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Improvement of Pollen Production and Seed Yield in Male Parent (CB 174R) and Rice Hybrid CORH 4

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ABSTRACT: The present investigation was undertaken to standardize techniques to improve the pollen production in male parent and to enhance the seed yield of rice hybrid CORH 4. The male parent of rice hybrid CORH 4 *i.e.*, CB 174R was given different methods of staggering and foliar sprays. Different staggering methods includes alternate planting of the staggered male parent (Planting of first staggered seedlings in 1st row at 30 cm spacing, planting of second staggered seedlings at 2nd row at 30 cm spacing and planting of third staggered seedlings in both the rows at 15 cm spacing), planting of three staggered seedlings by dividing the male rows into three equal parts and mixed planting of the male parent in single hill. Foliar sprayings were given as boron spray @ 0.02% and $ZnSO_4$ spray @ 0.5% at boot leaf stage. The result of this study indicated that among various staggering methods, mixed planting of three staggered seedlings in a single hill along with application of boron (0.02%) as foliar spray at boot leaf stage enhanced the pollen viability (97.5 %), pollen germination (66.6%) and pollen tube growth (150.6µ) in male parent. The seed yield recorded was maximum (1031.2 kg/ha) in mixed planting of the three staggered seedlings coupled with the foliar spraying of boron (0.02 %), as compared to control (610.2 kg/ha).

Key words: Synchronization, Staggering, Male parent and Pollen production

INTRODUCTION

In India, rice is the staple food for nearly 65 per cent of the population. In order to meet out the needs of the burgeoning population, hybrid seed production technology is essential The hybrid technology is acknowledged as the most adoptable genetic option, but itis restricted by the yield barrier, due to lack of synchronization in flowering of male and female parent and the period of anthesis, pollen production, pollen fertility and pollen germination. The seed set on male sterile line mainly depends on cross pollination, under scoring the importance of synchronization of the parental lines [1].

Maximization of cross pollination is achievable through many techniques. The importance of staggered sowing of parental lines in order to achieve higher seed set has been reported by earlier workers [2]. The percentage of seed set in male sterile lines of hybrid rice is wholly dependent on cross pollination with male parent which in turn is dependent on synchronization of flowering, period of anthesis, pollen production and the prevailing weather conditions [3].

High crop yield generally depends on viable pollen grains, pollen fertility and pollen germination in any hybridization programme. The pollen grains are the sexual reproductive unit and the carrier of male genetic material in higher plants. Pollen tube growth can be affected by many factors, including temperature, availability of calcium, zinc and boron [4, 5], among which boron deficiency significantly influences the performance of pollen grains [6].

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MATERIALS AND METHODS

Rice hybrid CORH 4, seeds of parental lines *viz.*, COMS 23A and CB 174R and hybrids were collected from Paddy Breeding Station, and the field was raised in Wetland, Tamil Nadu Agricultural University, Coimbatore for this study. The experiment details are furnished below.

Staggered sowing of the male parent

In the first staggering, the seeds of male parent were sown five days before the sowing of female parent, second staggering was simultaneous sowing of both male and female parents and the third staggering was five days after the sowing of female parent.

Factor 1: Staggering of the male parent.

S1 – Alternate planting of the staggered male parent

a) Planting of first staggered seedlings in 1^{st} row at 30 cm spacing

b) Planting of second staggered seedlings in 2^{nd} row at 30 cm spacing

c) Planting of third staggered seedlings in both the rows at 15 cm spacing

S2 – Planting the three staggered seedlings by dividing the male rows into three equal parts

S3 – Mixed planting of the male parent

Factor 2: Foliar sprayings in male parent

T1 - Control

T2 - Boron spray @0.02% at boot leaf stage.

T3 - $ZnSO_4$ spray @ 0.5% at boot leaf stage.

For hybrid rice seed production of CORH 4, the parental lines *viz.*, COMS 23A and CB 174R were raised with suitable staggering and the seedlings were transplanted as per the above treatment details at 15 ×10 cm spacing in the main field in split plot design with four replications. The following observations were recorded in randomly selected ten hills in each replication.

RESULTS

Pollen viability

The pollen grains were collected from each treatment in cavity slides and stained with Iodine- potassium iodide solution (0.44 g of iodine + 20.008 g of potassium iodide dissolved in 500 ml of 70 % alcohol). The viable pollen stained immediately as dark and the non – viable ones which remained colorless were counted using stereomicroscope. The viability percentage was calculated from the mean of twelve microscopic field counts for each treatment.

$$\text{Pollen viability (\%)} = \frac{\text{No. of viable pollen grains}}{\text{Total no. of pollen grains}} \times 100$$

POLLEN GERMINATION

Hanging drop culture [7]

At the time of flowering, anthers from freshly opened spikelet of each treatment were collected. The anthers were gently tapped with the help of a needle so that the pollen grains fall on a drop of the medium placed over the cover slip. The cover slip was then inverted over the cavity slide in such a way that the drop was suspended in the centre of the cavity. The cavity was sealed using petroleum jelly and kept at room temperature (28-30°C) using 1% acetocarmine stain and the cover slip was examined under microscope after incubation at different time intervals ranging from 0 to 80 minutes. Germinated pollen grains were counted 80 minutes after shedding of pollen grains on the medium. When the length of the pollen tube was twice the diameter of pollen grain, it was taken as germinated [8], and the other pollen grains with reduced pollen tube were considered as of doubtful for germination. In each observation, 200-300 pollen grains were counted and tube growth was measured. The pollen germination was computed as,

$$\text{Pollen germination (\%)} = \frac{\text{No. of germinated pollen grains}}{\text{Total no. of pollen grains}} \times 100$$

Pollen tube growth

For germinated pollen grains, the length of germinated tube was measured using stereomicroscope and expressed in microns.

Seed setting percentage

The number of filled seeds present in the total spikelet of the main tiller were counted in ten randomly selected plants and expressed as percentage.

Seed yield (Kg/ha)

The panicles harvested from each treatment plot were threshed separately, cleaned, dried to 12 % moisture content and seed weight was recorded in g/plot. Since the plot size varies among the treatments, the seed yield per hectare was calculated and expressed in kg/ha.

1000 seed weight (g)

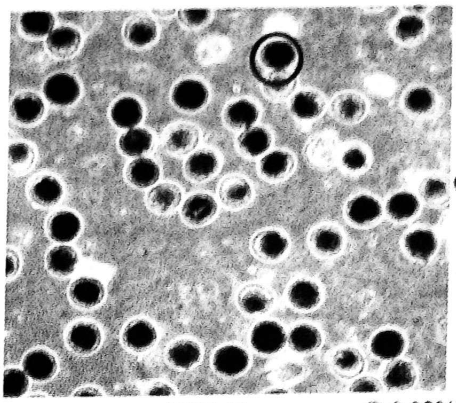
Eight replications of 1000 seeds were counted in each treatment and weighed in a precision balance to calculate the 1000 seed weight and expressed in gram.

RESULTS

The viability of pollen differed significantly due to different treatments. In male parent, the maximum pollen viability (97.5 %) was observed in S3T2 and S1T2 followed by S2T2 (97.0 %) and the minimum was recorded by S1T1 (90.0%). Among different treatments, T2 showed its superiority in exhibiting the highest pollen viability (Table 1) (Plate 1).

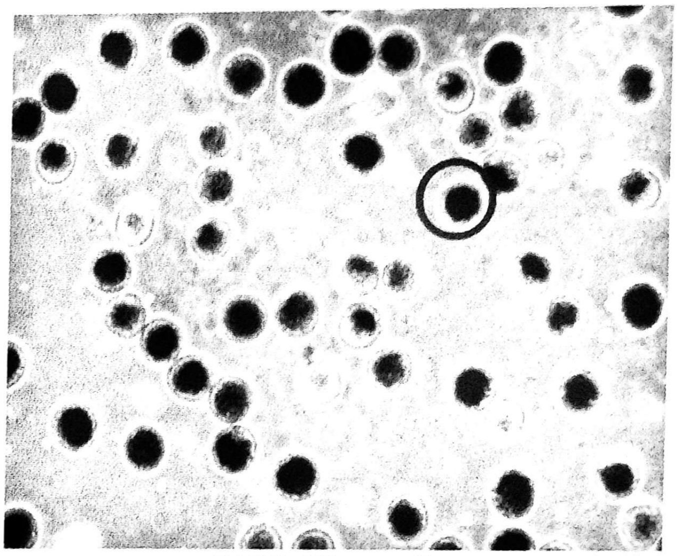
Significant differences were observed for *in vitro* pollen germination due to different staggering methods and foliar sprayings. S3T2 exhibited the highest pollen germination (66.6%) and the lowest was recorded by S2T1 (27.8%). Among different methods, T2 was found to be superior in recording the maximum germination percentage (64.7%) (Table 1).

The pollen tube growth differed significantly due to various staggering and foliar spraying methods. The highest pollen tube growth was recorded in S3T2 (150.6 μ) and the minimum was recorded by S3T1 (38.2 μ). Among different methods, T2 was found to be superior in recording the maximum pollen tube growth (145.0 μ) (Table 2).



-Indicates viable pollen grain

S3T2 (Boron spray @ 0.02%)



S1T1 (Control)

Fig. 1. Pollen viability of male parent of rice hybrid CORH 4

There was significant difference in the seed setting percentage for rice hybrid due to different staggering methods and foliar sprayings. The highest seed setting percentage was exhibited by S3T2 (37.3%). Among different methods of staggering, S3 recorded maximum seed setting percentage (36.5%) and in foliar spraying, T2 was found to be superior in recording maximum value (33.3%) (Table 3). In male parent, significant difference was obtained in seed setting percentage. The maximum was recorded by S3T2 (97.0%). S3 exhibited the highest seed setting

Table 1. Effect of staggering methods and foliar sprayings on pollen viability (%) and pollen germination (%) of male parent of rice hybrid CORH 4

S.No.	Treatments	Pollen viability (%)					Pollen germination (%)			
		T1	T2	T3	Mean		T1	T2	T3	Mean
1.	S190.0	97.5	95.5	94.3		S1	32.4(34.8)	63.7(53.4)	63.2(52.5)	53.1(46.9)
2.	S2	94.0	97.0	96.5	95.8	S2	27.8(31.6)	63.8(52.8)	60.1(50.8)	50.6(45.1)
3.	S3	90.5	97.5	95.0	94.3	S3	37.9(37.8)	66.6(53.4)	59.8(50.8)	54.8(47.3)
4.	Mean	91.5	97.3	95.7		Mean	32.7(34.7)	64.7(53.2)	61.0(51.3)	
			SEd		CD(p=0.05)		SEd		CD(p=0.05)	
	S	0.36			1.55			0.17		0.71
	T	0.73			1.79			0.22		0.53
	ST	1.09			2.90			0.35		1.00

(Figures in parentheses indicate arcsine values); S1– Alternate planting of the staggered male parent; S2- Planting the three staggered seedlings by dividing the male rows into three equal parts; S3 – Mixed planting of the staggered male parent; T1 – Control; T2-Boron spray @ 0.02%; T3-$ZnSO_4$ spray @ 0.5%

Table 2. Effect of staggering methods and foliar sprayings on pollen tube growth (μ) in male parent of rice hybrid CORH 4

S.No.	Treatments	Pollen tube growth (μ)			
		T1	T2	T3	Mean
1.	S1	42.1	142.8	99.1	94.7
2.	S2	57.6	141.7	98.6	99.3
3.	S3	38.2	150.6	122.9	103.9
4.	Mean	45.9	145.0	106.9	
	SEd		CD(p=0.05)		
S		1.29		2.74	
T		1.29		2.74	
ST		2.24		4.75	

S1-Alternate planting of the staggered male parent; S2-Planting the three staggered seedlings by dividing the male rows into three equal parts; S3 – Mixed planting of the staggered male parent

percentage (93.6%) among different staggering methods. For foliar spraying methods, T2 showed the maximum seed setting percentage (92.0%) (Table 3).

Significant differences were obtained in hybrid seed yield due to different treatments. The maximum seed yield was observed in S3T2 (1031.2 kg) followed by (940.0 kg) in S3T3 and the minimum (610.2 kg) in S1T1. Among foliar spraying methods, T2 recorded maximum seed yield (916.8 kg) and S3 recorded the highest values (863.8 kg) among various staggering methods (Table 4).

Among different treatments S3T2 recorded the maximum thousand seed weight (20.9 g) and the minimum was recorded by S3T1 (17.0 g). For foliar spraying, T2 exhibited highest thousand seed weight (18.8 g). Among different staggering methods, S3 recorded more seed weight (18.7 g) when compared to other methods. Similarly in male parent, significant difference was obtained for seed weight due to various treatments. The maximum was observed in S3T2 (18.8 g). S3 recorded the highest seed weight (16.8 g) among different staggering methods and T2 registered the maximum weight of 16.9 g (Table 4).

Table 3. Effect of staggering methods and foliar sprayings on seed setting percentage in rice hybrid CORH 4 and male parent

S.No.	Treatments	Seed setting (%)								
		Hybrid seeds					Male parent			
		T1	T2	T3	Mean		T1	T2	T3	Mean
1.	S1	28.0	30.9	33.9	30.9	S1	90.8	85.2	82.6	86.2
2.	S2	27.6	31.6	27.5	28.9	S2	84.8	93.9	93.2	90.6
3.	S3	35.0	37.3	37.1	36.5	S3	92.1	97.0	91.7	93.6
4.	Mean	30.2	33.3	32.8		Mean	89.2	92.0	89.2	
		SEd	CD(p=0.05)				SEd		CD(p=0.05)	
S		1.14	3.47				1.36		2.88	
T		1.14	3.47				1.36		2.88	
ST		2.83	6.01				2.35		4.99	

S1-Alternate planting of the staggered male parent; S2-Planting the three staggered seedlings by dividing the male rows into three equal parts; S3-Mixed planting of the staggered male parent

T1-Control; T2-Boron spray @ 0.02%; T3-nSO_4 spray @ 0.5%

DISCUSSION

Information on the flowering behaviour of seed parent and pollen parents with reference to time of sowing is a prerequisite for a successful hybrid rice seed production programme. Earlier studies conducted on the flowering behaviour of plants revealed that variations existed in flowering behaviour depending on the genotypes [9], environment [10] and due to the interaction of both.

With respect to pollen viability, among different staggering methods of male parent and the foliar spraying of boron and zinc sulphate, mixed planting of all the three staggered seedlings in a single hill with 15 cm spacing, along with boron(0.02%) foliar spray and alternate planting of staggered seedlings of male parent along with foliar spraying of boron (0.02%) were found to be superior (97.5%), followed by planting of the three staggered seedlings by dividing the male rows into three equal parts with foliar spraying of boron (97.0%). For foliar spraying methods alone, boron (0.02 %) exhibited the highest (97.3%) pollen viability percentage (Fig 1). The role played by micronutrients for enhancing pollen viability has been reported in wheat [11] and cotton [12]. Boron is essential for pollen tube formation. The present findings are in conformity with earlier reports in maize [13], and hybrid rice [14]. Among the treatments, the mixed planting of staggered seedlings of male parent with boron (0.02%) spray recorded maximum pollen germination percentage (66.6%) and pollen tube growth (150.6 μ)

The results of this study clearly revealed that for improved pollen production and enhanced pollen germination, the application of boron in the form of foliar spraying at boot leaf stage is very much essential. With regard to pollen production from different staggering methods, the mixed planting of the three staggered male parent transplanted in a single hill, produced maximum viable and vigorous pollen grain that ensured maximum pollen tube growth for assured fertilization and seed set. The effect of boron on

Table 4. Effect of staggering methods and foliar sprayings on seed yield (Kg / ha) and 1000 seed weight (g) in rice hybrid CORH 4 and male parent

S.No.	Treatments	Seed yield (Kg/ha) Hybrid seeds T1	T2	T3	Mean		1000 seed weight (g) Hybrid seeds T1	T2	T3	Mean		1000 seed weight (g) Male parent T1	T2	T3	Mean
1.	S1	610.2	850.0	931.2	797.1	S1	17.4	17.8	17.8	17.6	S1	15.4	15.9	16.1	15.8
2.	S2	731.0	869.2	730.9	777.0	S2	17.2	17.8	17.3	17.4	S2	15.8	16.1	15.7	15.8
3.	S3	620.1	1031.2	940.0	863.8	S3	17.0	20.9	18.3	18.7	S3	15.5	18.8	16.1	16.8
4.	Mean	653.7	916.8	867.4		Mean	17.2	18.8	17.8		Mean	15.6	16.9	15.9	

	SEd	CD(p=0.05)	SEd	CD(p=0.05)	SEd	CD(p=0.05)
S	3.88	8.24	0.51	1.07	0.29	0.62
T	3.87	8.24	0.50	1.06	0.29	0.62
ST	6.73	14.26	0.88	1.85	0.50	1.07

CV = 1.01%; S1-Alternate planting of the staggered male parent; S2-Planting the three staggered seedlings by dividing the male rows into three equal parts; S3- Mixed planting of the staggered male parent; T1-Control; T2-Boron spray @ 0.02%; T3- $ZnSO_4$ spray @ 0.5%

pollen germination is more significant than manganese and the viability and germination of pollen increased significantly with the application of foliar minerals [15]. Boron deficiency resulted in low pollen viability, pollen germination and reduced pollen tube growth and boron is required for stigma receptivity and pollen tube extension by formation of boron – sorbitol complex that promotes absorption, translocation and metabolism of sugar in pollen and synthesis of pectin material for the cell wall of growing pollen tube [16].

The seed setting percentage observed was found to be maximum (37.3 %) in mixed planting of three staggered seedlings combined with the foliar spraying of boron in seed parent while the control recorded the minimum seed setting percentage (27.5%). In male parent also, mixed planting of three staggered seedlings combined with the boron @ 0.02 % foliar spray recorded maximum seed setting percentage (97.0 %) [14].The reason might be due to the better utilization of boron made available through exogenous application.

The seed yield recorded was maximum (1031.2 kg/ha) in mixed planting of the three staggered seedlings coupled with the foliar spraying of boron (0.02 %). The control recorded a minimum seed yield (610.2 kg/ha). Application of micronutrient in the form of foliar spray always has a positive influence on pollen production and subsequent seed set and seed yield. Boron helps improvement of the seed setting due to enhanced sugar translocation from complex compounds like carbohydrates and it gets translocated at greater ease. Boron plays a greater role in nitrogen based synthesis or utilization and is involved in RNA metabolism. Similar results were reported by Dileepkumar *et al.* (2009) in cowpea [17]. In the present study, as compared to zinc sulphate @ 0.5 %, application of boron @ 0.02 % during boot leaf stage improved pollen production, pollen germination and seed yield attributes. On the contrary, application of zinc enhanced the seed yield in soybean [18], wheat [11], rice [3] and cotton [12]. In the present investigation, both the male parent and hybrid seeds registered maximum 1000 seed weight (20.9 g) in mixed planting of three staggered seedlings with foliar spraying of boron [19]. Vimala (1997) also reported that thousand grain weight was largely altered by the nutrients sprayed on the parental lines [18].

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