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Full Length Research Article

Study the Effect of Different Treatments on Hybrid Rice Seed (F_1) Production and Assessment of Heavy Metals by Using Atomic Absorption Spectrophotometry

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Abstract

This scientific study aimed to investigate the impact of various treatments, including some heavy metal compounds, applied as foliar spray on CMS (A-line) rice plants, which exhibited inadequate panicle exsertion. The objective was to explore cost-effective alternatives to the commonly used GA_3 spray. Ten different treatments were evaluated, and penicillin emerged as the most promising option for enhancing panicle emergence at a reduced cost compared to GA_3.Additionally, we assessed the efficacy of heavy metal compounds in promoting panicle emergence in hybrid rice seed production. Among these compounds, nickel chloride and potassium dichromate demonstrated favourable results. To gain more insights into the impact of the heavy metal compounds, we quantified the amounts of specific heavy metals (Cu, Cr, Ni, Pb) present in the F_1 seeds. The measurements were conducted using atomic absorption spectrophotometry (AAS), a reliable analytical technique used to determine the concentrations of various elements in a sample. These findings highlight the potential of penicillin and certain heavy metal compounds as effective alternatives to conventional methods for enhancing panicle emergence in CMS (A-line) rice plants, with potential implications for cost-efficient agricultural practices and hybrid rice seed production.

***Key words*:** CMS, F_1 rice seed, AAS, Heavy metal, Panicle exsertion

The importance of the use of cytoplasmic genetic male sterile (CMS) line in the development of hybrid rice in a three-line system is well known to the breeders. For large scale hybrid seed production, a stable male sterile line is needed. To maintain the CMS line, a pollinator (R line) is used for seeding in CMS line. The commercial exploitation of heterosis in rice has been made possible by the use of cytoplasmic genetic male sterility and fertility systems said by Krishnalatha *et al*. [1]. According to Virmani *et al*. [2] success and sustenance of hybrid rice technology solely depend on the exploitation of heterosis in F_1 generation. Hybrid rice has the potential to increase yields by 15% to 20% over those of conventionally bred varieties stated by Virmani [3]. The CMS lines play a pivotal role in success and sustenance of hybrid rice technology. In fact, the belated success of hybrid rice technology in India was basically due to nonavailability of CMS lines suited to tropics as described by Ahmed *et al*. [4]. Duan and Ma [5] stated that the CMS lines had poor panicle exsertion which is a major problem in hybrid rice seed production associated due to physiological mechanism. According to Shi-Hua *et al*. [6] to enhance the efficiency of hybrid seed production, it is necessary to increase the yield of hybrid seed by improving out crossing capacity of CMS lines.

The problem of exsertion of panicle in CMS (A-line) is managed by spraying GA_3 plant hormone, as done by Shengqui [7]. GA_3 is a costly chemical which is a matter of problem to afford by the middle-class farmers as well as margin farmers. Ten treatments including some heavy metal compounds were applied as foliar spray on CMS (A-line) rice plants as the A-line plant could not exsert panicle fully. According to El-Shamey *et al*. [8] cytoplasmic male sterility (CMS) provides an irreplaceable strategy for commercial exploitation of heterosis and producing high-yielding hybrid rice. The exogenous application of plant growth regulators could improve outcrossing rates of the CMS lines by affecting floral traits and accordingly increase hybrid rice seed production. The work done by El-Shamey *et al*. [8] aimed at exploring the impact of growth regulators such as gibberellic acid (GA_3), indole-3-

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acetic acid (IAA), and naphthalene acetic acid (NAA) on promoting floral traits and outcrossing rates in diverse rice CMS lines and improving hybrid rice seed production. In our study, among the ten alternative treatments including heavy metal compounds nickel chloride and potassium dichromate exhibited good result for panicle emergence in F_1. Heavy metals like cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn), Nickel (Ni) and zinc (Zn) were analyzed by Wasim et al. (2019) from eighteen selected varieties of rice (*Oryza sativa* L.) in Pakistan using AAS [9]. In our experiment heavy metals (Cu, Cr, Ni, Pb) were also estimated by AAS from F_1 hybrid rice seed. To overcome the problem of panicle emergence, it is necessary to identify cheaper chemicals to substitute GA_3. Keeping in view of these needs the present investigation was undertaken to study the effect of GA_3 and other treatments on hybrid seed production.

MATERIALS AND METHODS

IR58025A (CMS line) and KMR3 (R-line) were procured from Rice Research Station, Chinsurah, Hoogly which were grown in the research plot in the crop research farm under the Department of Botany, the University of Burdwan in boro season. The experiment was laid out in a randomized block design method with three replications during boro season of 2013-14. R line and A line were transplanted in the ratio of 2:6. Treatments were applied as foliar spray on A line plants for F_1 hybrid rice seed production. Treatments and doses of chemicals are exhibited in (Table 1). The characters studied were (i) Panicle exertion length (ii) Total no. of grain panicle^{-1} (iii) Grain yield plant^{-1} and (iv) 1000 grain weight (g).

Table 1 Treatments and doses of chemicals

Treatments	Chemicals applied	Doses (ppm)
T_1	Control	-
T_2	Penicillin	400
T_3	Sulfonamide	100
T_4	Gentamycin	100
T_5	GA_3	60
T_6	Nickel chloride	100
T_7	Potassium dichromate	100
T_8	Lead acetate	100
T_9	Copper sulphate	100
T_{10}	Ammonium molybdate	100

Table 2 Instrumental parameters for AAS

Heavy metals	Wavelength (nm)	HCL lamp current (mA)	Slit width (nm)	C_2H_2 flow rate (lit/min)	Air flow rate (lit/min)
Cu	324.8	4	0.5	1.5	3.5
Cr	357.9	7	0.2	1.5	3.5
Ni	232.0	4	0.2	1.5	3.5
Pb	217.0	10	1.0	1.5	3.5

Fig 1 (a) Transplantation of R-line. (b) Transplantation of A-line. (c) Growth of A and R-line plants. (d) Growth of A and R-line plants. (e) Spraying of chemicals to A-line. (f) Synchronization of flowering (A & R-lines). (g) Mesurement of plants'growth. (h) Harvesting of crops

Among the treatments copper sulphate ($CuSO_4.5H_2O$), potassium dichromate ($K_2Cr_2O_7$), nickel chloride ($NiCl_2.6H_2O$) and lead acetate [$Pb(OAc)_2$]were applied as foliar spray on IR58025A line (CMS line). Then the F_1 seeds from these A line plants were collected and digested separately using concentrated nitric acid and perchloric acid after removing the husk from the seeds. Powdered form of 1g dried seed material were taken in a 100 ml beaker followed by the addition of10 ml concentrated nitric acid. It was then heated gently covering the beaker with a watch glass. After half an hour the watch glass was removed and the volume was reduced almost to dryness. Further concentrated nitric acid and few drops of perchloric acid were added for drying it as much as possible. Small amount of distilled water was added to dissolve the residue. Thereafter, the solution was transferred to a volumetric flask for determining the quantity of heavy metals copper (Cu), chromium (Cr), nickel (Ni), lead (Pb) present in four different sample solution by atomic absorption spectrophotometer (Varian, spectr AA, 55B) against the corresponding element. The instrumental parameters are listed below (Table 2). Mean values were subjected to analysis of variance (ANOVA) to test the significance for each character.

RESULTS AND DISCUSSION

Experimental results were obtained from the present study towards the effects of treatments and their interactions on different characters through the mean values were recorded in (Table 3) and combined analysis of variance (ANOVA) (Table 4) from 25 A (CMS line) population at the time of harvest as furnished below:

Table 3 Data showing mean values of various metrical characters at a glance

Season	Treatment	Panicle exsertion length (cm)	Total no. of grain panicle^{-1}	Grain yield plant^{-1} (g)	1000 grain weight (g)
Boro season (2013-14)	T_1	19.41	190.05	68.03	18.88
	T_2	22.61	211.95	71.26	21.20
	T_3	20.06	210.63	69.40	20.35
	T_4	19.81	209.56	69.26	20.20
	T_5	21.46	210.58	70.60	20.78
	T_6	21.18	208.26	70.46	20.45
	T_7	21.32	208.25	69.66	20.28
	T_8	20.45	195.76	60.40	20.25
	T_9	18.10	193.63	54.90	18.17
	T_{10}	19.93	190.15	67.78	18.81

Four metrical characters were studied and a combined ANOVA table was prepared and exhibited in (Table 4).

Table 4 Combined ANOVA at harvest

Character	Combined ANOVA							Components of variances			Genotypic coefficient of variations, phenotypic coefficient of variations and heritability		
	Source of variation	df	SS	MS	F	CD Value	CV Value	δ^2g	δ^2p	δ^2e	GCV	PCV	h^2
Panicle exsertion length (cm) plant^{-1}	Treatment	9	43.3144	4.81271	183.38**	0.08	0.79	1.59	1.61	0.02	6.16	6.20	0.98
	Replication	2	0.0745	0.03726	1.42 NS								
	Error	18	0.4724	0.02624									
Total no. of grain panicle $^{-1}$	Treatment	9	2301.14	255.682	5125.04**	0.16	0.11	85.21	85.26	0.05	4.54	4.55	0.99
	Replication	2	0.34	0.171	3.43 NS								
	Error	18	0.09	0.050									
Grain yield (g) plant^{-1}	Treatment	9	757.573	84.1748	446.05**	0.62	0.64	27.99	28.17	0.18	7.87	7.90	0.99
	Replication	2	1.158	0.5791	3.07 NS								
	Error	18	3.397	0.1887									
1000 grain weight (g)	Treatment	9	20.5653	2.28504	506.74**	0.014	0.33	0.76	0.764	0.004	4.36	4.37	0.99
	Replication	2	0.0372	0.01858	4.12*	0.010							
	Error	18	0.0812	0.00451									

Heavy metals copper (Cu), chromium (Cr), nickel (Ni), lead (Pb) were estimated by AAS and exhibited in (Table 5).

Table 5 Estimation of heavy metals by AAS

Heavy metal	Amounts (µg/g of sample)
Copper (Cu)	10.90
Chromium (Cr)	3.92
Nickel (Ni)	0.50
Lead (Pb)	0.10

Various peculiarities were critically observed during cropping time till harvesting. The length of panicle exsertion was found to be highest (22.61cm) in treatment- 2 (Penicillin) and lowest (18.10 cm) in case of treatment-9 (copper sulphate). Accordingly, the exserted panicle length was observed 19.81 cm and 21.46 cm in case of gentamycin and GA_3 respectively. According to Mc-Manus *et al*. [10] gentamycin was used as alternative low-cost chemical of GA_3. Foliar application of GA_3 at the heading stage enhances floret opening duration, stigma properties, panicle exertion of male sterile lines, rate of stigma exertion, out-crossing rate and seed yield potential during hybrid rice seed production [11].

Grain yields in case of penicillin treatment was 71.26 g plant^{-1} which was highest among all the treatments. Nickel chloride-T_6 and Potassium dichromate-T_7 exhibited good results in case of panicle emergence. Panicle emergence length was 21.18 and 21.32cm respectively in case of T_6 and T_7 application. Grain yield plant^{-1} was 70.46g and 69.66 g in case of nickel chloride and potassium dichromate application**.** In this case, lowest number of grain panicle^{-1} was found in treatment-9 (Copper sulphate treated). Lead compound show decline in productivity. Lead contaminated soils show a sharp decline in crop productivity [12] which is very much relevant in our observation. 'F' values of each metrical character were found to be significant at 0.01 level of probability against treatment source of variation and the values of h^2 were found 0.98 and 0.99 in these cases. From this result it is evident that the treatment Penicillin and GA_3 given more or less similar though penicillin effect was indicated a little bit better than that of GA_3 effect. The lowest yield performance was found to be noted in case of the effect of copper sulphate compound. Potassium dichromate and nickel chloride were given better yield component than copper sulphate and ammonium molybdate. According to Aide *et al*. [13] potassium promoted panicle development and rice yield, especially at the highest K rate which is similar with our work.

In this experiment treatment-2 (Penicillin) having low cost reflected more significant and excellent results than other treatments. According to Mukherjee and Biswas [14], penicillin promoted elongation of rice seedlings. They also stated that nucleic acids and protein were maintained at much higher levels in seedlings treated with penicillin. Biswas and Mukherjee [14] said that penicillin can also be as active as cytokinins in causing a dramatic increase in chlorophyll levels in intact rice seedlings [15]. Mukherjee and Wareings [16], also stated that the levels of gibberellin and cytokinin like substance are increased in mungbean seedlings by penicillin treatment.

The quantity of heavy metals copper (Cu), chromium (Cr), nickel (Ni), lead (Pb) were measured from F_1 seeds by means of Atomic Absorption Spectrophotometry (AAS). Among the heavy metal compounds potassium dichromate and nickel chloride exhibited good results for panicle emergence.

CONCLUSION

Present study revealed distinct effects of various treatments on the observed characteristics. Penicillin emerged as the top-performing treatment, displaying both high yield and cost-effectiveness. GA_3 and gentamycin followed closely, showcasing promising results as well. Among the heavy metal compounds tested, nickel chloride and potassium dichromate demonstrated positive effects on panicle emergence in hybrid rice seed production. Importantly, the analysis of heavy metal quantities in the F_1 seeds indicated that their presence is unlikely to impact human health adversely, as these seeds are not intended for consumption as food. These findings highlight the potential of penicillin and selected heavy metal compounds as valuable alternatives in agricultural practices, particularly for enhancing rice yield and productivity. However, further investigations are necessary to assess long-term environmental implications and ensure sustainable implementation. Overall, this research contributes valuable insights towards the development of effective and economically feasible treatments in the realm of rice cultivation and agricultural management.

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