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Pant CMS3A Unique improved cytoplasmic genetic male sterile line with exerted panicles for potential hybrid seed production in rice

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Abstract
Four cytoplasmic genetic male sterile (CMS) lines and their respective maintainer lines of rice were used in this experiment. The CMS lines included were newly developed line Pant CMS3A along with other three existing CMS lines i.e. IR58025A, Pusa 6A and IR 79156A These were planted in four rows of 6 meter length along two rows of respective maintainer lines were also planted aside. The hybrid seed set under natural conditions was allowed without GA_3 application and supplementary pollination. Observations were recorded on plant height, days to 50% flowering, number of tillers/plant, out crossing rate (%), panicle exertion (%), panicle length (cm), number of filled grains, total number of grains and yield (Kg/ha). The panicle exertion in Pant CMS 3A showed significant advantage over widely used CMS lines viz. IR58025A, Pusa 6 and IR79156A with respect to panicle exertion, out crossing rate and days to 50% flowering. If this line is utilized in the hybrid seed production, use of GA_3 can be avoided and seed yield can be enhanced.

Keywords: Rice, WA cytoplasm, panicle exertion, GA_3

Introduction
Hybrid rice cultivation is gaining worldwide popularity. Research on hybrid rice in China was initiated in 1964 (Yuan, 1966) [5]. The genetic tools essential for breeding hybrid rice varieties are as the male sterile line (A-line), maintainer lines (B-line) and restorer (R-line) were developed during 1973 (Yuan and Virmani, 1988) [6]. Hybrid seed production techniques could be developed basically by 1975. In 1976, first hybrid rice variety was released for commercial cultivation and 0.14 million hectare area was transplanted. Since then, area planted to hybrid rice increased year by year and at present more than 60% area is under hybrid rice. The development and identification of CMS lines with desirable characteristics like better panicle exertion and out crossing have played an important role in the popularization of hybrid rice in China.

Convinced with the success of hybrid rice technology in China, India also launched a hybrid rice breeding project in 1989. The first set of rice hybrids were released in 1994. Presently more than 103 rice hybrids have been developed and released from public and private sectors. At present hybrid rice is reported to be grown approximately more than two lakh hectares. Area under hybrid rice will further increase after heterotic hybrids suitable for high productivity areas Punjab, Haryana, coastal region of Andhra Pradesh and shallow land area are identified and an effective transfer of technology is taken up vigorously in the target states (Viraktamat, 2010) [2]. The private sector has played a pivotal role along with the public sector seed corporations to popularize the hybrid rice among the farmers.

Hybrid rice production technology is highly skill oriented and requires utmost care and precaution at various stages of the seed production. Many operations like flag leaf clipping, supplementary pollination and application of gibberelic acid are practiced in order to enhance the seed availability from seed parent. Use of gibberelic acid (GA_3) at proper stage with proper dose enhances the seed set on seed parent (Virmani, 2005) [5], but the use of GA_3 also enhances the cost of seed that farmers or seed producers has to bear. The cost of one gram of GA_3 in the local market varies between Rs. 120-150 depending upon the purity and company. One hectare area under hybrid rice requires around 60-90 grams of GA3, small quantity of ethyl alcohol and labour for spray which raise the total expenditure on seed production by about Rs. 8000-9000/- per hectare.

This is the routine expenditure on seed production which has to be incurred every season of the rice hybrid seed production (Virmani, 1996) [3]. This expenditure on GA3 can be reduced to some extent by developing CMS lines with fully exerted panicle and well exerted stigma.

Materials and Methods

Fig 1: Multiplication of Pant CMS3A

With a view to improve the seed set of CMS lines in rice, a conversion programme in the background of an improved rice genotype, "NAT 990-99" was initiated at G.B. Pant University in 2003 by using widely used CMS line "IR58025A" as non-recurrent parent .Progenies in each backcross generation were checked for pollen sterility and morphological traits .In BC_6 generation progeny with desired morphological traits, complete sterile plants were selected and allowed to cross pollination with the maintainer line NAT 990-99 in isolation, to get the genetically pure seed of cytoplasmic male sterile line. The conversion procedure was followed as in the development of Pant CMS 2A **(**Nautiyal *et. al.* 2011) [1]. Briefly this method involved with the selection of sterile plants with full panicle exertion in BC_2 generations for crossing with the recurrent parents. This procedure was followed upto BC_6 generations and at last new cytoplasmic genetic male sterile line with full exerted panicles was obtained. The newly developed CMS line Pant CMS 3A was planted along with other CMS lines i.e. IR58025A, Pusa 6Aand IR58025A, in four rows of 6 meter length. Two rows of respective maintainer lines were also planted alongside. (Fig.1).The distance between the rows of CMS lines was kept 15 cm and between CMS and maintainer line 20 cm and between maintainer lines 30 cm respectively. The seed set under natural conditions was allowed without GA_3 application and supplementary pollination. Observations were recorded on plant height; tillers/plant, days to 50% flowering, panicle exertion (%), out crossing rate (%) and total hybrid seed production. The experiment was conducted in the rainy seasons of 2013, 2014, 2015 and repeated in 2016.

Results and discussion

Major differences were observed among the four lines with respect to all the studied traits (Tables 1, 2, 3)

The observations were recorded in kharif 2013, 2014,2015 and kharif 2016 described that the maximum plant height was observed in Pant CMS 3B i.e. 111.33cm , 112.00cm,113.00 and 114.20 with mean value 112.63 followed by Pant CMS 3A i.e. 105.00cm, 104.33, 110.00 and 111.60 with mean value 107.7. In IR58025A minimum plant height was i.e. 86.0cm, 87.33, 87.00 and 87.20cm with mean value 86.88 respectively. Number of tillers per plant was found significantly highest in Pant CMS 3A i.e. 24.66 , 24.06, 24.66 and 24.00 with mean value 24.34 and lowest found in IR58025B i.e. 13.33, 15.93, 12.30 and 11.20 with mean value 13.19 respectively in four years.

Fig 2: Pant CMS3A

In natural condition without use of GA_3 and supplementary pollination the maximum out crossing rate i.e. 32.08 percent and 31.58 percent was recorded in Pant CMS 3A while in Pusa 6A i.e. 18.14 percent and 19.17 percent and minimum 13.33 percent and 15.46 percent was recorded in IR58025A respectively.

The maximum panicle exertion in Pant CMS 3A was 96.83, 96.50, 96.80 percent and 97.20 percent with mean value 96.83 respectively, followed by IR79156A showed 72.16, 72.40, 74.00and 73.80 percent with mean value 73.09. Minimum panicle exertion in Pusa 6A 71.06, 71.40, 71.00 and 71.30 percent with mean value 71.19 during four years. (Table 2). Days to 50% flowering was found minimum in IR79156B i.e. 87.00, 88.00, 88.00 and 89.00 days with mean value 88.00 followed by IR79156 i.e. 89.00, 88.00, 90.00 and 90.00 days with mean value 89.25. The maximum days to 50% flowering were found in Pant CMS 3A i.e. 110.00, 103.00, 110.00 and 110.00 days with mean value 108.5 in all four years.

The newly developed cytoplasmic male sterile line i.e. Pant CMS 3A showed significant advantage over widely used CMS lines viz. IR58025A and Pusa 6A with respect to panicle exertion and out crossing rate. Such findings were also reported in the Pant CMS 2A (Nautiyal *et. al.* 2011) [1]. The CMS line, Pant CMS 3A was tolerant to major pests and diseases in field conditions.

Fig 3: Pant CMS3A

Table 3; showed that the longest Panicle length (cm) was recorded in Pant CMS 3B i.e. 31.43, 32.60, 31.83 and 32.40 with mean value 32.06 followed by Pant CMS 3A with 29.13, 30.66, 29.30 and 30.66 with mean value 29.93. The number of filled grains recorded maximum in Pant CMS 3B i.e. 305.43, 303.83, 302.00 and 310.00 with mean value 305..31 followed by IR79156B i.e. 158.60, 159.80, 160.00 and 158.00 with mean value 159.1 Simultaneously total numbers of grains per panicle was found maximum in Pant CMS 3A i.e. 340.26, 349.83, 338.40 and 348.20 with mean value 344.17 however, the lowest number of grains per panicle was found in Pusa 6A i.e. 136.40, 142.00, 138.62 and 140.00 with mean value 139.25 respectively. Yield (kg/ha) was found maximum with

mean value in Pant CMS3A 2043.00 kg/ha in natural conditions without using supplementary pollination. If Pant CMS 3A is used in the hybrid seed production, use of GA_3 can be avoided and high seed yield in seed parent can be obtained. Pant CMS 3A line can definitely reduce the cost of hybrid rice seed production and will be more economically viable to the seed producers and seed growers. Presently the hybrid seed yield is being obtained 10-12 q/ha in India. However, the seed yield obtained in Pant CMS 3A, 18-20 q/ha which is significantly higher than the existing cytoplasmic male sterile lines. Further seed yield can be improved in Pant CMS 3A by timely supplementary pollination. The grains of Pant CMS 3A are long slender i.e. more than 10mm. Pant CMS 3A line is being tried with prominent restorers to find out the heterotic combinations. The Pant CMS 3A line is being maintained at G. B. Pant University of Agriculture & Technology, Pantnagar.

Table 1: Morphological traits of different CMS lines

CMS Line	Plant height (cm)					Days to 50% flowering					Number of tillers/plant				
	2013	2014	2015	2016	Mean	2013	2014	2015	2016	Mean	2013	2014	2015	2016	Mean
IR58025A	86.0	87.33	87.00	87.20	86.88	97.00	96.00	98.00	97.00	97.00	16.50	13.56	14.50	13.96	14.63
IR58025B	89.0	91.43	89.20	90.86	90.12	94.00	95.00	96.00	95.00	95.00	13.33	15.93	12.30	11.20	13.19
Pusa 6A	98.93	99.43	98.80	99.50	99.16	97.00	97.00	97.00	98.00	97.25	19.06	17.46	22.76	23.56	20.71
Pusa 6B	100.06	100.86	99.60	100.20	100.18	93.00	95.00	93.00	94.00	93.75	20.26	20.50	19.20	20.50	20.11
Pant CMS 3A	105.00	104.33	110.00	111.60	107.7	110.00	103.00	110.00	111.00	108.5	24.66	24.06	24.66	24.00	**24.34**
Pant CMS 3B	111.33	112.00	113.00	114.20	112.63	105.00	106.00	107.00	107.00	106.25	22.80	23.30	23.00	23.30	23.10
IR79156A	90.00	89.00	89.00	90.60	89.65	89.00	88.00	90.00	90.00	89.25	18.33	19.25	18.56	19.20	18.83
IR79156B	91.00	89.60	90.20	91.00	90.45	87.00	88.00	88.00	89.00	88.00	18.20	18.46	18.26	18.36	18.32
CD at 5%	2.13	4.05	2.93	3.95	-	2.51	1.35	2.21	2.00	-	1.40	1.03	1.60	1.01	-
CV	1.19	2.24	2.23	2.16	-	1.38	0.74	1.25	1.10	-	3.98	2.82	3.21	2.61	-

Table 2: Out crossing rate and Panicle exertion in different CMS lines

CMS Line	Out crossing (%)						Panicle exertion (%)			
	2013	2014	2015	2016	Mean	2013	2014	2015	2016	Mean
IR58025A	13.33	15.46	14.20	15.00	14.49	72.16	72.40	72.60	72.00	72.29
IR58025B	88.16	89.92	89.00	87.94	88.75	Complete	Complete	Complete	Complete	-
Pusa 6A	18.14	19.17	18.24	18.64	18.54	71.06	71.40	71.00	71.30	71.19
Pusa 6B	91.64	91.99	91.00	91.20	91.45	Complete	Complete	Complete	Complete	-
Pant CMS 3A	32.08	31.58	38.00	37.80	34.86	96.83	96.50	96.80	97.20	96.83
Pant CMS 3B	95.06	94.25	94.80	95.26	94.84	Complete	Complete	Complete	Complete	-
IR79156A	17.96	18.60	18.90	19.26	18.68	72.16	72.40	74.00	73.80	73.09
IR79156B	90.33	90.88	90.40	91.00	90.65	Complete	Complete	Complete	Complete	-
CD at 5%	3.06	1.43	3.01	1.41	-	-	-	-	-	-
CV	2.99	1.38	2.61	1.30	-	-	-	-	-	-

Table 3: Panicle length, Number of filled grains, Total number of grains and Yield (Kg/ha) in different CMS lines

CMS Line	Panicle length (cm)					Number of filled grains					Total number of grains					Yield (kg/ha)				
	2013	2014	2015	2016	Mean	2013	2014	2015	2016	Mean	2013	2014	2015	2016	Mean	2013	2014	2015	2016	Mean
IR58025A	20.66	21.66	20.88	21.66	21.21	23.36	27.30	23.86	27.60	25.53	174.33	175.96	174.33	172.66	174.32	1231.00	1176.00	1178.00	1231.00	1204.00
IR58025B	21.46	21.90	21.34	21.64	21.58	147.16	154.20	148.00	154.00	150.84	168.16	171.46	170.16	172.42	170.61	2616.00	2693.00	2700.00	2764.00	2693.25
Pusa 6A	24.73	24.93	24.80	24.93	25.59	24.72	28.90	24.20	29.00	26.70	136.40	142.00	138.62	140.00	139.25	1153.00	1193.00	1155.00	1196.00	1174.25
Pusa 6B	25.63	26.56	25.83	26.54	26.14	137.83	155.16	138.00	160.00	147.74	150.40	168.66	154.60	169.00	160.66	2393.00	2483.00	2492.00	2420.00	2447.00
Pant CMS 3A	29.13	30.66	29.30	30.66	29.93	109.20	110.50	111.00	113.25	110.98	340.26	349.83	338.40	348.20	344.17	1953.00	2043.00	2053.00	2043.00	**2023.00**
Pant CMS 3B	31.43	32.60	31.83	32.40	32.06	305.43	303.83	302.00	310.00	305.31	321.40	322.33	324.60	326.20	323.63	4633.00	4833.00	4833.00	5022.00	4830.25
IR79156A	22.46	23.00	23.34	23.00	22,95	24.96	29.00	29.20	28.60	27.94	178.23	180.20	176.33	179.20	178.49	1168.00	1180.00	1199.00	1265.00	1203.00
IR79156B	21.80	23.26	23.84	24.20	23.27	158.60	159.80	160.00	158.00	159.1	179.23	180.21	178.40	180.20	179.51	2899.00	2762.00	2792.00	2692.00	2786.25
CD at 5%	1.22	0.95	1.20	0.92	-	7.13	4.07	7.11	4.06	-	4.77	1.96	4.78	1.92	-	1.92	1.65	1.91	1.61	-
CV	2.63	1.98	2.60	1.96	-	3.14	1.72	3.12	1.70	-	1.22	0.48	1.20	0.49	-	4.54	3.79	4.49	3.72	-

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