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Influence of GA₃ on seed multiplication of CMS lines used for hybrid rice development

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In Pakistan, the F₁ seed production ranks very low in the three-line system. One of the reasons is poor panicle exertion and less out crossing rate in the female lines. Gibbrillic Acid (GA₃) increase the seed and improve panicle exertion as well as outcrossing. This study was designed to identify the suitable doses of GA₃ application in the seed multiplication block of different CMS lines. The material comprising both the parental lines (cytoplasmic male sterility and maintainer) used in the development of hybrid rice. GA₃ powder used in the experiment is 90% concentration in three level concentration that is 0, 100, 200, ppm. The outcomes indicated that GA₃ positively increased plant height, stigma exertion, panicle exertion, duration of floret opening, angle of floret opening and panicle length. Increased seed production is 0.3 to 1 t/ha compared to the control (0 ppm GA₃). Application of GA₃ concentration at the rate of 200ppm gave the best results as compared to the other treatments like control and 100 ppm in all the tested CMS lines increased productivity. So, 200 ppm concentration is recommended in seed multiplication of parental lines used in hybrid rice development.

Key words: Hybrid rice, CMS lines, GA₃, seed multiplication.

INTRODUCTION

Rice is a significant crop for food security everywhere in the world. Rice demands are very high since maximum of the people consumes rice. There are two ways to meet the rice needs: intensifying the rice planting area and increased production, or together. But in the upcoming time, expansion in area will be tougher and non-economical, significant advancement can be done over the implementation of hybrid rice (Nguyen, 2010). Amongst the several possible methods, hybrid rice cultivation is the most reasonable and practical one in estimation of its 10-15% yield benefit over the high

yielding conventional varieties. The accomplishment in growing rice production through hybrid rice has been proven in China. The rise in production is 15-20% greater than the best commercial inbred rice, with a planting area grasped more than 50% of the total rice cultivated area (You et al., 2006). Hybrid rice has added meaningfully to food safety in China in the last 25 years. Rice is autogami plant so that the level of cross breeding is naturally low (Sheeba et al., 2006), so the hybrid rice is developed by male sterility system. The genetic systems known for developing rice hybrids are two type systems. First,

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cytoplasmic-genetic male sterility (CMS) for the development of three-line hybrid rice. Second, photo/thermo-sensitive genetic male sterility (PGMS or TGMS) for developing two-line hybrid rice (Yuan et al., 2003).

In Pakistan, hybrid rice is developed by the three line systems. The drawback of F_1 system level for seed production is very low in this system. Poor panicle exertion, due to which about 25 to 30% panicle remained inside sheath of flag leaf and low outcrossing rate are also the main reasons of low seed production. Gibberellic Acid application is an effective means to increase seed production rate by coping the problems; it also enhances cell elongation. In hybrid rice seed production, GA_3 plays a vital role to obtain high seed yield. In China, application of Gibberellic acid (GA_3) at fairly high concentration (150-225 g/ha) plays a significant role in solving the problem of poor panicle exertion, better stigma exertion and stigma receptivity; besides enhancing wider glume opening, thereby increasing the outcrossing rate (Duan and Ma, 1992).

It can upsurge the rate of stigma exertion, increase the duration of floret opening, increase the rate of panicle exertion from the flag leaf sheath, modify plant height, and make the later taller and productive (Virmani and Sharma, 1993; Yuan et al., 2003; Viraktamath and Ilyas, 2005; Gavino et al., 2008). The behavior of GA_3 on development and growth of plants is determined by environment and varieties. In a new growing environment of hybrid rice adoption in Sindh province of Pakistan, it is essential to recognize the suitable concentration of GA_3 for some hybrid rice varieties. Thus, it is required to conduct a research to study the impact/effect of GA_3 different concentration on some hybrid rice varieties. This research was expected to provide some information regarding appropriate GA_3 concentration levels to support the process of pollination between male sterile lines with maintainer, in Pakistan

MATERIALS AND METHODS

The experiment was conducted at Rice Research Institute, Kala Shah Kaku, during kharif seasons, 2016 and 2017, in randomized complete block design with 3 replications. The material comprise both the CMS lines and their maintainer lines. The GA_3 powder was used in the experiment with 90% concentration in three levels, that is 0, 100, 200 ppm. The age of seedling for transplanting was 25 days for ten CMS (A), named KSK and maintainer (B) lines. B lines or maintainer lines are morphologically similar to the A line expect fertility, due to which it produce the pollens to pollinate the A line. Both A lines and B-lines were sown on 3 days intervals. Then, the equal proportion of seedlings of B lines transplanted on two different dates were mixed equally before transplanting. Maintainer lines were transplanted in the pured rows with two/three seedlings per hill keeping 30x15 cm spacing and A line was 15x15 cm and space was 20 cm between A and B line, in a plot of 4x5 m. The row ratio of B:A was 2:6 used in the experiments. Each CMS lines along with maintainer were individually positioned out in the field. The polyethylene plastic was used in testing the area as barrier to isolate different CMS lines and other experimental material at 2.5 m height, during the flowering stage confirm the F_1 seed purity. Every

plot of CMS line was sprayed with GA_3 in two spraying, using knapsack sprayer. Initially sprayed, A lines were at 5-10% heading stage. Second time spraying was done three days after first time spraying. One, which remained untreated, was used as the control. The data were recorded on 5 randomly selected plants from each replication for quantitative characters. The characters studied were plant height (cm), productive tillers per plant, panicle length (cm), panicle exertions of male sterile lines (%), stigma exertions of male sterile line (%), seed set (%), angle of floret opening ($^\circ$) and seed yield (kg/ha). The over-all reference for data assortment was by standard evaluation system for rice (SES) (IRRI, 2002). The data assembled was statistically investigated, using the analysis of variance (ANOVA) in Split Plot Design (parental lines were in main plot and sub plot was dosages of GA_3) to the test the significance for each trait. On the significance of results, the treatments were compared using 5% level of significance of Duncan's Multiple Range Test (DMRT) (Steel and Torrie, 1993). The list of plant material, including CMS lines along with their maintainer, is as shown in Table 1.

RESULTS AND DISCUSSION

The GA_3 concentrations significantly affect all studied parameters except the number of productive tillers per plant and 50% flowering age in the analysis of variance. The interface between CMS lines and GA_3 concentration revealed in panicle exerts plant height panicle length, stigma exertion, and seed set and seed yield (Biradarpatil and Shekhargouda, 2006; Tiwari et al., 2011; Susilawati et al., 2014). The panicle exertion is also affected by the application of GA_3 (Gavino, 2008; Rumanti, 2012; Susilawati et al., 2014). Plant height of the CMS lines increases with the increase in dose from 0-200 ppm. It increases on application of 100 ppm GA_3 , with range of 8-10 cm. GA_3 concentration of 200 ppm causes the increase in plant height from 7 to 18 cm (Table 1). The rise in plant height was due owing to improved action of cells division, elongation and enlargement. Regulation of several processes of plant growth and development is censored by the Gibberline, which is a hormone important for cell elongation also (Hedden and Phillips, 2000; Sakamoto et al., 2004; Sun, 2004; Tiwari et al., 2011). Values with different letter(s) within a column differ significantly at 5% level probability (LSD).

Panicle length and panicle exertion influenced by the interaction between varieties with GA_3 spraying at 100, 200 ppm significantly improved the panicle length linked to controls on all CMS lines (Table 1). Impact on panicle elongation due to GA_3 spraying is from 12cm-18cm within application of GA_3 , which rises panicle length due to elongation and cell division (Yuan et al., 2003; Tiwari et al., 2011). The point from where the panicle comes out from the flag leaf sheath to total panicle length in the panicle exertion is measured. All the parental lines tested in this experiment of GA_3 application showed significant increase in panicle exertion rate. On both treatments of 100 and 200 ppm, the values showed the GA_3 at 100 and 200 ppm, which shows the highest effect (87 to 89%) compared to controls treatment (74 to 79%). Yin et al.,

Table 1. List of Cytoplasmic CMS lines and their maintainer lines used in seed multiplication.

S/N	Name of A line and B lines	Code name
1	KSK1310A	A1
2	KSK1310B	B1
3	KSK1315A	A2
4	KSK1315B	B2
5	KSK1313A	A3
6	KSK1313B	B3
7	KSK1401A	A4
8	KSK1401B	B4
9	KSK1402A	A5
10	KSK1402B	B5
11	KSK1317A	A6
12	KSK1317B	B6
13	KSK1301A	A7
14	KSK1301B	B7
15	KSK1319A	A8
16	KSK1319B	B8
17	KSK1302A	A9
18	KSK1302B	B9
19	KSK1318A	A10
20	KSK1318B	B10

(2007) study has presented the influence of GA₃ on panicle base elongation of CMS lines and the panicle inside flag leaf sheath was filled out, so the chances of grain filling increases in the rise of panicle exertion. In this research finding, the panicle exertion doses not reach 100% while the concentration of GA₃ application of 200 ppm on all spikelets emerged fully. For study one, CMS lines A8, A9, and A10 showed the maximum panicle exertion on 200 ppm, although the panicle is not fully exerted but all the spikelets were out of flag leaf sheath. However, in stiff panicles the percentage of panicle exertion got to 100%, but also lowered the yield of plant. The percentage of panicles exertion, 100%, can result in brittle panicle by wind and rain; thus, it will lower the yield, particularly in the rainy weather (Gavino et al., 2008). Improved panicle exertion positively increase seed set. Chances of outcross breeding also increased if the panicle exertion is increased by a CMS line/variety, ultimately seed set improved. Application of GA₃ increased the panicle exertion by 20 to 30%, and rise in yield as much as 35 to 60% (Jagadeeswari et al., 1998). In this study, increase in seed set due to GA₃ spraying ranged from 5 to 40% (Table 2).

The angle of floret opening is significantly affected by spraying of GA₃ application as linked to the zero application (control) of GA₃ in spraying. The proper angel of floret opening is also helpful in more seed setting due to cross pollination, ultimately the seed setting will be high. Proceeding exhibited that high degree of cross-

pollination in hybrid rice is affected by the angle of floret opening, large stigma surface and long duration of floret opening (Singh and Shirisha, 2003; Biradarpatil and Shekhargouda, 2006; Gavino et al., 2008; Susilawati et al., 2014), save for the lengthier pollination activities.

Values with dissimilar letter(s) within a row vary significantly at 5% level of probability (LSD). Seed yield indicated that all CMS tested for seed multiplication by application of GA₃ resulted in higher productivity as compared to control treatments values (Table 3). The GA₃ application with the doses of 100 and 200 ppm gave the increased yield. In previous studies, it is shown that the treatment of 200 ppm gave the highest seed yield as compared to 300ppm, because by application of 300 ppm the plant height of cms lines increased panicle stalk broke in rainy season and faced yield loss due to wind (Yuan 1985; Prasad et al., 1988; Gavino et al., 2008; Susilawati et al., 2014). Consequently, application of GA₃ must be improved with responsiveness of cm line, seasons and agro-ecological conditions and.

Conclusion

Treatment using two dissimilar GA₃ concentration positively increase plant height, panicle length, panicle exertion, angle of floret opening and enhanced some growth and flowering traits which are involved the rise in seed set/seed production of CMS lines by 0.3 to 1.2 t/ha.

Table 2. Agronomic traits of ten male sterile lines in several different applications of GA3 Concentration (Pooled data of two rice crops/seasons).

Parameter	Concentration of GA3 (ppm)			
	Plant Height			
	0	100	200	Average
A Lines				
A1	97	110	127	111
A2	92	105	121	106
A3	93	108	115	104
A4	105	115	132	117
A5	102	111	123	112
A6	75	87	105	89
A7	88	98	117	101
A8	100	104	125	110
A9	101	112	125	113
A10	95	105	115	
Average	95^c	105^b	110^a	
Number of productive tillers/plant				
A1	9	10	11	10
A2	13	12	10	12
A3	13	15	17	15
A4	17	16	16	16
A5	9	8	12	10
A6	15	13	12	13
A7	12	14	16	14
A8	16	14	17	16
A9	18	17	15	17
A10	13	11	17	14
Average	13	13	14	
Panicle Length (cm)				
A1	23	24	25	24
A2	28	29	30	29
A3	30	32	33	32
A4	24	26	27	26
A5	22	24	25	24
A6	19	23	25	22
A7	22	24	25	24
A8	26	28	29	28
A9	26	27	28	27
A10	22	23	24	23
Average	24^c	26^b	27.1^a	
Flowering age 50%				
A1	79	79	79	79
A2	75	75	75	75
A3	78	78	78	78
A4	75	75	75	75
A5	76	76	76	76
A6	71	71	71	71
A7	72	72	72	72

Table 2. Contd.

A8	76	76	76	76
A9	75	75	75	75
A10	73	73	73	73
Average	75	75	75	

Table 3. Panicle exertion, angle of floret opening, seed set, and seed yield of four male sterile lines in several different applications of GA3 concentration (Pooled data of two rice crops/seasons).

Parameter	Concentration of GA3 (ppm)			
	Panicle exertion			
	0 ppm	100 ppm	200 ppm	Average
CMS line				
A1	75	82	84	80
A2	74	82	85	80
A3	79	84	86	83
A4	76	85	85	83
A5	71	83	84	80
A6	75	87	83	82
A7	76	85	86	82
A8	79	86	88	85
A9	77	85	87	83
A10	75	84	89	83
Average	75.7	84.3	86.1	
Angle of floret opening				
A1	22.5	26.4	28.4	26
A2	24.7	28.2	30.1	28
A3	23.3	27.1	29.2	27
A4	22.4	26.2	27.8	25
A5	23.1	27.4	29.5	27
A6	24.3	28.5	30.7	28
A7	25.2	29.2	31.1	29
A8	23.5	26.7	28.7	26
A9	24.4	27.7	29.4	27
A10	22.5	26.5	28.7	26
Average	23.59	27.39	29.36	
Seed set %				
A1	17	25	26	23
A2	13	23	25	20
A3	16	32	34	27
A4	20	30	34	28
A5	19	21	23	21
A6	22	28	30	27
A7	24	31	35	30
A8	21	31	34	29
A9	23	31	33	29
A10	23	28	32	28
Average	20	28	31	

Table 3. Contd.

Seed Yield kg/ha				
A1	750	1120	1433	1434
A2	325	520	820	588
A3	640	841	1140	1040
A4	870	1015	1431	1472
A5	810	912	1550	1291
A6	590	740	914	650
A7	690	830	1017	1320
A8	810	1012	1387	1379
A9	780	920	1360	1110
A10	810	1017	1623	1217
Average	777.5 ^c	962.7 ^b	1710.4 ^a	

Application of GA₃ concentration at the rate of 200 ppm gives the best results as compared to other treatments like control and 100 ppm, in all the tested CMS lines productivity increased. Thus, concentration of 200 ppm is recommended in seed multiplication of parental lines for hybrid rice development.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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