to improper pollination resulting in poor seed setting. So in order to enhance out crossing rate we adopt the technique of supplementary pollination which includes shaking of pollen parent with the help of a rope, rod or bamboo stick so that the pollen grains are shed and effectively dispersed over the A-line plant.

Materials and Methods
In Kharif (2017) pollen parent PRR 78 were transplanted along with seed parent Pusa 6A in split plot design in two planting ratio (2:6; 2:8) and five treatments combination (Control, Rope pulling; Rope pulling plus GA_3 (60ppm); Rope pulling plus flag leaf clipping; Rope pulling plus GA_3 (60ppm) along with flag leaf clipping).

Methodology
Rope pulling
Rice is a self-pollinated crop and hence there is need for supplementary pollination for enhancing out crossing. This was done with the help of rope pulling in all DOS. The male parent was shaken at on half hour interval at peak anthesis time between 9.30 am to 11.30 a.m.

Gibberellic acid (GA_3)
GA_3 powder of high media company (HMC) was bought. Its name was RM9157 with molecular weight of 346.37.

Spray of GA_3 was done when 5% of tillers had started heading and it was done during evening. GA_3 (60 ppm) was applied at the rate of 30 gm per ha. Out of total quantity of GA_3, 40 percent sprayed on first day and remaining 60 per cent on next day.

Flag leaf clipping
Flag leaf was clipped when primary tillers are at booting stage. This will enhance uniform pollen movement and wide dispersal of pollen grains to give higher seed set.

Observation:
Plant height (cm)
Plant height was measured in centimetres from the ground level to the tip of the plant in both the parents, at grain maturation time.

Days to panicle initiation
The number of days from sowing till emergence of panicle is calculated for both A and R line.

Number of effective tillers
Total number of fertile panicles/plant in five randomly selected plants in each treatment was counted from the labelled plant to calculate the mean number of productive tillers per plant.

Panicle length (cm)
The length of panicle was measured in centimetres from the tip of the panicle to the ciliate ring at the base. Six panicles, comprising two each from main tillers, mid tillers and lower tillers were selected randomly of each treatment and the length was measured from last node up to the top of panicle.

Days to 50 per cent flowering
The number of days taken from sowing to 50 per cent of the plants in the plot to flower was recorded as days to 50 per cent flowering.

Panicle exertion percentage
Panicle exertion percentage is calculated using formula:-

Panicle exertion (%) = (Length of exerted panicle/Total length of panicle) × 100

Seed setting (%)
For determining the per cent seed set/row, six panicles were selected randomly from each row of each plot. The filled spikelet was separated from the unfilled spikelet and was counted.
The seed set per cent was calculated by using the following formula and expressed as seed set in percentage.

Seed setting (%) = (No of filled spikelet/Total number of spikelet) × 100

Seed yield (kg/ha)
Seed harvested from seed parent (A line) was taken and yield was calculated as kg seed per ha.

Results and Discussion
The CMS lines/seed parent based on WA cytoplasm generally have imperfect panicle exertion, in which certain portion of the panicle are covered within leaf sheath. It results into no pollination and fertilization of enclosed spikelet. The seed parent Pusa 6A having WA cytoplasm facing the same problem of poor panicle exertion as reported by Thangapandian *et al*. (2018) [7]. There were several reports on application of GA_3 and flag leaf clipping for enhancing panicle exertion as reported by Rahman *et al*. (2013) [5]. Gibberellic acid (GA_3) is a diterpenoid carboxylic acid which plays an important role in rice hybrid seed production. It is an effective and efficient growth hormone, which stimulates the cell elongation and thus causes panicle exertion. GA_3 alone was not sufficient in enhancing the seed yield at maturation, but found effective when applied in combination with flag leaf cutting and rope pulling. The flag leaf is the last formed leaf and is erect, longer than the panicle which emerges along with it. The function of flag leaf is required only during the last part of the plant life cycle. It contributes to not less than ten per cent to the yield. Enhancing out crossing rate is one of the key factors to increase the seed setting in hybrid rice production, which is achieved by flag leaf clipping. It increases uniform pollen movement and wide dispersal of pollen grains to give higher seed setting**.** Data present in table-1 shows that the treatment has significant effect on plant height for both the parents, but mean height of pollen parent is still higher than seed parent. Maximum plant height in seed parent (Pusa 6A) were recorded in T_5 (rope pulling + GA_3 + flag leaf clipping) with 89.45 cm followed by T_3 (rope pulling + GA_3) with 87.68 cm. No significant difference was observed between T_1 (control) with 75.78 cm and T_2 (rope pulling) with 75.64 cm. Also, from as presented in table-2 & 3 spikelet opening angle as well as flag leaf angle was significantly affected by various treatment combinations.
Data presented in table -4 revealed the significant and positive effect of GA_3 application and flag leaf clipping on panicle exertion in seed parent. The panicle exertion was significantly highest in T_5 (93.45%) followed by T_3 (92.97%) and T_4 (82.80%) than control T_1 (79.12%). Suralta and Robles (2003) [6] showed that GA_3 application at 150 g/ha in two split doses at the beginning of 5-10 per cent panicle initiation of the female parent population, results in increased percentage of panicle exertion from the flag leaf sheath by a maximum of around 80 per cent. Seed setting percentage is one of the most yield attributing characters. In present experiment, we have found that seed setting percent was significantly highest in T_5 (18.96%) followed by T_3 (13.13%), T_4 (12.75%), T_2 (9.25%) than control (4.21%). There was also significant effect of staggered sowing reported. Similar trend were observed on effect of treatment and staggered sowing hybrid seed per ha. The highest hybrid seed yield was reported in T_5 (1113.49 kg/ha) followed by T_3 (774.64 kg/ha), T_3 (752.23 kg/ha), T_2 (554.04 kg/ha) than control (226.74 kg/ha). Similarly, Ponnuswamy *et al*. (1998) [4] suggested that application of GA_3 (125 g/ha) at the 15-20 per cent panicle exertion stage increased plant height, panicle exertion, flag leaf angle, seed setting percentage and seed yield.

Table 1: Effect of supplementary pollination along with treatment combination on plant height of seed parent and pollen parent

Treatments	Plant Height (cm)									
	Seed Parent (Pusa 6A)					Pollen Parent (PRR 78)				
	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean
T1: Control	75.72	76.05	75.72	75.63	75.78	99.17	104.18	103.8	101.17	102.08
T2: Rope pulling	75.65	75.65	75.65	75.62	75.64	101.18	105.18	104.27	102.32	103.24
T3: Rope pulling +GA3	87.35	87.30	87.35	88.73	87.68	109.92	110.85	114.68	114.03	112.37
T4: Rope pulling +Flag leaf clipping	77.10	77.15	77.12	77.11	77.12	104.12	104.52	104.52	102.52	103.92
T5: Rope pulling+GA3+Flag leaf clipping	89.48	89.00	89.50	89.82	89.45	112.28	115.02	116.02	114.2	114.38
Mean	81.06	81.02	81.07	81.38		105.33	107.95	108.66	106.85	
CD (P=0.05)										
T	1.44					1.85				
S	NS					1.71				
T x S	NS					NS				

S1: 15 days seeding interval between Pollen parent & Seed Parent
S2: 10 days seeding interval between Pollen parent & Seed Parent
S3: 5 days seeding interval between Pollen parent & Seed Parent
S4: 0 days seeding interval between Pollen parent & Seed Parent
S: Staggered Sowing; T: Treatment (Supplementary Pollination)

Table 2: Effect of supplementary pollination along with treatment combination on spikelet opening angle of seed parent and pollen parent

Treatments	Spikelet Opening Angle (º)									
	Seed Parent (Pusa 6A)					Pollen Parent (PRR 78)				
	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean
T1: Control	18.05	18.43	18.47	17.97	18.23	17.00	15.35	14.87	15.25	15.62
T2: Rope pulling	18.30	19.90	19.27	19.21	19.21	16.87	16.28	15.07	16.38	16.15
T3: Rope pulling +GA3	21.08	21.53	21.62	21.53	21.53	23.08	22.13	22.00	19.58	21.70
T4: Rope pulling +Flag leaf clipping	17.70	18.25	19.12	18.47	18.47	16.63	15.48	15.76	16.70	16.14
T5: Rope pulling+GA3+Flag leaf clipping	21.55	21.85	22.25	21.93	21.93	24.22	22.15	23.07	18.95	22.09
Mean	19.34	19.99	20.14	20.02		19.56	18.28	18.15	17.37	
CD (P=0.05)										
T	1.14					1.13				
S	NS					0.86				
T x S	NS					2.26				

S1: 15 days seeding interval between Pollen parent & Seed Parent
S2: 10 days seeding interval between Pollen parent & Seed Parent
S3: 5 days seeding interval between Pollen parent & Seed Parent
S4: 0 days seeding interval between Pollen parent & Seed Parent
S: Staggered Sowing; T: Treatment (Supplementary Pollination)

Table 3: Effect of treatments and staggered sowing on flag leaf angle of seed parent and pollen parent

Treatments	Flag Leaf Angle (º)									
	Seed Parent (Pusa 6A)					Pollen Parent (PRR 78)				
	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean
T1: Control	23.08	22.57	23.70	22.40	22.94	22.00	21.15	23.88	22.12	22.29
T2: Rope pulling	24.67	25.88	25.18	24.90	25.16	22.58	23.90	23.87	22.25	23.15
T3: Rope pulling +GA3	30.48	32.35	31.80	30.82	31.36	30.30	31.25	32.07	33.35	31.74
T4: Rope pulling +Flag leaf clipping	23.95	24.47	23.92	23.58	23.98	21.73	23.35	24.80	22.70	23.15
T5: Rope pulling+GA3+Flag leaf clipping	29.58	31.37	31.48	30.68	30.78	33.50	31.97	33.68	30.02	32.29
Mean	26.35	27.33	27.22	26.48		26.02	26.32	27.66	26.09	
CD (P=0.05)										
T	1.14					1.19				
S	NS					1.24				
T x S	NS					NS				

S1: 15 days seeding interval between Pollen parent & Seed Parent
S2: 10 days seeding interval between Pollen parent & Seed Parent
S3: 5 days seeding interval between Pollen parent & Seed Parent
S4: 0 days seeding interval between Pollen parent & Seed Parent
S: Staggered Sowing; T: Treatment (Supplementary Pollination)

Table 4: Effect of supplementary pollination along with treatment combination on panicle exertion of seed parent

Treatments	Panicle Exertion (%)				
	S1	S2	S3	S4	Mean
T1: Control	78.88	79.05	79.52	79.03	79.12
T2: Rope pulling	79.28	78.95	80.83	80.35	79.85
T3: Rope pulling +GA3	92.63	93.45	92.9	92.90	92.97
T4: Rope pulling +Flag leaf clipping	82.48	82.48	82.48	83.75	82.80

T_5: Rope pulling+GA_3+Flag leaf clipping	93.97	93.13	93.10	93.58	93.45
Mean	85.45	85.41	85.77	85.92	
CD (P=0.05)					
T	1.33				
S	NS				
T x S	NS				

S: Staggered Sowing; T: Treatment (Supplementry Pollination); S_1: 15 days seeding interval between Pollen parent & Seed Parent; S_2: 10 days seeding interval between Pollen parent & Seed Parent; S3: 5 days seeding interval between Pollen parent & Seed Parent S_4: 0 days seeding interval between Pollen parent & Seed Parent;

Table 5: Effect of supplementary pollination along with treatment combination on yield of hybrid seed production

Treatments	Seed Setting (%)					Hybrid Seed Yield (kg per ha)				
	S_1	**S_2**	**S_3**	**S_4**	**Mean**	**S_1**	**S_2**	**S_3**	**S_4**	**Mean**
T_1: Control	4.333	6.333	4.500	1.667	4.208	234.447	337.700	250.648	84.167	226.740
T_2: Rope pulling	10.000	12.833	10.167	4.000	9.250	591.837	755.260	600.295	228.803	544.049
T_3: Rope pulling +GA_3	13.333	17.167	14.500	7.500	13.125	790.022	1,006.982	861.455	440.107	774.641
T_4: Rope pulling +Flag leaf clipping	15.667	15.833	13.000	6.500	12.750	925.188	940.733	761.668	381.357	752.237
T_5: Rope pulling+GA_3+Flag leaf clipping	21.500	23.833	21.333	9.167	18.958	1,263.860	1,397.607	1,254.497	538.025	1,113.497
Mean	12.967	15.200	12.700	5.767		761.071	887.656	745.713	334.492	
CD (P=0.05)										
T	0.483					28.001				
S	0.454					24.476				
T x S	0.966					56.002				

S: Staggered Sowing; T: Treatment (Supplementry Pollination) S_1: 15 days seeding interval between Pollen parent & Seed Parent; S_2: 10 days seeding interval between Pollen parent & Seed Parent; S3: 5 days seeding interval between Pollen parent & Seed Parent S_4: 0 days seeding interval between Pollen parent & Seed Parent T_1: Control; T_2: Rope pulling; T_3: Rope pulling plus application of GA_3; T_4: flag leaf cutting along with Rope pulling; T_4: flag leaf cutting along with Rope pulling plus application of GA_3

Table 6: Effect of treatments on planting ratio on hybrid seed setting percent and yield

Treatment	Seed Setting (%)			Hybrid Seed Yield (Kg per ha)		
	P_1	**P_2**	**Mean**	**P_1**	**P_2**	**Mean**
T_1: Control	4.417	4.000	4.208	250.453	203.028	226.740
T_2: Rope pulling	9.333	9.167	9.250	525.240	562.858	544.049
T_3: Rope pulling +GA_3	13.417	12.833	13.125	750.938	798.344	774.641
T_4: Rope pulling +Flag leaf clipping	13.250	12.250	12.750	742.688	761.786	752.237
T_5: Rope pulling+GA_3+Flag leaf clipping	19.583	18.333	18.958	1,092.616	1,134.378	1,113.497
Mean	12.000	11.317		672.387	692.079	
CD (P=0.05)						
T	0.483			28.001		
P	0.306			17.709		
T x P	NS			39.600		

S: Staggered Sowing; P: Planting Ratio; T: Treatment (Supplementry Pollination) T1: Control; T_2: Rope pulling; T_3: Rope pulling plus application of GA_3; T_4: flag leaf cutting along with Rope pulling; T_4: flag leaf cutting along with Rope pulling plus application of GA_3; P_1: Planting ratio 6:2 (Female: male); P_2: Planting ratio 8:2 (Female: male)

Conclusion

It is concluded from the above studies that for successful production of hybrid rice PRH 10 in Sabour (Bihar) conditions. Application of GA_3 at 5 per cent flowering stage having significantly and positive effect on enhancing the panicle exertion alone or in combination with flag leaf clipping. Supplementary pollination with rope pulling alone have significantly increases the seed setting percentage by 5.04 per cent (9.25; 554.04 kg/ha) over control (4.21; 226.74 kg/ha). The application of GA_3, in combination with flag leaf clipping and rope pulling was found best treatment for hybrid seed yield and its attributing traits such as, panicle length, panicle exertion percentage, spikelet opening angle and flag leaf angle.

Application of research

For achieving maximum hybrid seed setting percentage and hybrid seed yield per ha, application of GA_3 in combination with flag leaf clipping and supplementary pollination (rope pulling) was found best in sabour (Bihar) condition.

Research Category: Seed Technology

Abbreviations

S: Staggered Sowing; T_0: control; T_1: rope pulling; T_2:rope pulling along with GA_3 application; T_3: rope pulling along with flag leaf clipping T_4: rope pulling along with GA_3 application as well as flag leaf clipping

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