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Analysis of Trait Variation and Correlations in Hybrid Rice Seed Production Using CMS Breeding Techniques

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Abstract

Imperfect panicle emergence in Cytoplasmic Male Sterile (CMS) lines is a hindrance for hybrid rice seed (F_1) production. In CMS lines 10-15% of spikelets remaining enclosed within the flag leaf. This limits their availability for outcrossing, thereby reducing the efficiency of hybrid rice seed production. To mitigate this issue, the application of gibberellic acid (GA_3) is a common practice. However, the high cost of GA_3 poses a significant barrier for middle-class farmers involved in hybrid rice seed production. This study aims to identify low-cost chemical alternatives to GA_3. We evaluated ten different treatments, including a control, on CMS (A) line rice plants. Our findings indicate that penicillin is the most effective treatment, offering high yield at a reduced cost. According to the yield component, treatment^{-2} (penicillin) reflected grain yield 71.26 g plant $^{-1}$ which is highest among all the treatments. Potassium dichromate also performed well in promoting panicle emergence. Characters showing positive and significant correlations among different traits are expected to improve yield and can be selected for further advancement in hybrid rice seed production.

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Introduction

Essentially, hybridization is the most potential breeding method for improvement of crops. Hybrids are effective only when such product shows heterosis in yield over traditional popular varieties to become commercially successful. The three-line hybrid rice system involves three distinct parental lines: The A line (Cytoplasmic Male Sterile, CMS line), the B line (Maintainer line), and the R line (Restorer line). Each line plays a critical role in the production of hybrid rice, contributing to sterility, maintenance, and fertility restoration, respectively. Hybrid rice is a crop of cross between two genetically dissimilar parents. The foundational work on the development and utilization of hybrid rice technology in India began in the 1970s. A significant milestone was achieved

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in December 1989, when the Indian Council of Agricultural Research (ICAR), New Delhi, launched a time-bound, goal-oriented network project, prioritizing hybrid rice in the national agricultural agenda. Improving the efficiency of hybrid seed production requires enhancing the outcrossing capacity of Cytoplasmic Male Sterile (CMS) lines to increase hybrid seed yield.[1]

Gibberellic acid (GA_3) significantly enhances stigma exsertion rates, extends the duration of floret opening, and improves panicle exsertion from the flag leaf sheath. These effects contribute to improved outcrossing efficiency, making GA_3 a valuable growth regulator in hybrid rice seed production.

Additionally, GA_3 influences plant height, often resulting in taller plants with improved productivity, making it a crucial growth regulator in hybrid rice cultivation.[2-5] Gibberellic acid (GA_3) is an effective but costly chemical, posing a challenge for adoption by middle-class farmers. Growth regulators such as GA_3, indole-3-acetic acid (IAA), and naphthalene acetic acid (NAA) have been shown to enhance floral traits and improve outcrossing rates in various Cytoplasmic Male Sterile (CMS) rice lines, thereby boosting hybrid rice seed production.[6] The application of GA_3 alone led to the highest plant height, panicle length, higher seed yield followed by the combination of Brassinolides and GA_3, indicating their effectiveness in enhancing panicle exsertion and then Brassinolides alone across with CMS lines in both the seasons. Hybrid rice seed yield reached up to 276 g in treated plants compared to 73 g in the control, with the increase over control being higher in CRMS 32A (80%) than in IR 62829A (54%). Additionally, seed set percentage showed a significant positive correlation with seed yield.[7] The treatment combination T26 (GA_3 45 kg + Urea 10 g + Boric Acid 2 g + $ZnSO_3$ + K_2PO_4 2 g) yielded optimal results, suggesting it as an effective approach to enhance hybrid rice seed production. This treatment combination has potential as a substitute for GA_3 in India and other hybrid rice-producing countries.[8] The highest seed yield in CMS lines could be achieved with the application of GA_3 at 30 g ha^{-1} combined with Nutragold, highlighting its effectiveness in promoting hybrid rice seed production.[9] For improving yield, correlation is instrumental in identifying suitable selection criteria and yield-based direct selection may be beneficial. So, it is essential to know the relationship between morpho-agronomic characteristics and seed production for a successful selection process.[10]

The aims and objectives of this study is to explore low cost alternatives other than GA_3 to combat the unexsertion of panicle of CMS (A) line and enhance hybrid rice seed production.

Materials and Methods

IR58025A (CMS line) and KMR3 (R-line) were procured from the Rice Research Station, Chinsurah, Hooghly, and grown in the research plot at the Crop Research Farm under the Department of Botany, University of Burdwan, Tarabag, Bardhaman, West Bengal during the boro season.The experiment was conducted using a randomized block design (RBD) with three replications during the boro season of 2013-14. In this study, the R line and A line were transplanted at a 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) plant height (cm) (ii) no. of tiller $plant^{-1}$ (iii) total panicle length $plant^{-1}$ (cm) (iv) panicle exertion length (cm) (v) total no. of grain $panicle^{-1}$ (vi) no. of fertile grain $panicle^{-1}$ (vii) grain yield $plant^{-1}$ and (viii) 1000 grain weight (g). All the characters were also taken into account for calculating its analyses of variance (F value), its critical difference (CD) and coefficient of variation (CV) values. Similarly, other components of variances like genotypic variance (δ^2g), phenotypic variance (δ^2p),environmental variance (δ^2e), genotypic and phenotypic coefficient of variations i.e. GCV and PCV including heritability (h^2) were also estimated. For analysing correlation values (r), some metrical characters viz. plant height (cm), total panicle length $plant^{-1}$ (cm), panicle exsertion length $plant^{-1}$ (cm.), grains $panicle^{-1}$ (no.), fertile grain $panicle^{-1}$ (no.), 1000 grain weight (g) were selected.

By the assessment of value of variance i.e. 'F' value was noted whether it is significant or not. When the 'F' value was significant in order to compare the error mean square (EMS) and the degree of freedom (df) we calculated the critical difference (CD) by the following formula

CD = SE × t ...(1)

Where, SE is standard error of the difference of the treatment means to be compared, and is equal to

$SE = (2MS_e / r)^{1/2}$ ...(2)

With MS_e as error mean sum of square and r as the number of replications, and 't' is the tabulated value at 5% or 1% level of significance for the degree of freedom of error mean square. Thus,

$CD = (2MS_e / r)^{1/2} \times t$ ...(3)

The coefficient of variation (CV) was also calculated by the following formula

$CV = \frac{S.D}{\bar{X}} \times 100$ ...(4)

Accordingly, the components of variances were also calculated by the following equation

$E (MS_v) = \delta^2 e + r\, \delta^2 g$ ...(5)

$E (MS_e) = \delta^2 e$ ...(6)

And, therefore,

$\delta^2 g = \frac{MS_v - MS_e}{r}$ ...(7)

Thus, the genotypic variance being $\delta^2 g$ and the environmental variance as $\delta^2 e$, the phenotypic variance, i.e.

$\delta^2 p = (\delta^2 g + \delta^2 e)$ ...(8)

The components viz. $\delta^2 p$, $\delta^2 g$ and $\delta^2 e$ were used for estimation of other useful statistics such as phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), as followed by Singh and Chaudhary.[11] The broad sense heritability (h^2) was also calculated in all metrical character by the formula as

$h^2 = \delta^2 g / \delta^2 p.$ ...(9)

Pearson's correlation coefficient is given by

$r_{xy} = \frac{\sum(x_i-\bar{x})(y_i-\bar{y})}{\sqrt{\sum(x_i-\bar{x})^2 \sum(y_i-\bar{y})^2}}$ ...(10)

r_{xy}= correlation coefficient between x and y (Pearson's correlation coefficient)
x_i= the values of x within a sample
y_i= the values of y within a sample
$\bar{x}$= the average of the values of x within a sample
$\bar{y}$= the average of the values of y within a sample

Results
Mean values of various metrical characters are exhibited in (Table 2).

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	GA3	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: Data showing mean values of various metrical characters at a glance

Season	Treatment	Plant height (cm)	No. of tiller plant^{-1}	Total panicle length (cm) plant^{-1}	Panicle exsertion length (cm)	Total no. of grain panicle^{-1}	No. of fertile grain panicle^{-1}	Grain yield plant^{-1} (g)	1000 grain weight (g)
(Boro-	T1	79.66	10.16	22.81	19.41	190.05	75.55	68.03	18.88
2013	T2	83.78	11.53	25.75	22.61	211.95	81.81	71.26	21.20
-14)	T3	79.73	10.74	23.76	20.06	210.63	78.95	69.40	20.35

T4	79.23	10.87	23.91	19.81	209.56	79.04	69.26	20.20
T5	82.61	11.32	24.76	21.46	210.58	80.70	70.60	20.78
T6	82.76	11.10	24.50	21.18	208.26	79.85	70.46	20.45
T7	81.66	10.83	24.68	21.32	208.25	79.76	69.66	20.28
T8	80.95	10.71	24.51	20.45	195.76	78.47	60.40	20.25
T9	79.03	10.72	22.96	18.10	193.63	76.95	54.90	18.71
T10	76.46	10.17	23.25	19.93	190.15	75.56	67.78	18.81

Table 3: Combined ANOVA for various metrical characters

Character	Combined Anova							Components of Variances			Genotypic Coefficient of Variations, Phenotypic Coefficient of Variations and Heritability		
Plant height (cm)	Source of variation	df	SS	MS	F	CD Value	CV Value	δ²g	δ²p	δ²e	GCV	PCV	h²
	Treatment	9	129.550	14.3945	110.59**	0.43	0.44	4.75	4.88	0.13	2.70	2.74	0.97
	Replication	2	1.137	0.5686	4.37*	0.31							
	Error	18	2.343	0.1302									
No. of plant^{-1}	Treatment	9	5.15299	0.572554	726.11**	0.002	0.25	0.19	0.1907	0.0007	4.03	4.037	0.99
	Replication	2	0.00014	0.000070	0.09^{ns}								
	Error	18	0.01419	0.000789									
Total panicle length (cm) plant^{-1}	Treatment	9	22.9170	2.54633	84.41**	0.10	0.72	0.83	0.86	0.03	3.78	3.84	0.96
	Replication	2	0.1287	0.06433	2.13^{ns}								
	Error	18	0.543	0.03017									
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	6.2	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	0.05									
No. of fertile grain panicle^{-1}	Treatment	9	118.956	13.2174	1686.61**	0.02	0.11	4.40	4.407	0.007	2.66	2.668	0.99
	Replication	2	0.002	0.0010	0.13^{ns}								
	Error	18	0.141	0.0078									
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	3.07ns								
	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*	0.01							
	Error	18	0.0812	0.00451									

(**) – Significant at 1% level of probability, (*) – Significant at 5% level of probability, ns – Not Significant.

Table 4: Correlation values (r) at a glance

Sl. No.	Characters	Correlation values (r)	Remarks
1.	Plant height (cm) vs. total panicle length plant^{-1}(cm)	0.8395	Positively correlated and significant at 1%
2.	Total panicle length (cm) plant^{-1} (cm) vs. fertile grains panicle^{-1} (no.)	0.6533	Positively correlated and significant at 1%
3.	Total grains panicle^{-1} (no.) vs. grain yield plant^{-1} (no.)	0.6108	Positively correlated and significant at 1%
4.	Total panicle length plant^{-1} (cm) vs. panicle exsertion length plant^{-1}	0.9277	Positively correlated and significant at 1%
5.	Fertile grains panicle^{-1} (no.) vs.1000 Grains weight (g.)	0.9570	Positively correlated and significant at 1%

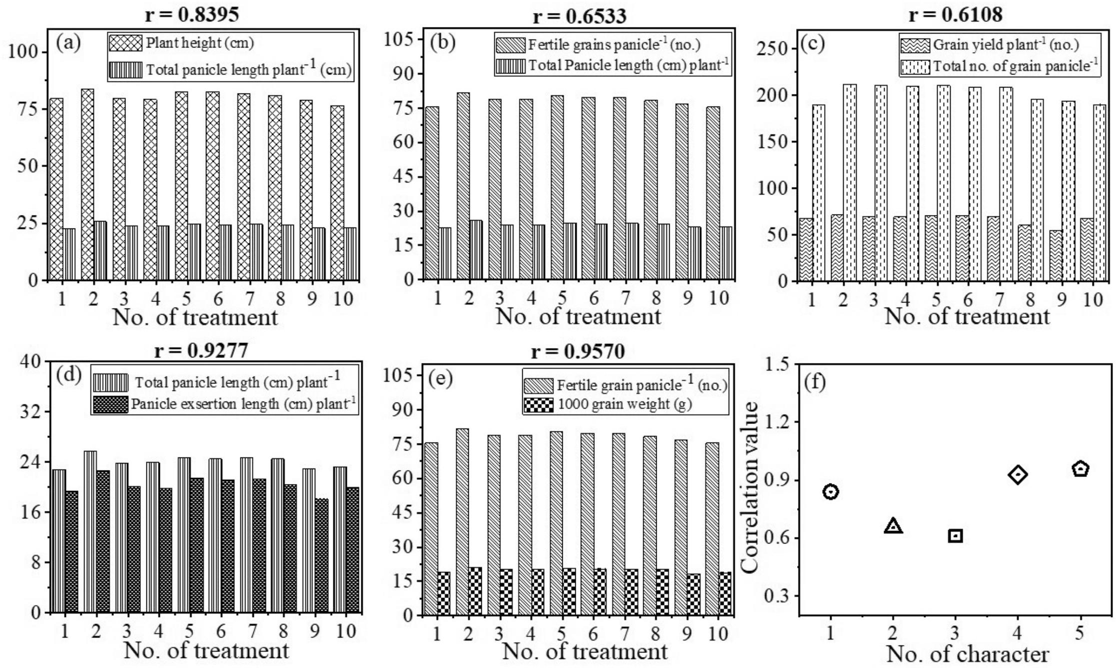

Fig. 1: Graphical representation of correlation values (r):(a) Plant height (cm) vs. total panicle length plant^{-1}(cm), (b) fertile grains panicle^{-1} (no.) vs. total panicle length plant^{-1} (cm) (c) grain yield plant^{-1} vs. total no.of grain panicle^{-1} (d) total panicle length plant^{-1} (cm) vs. panicle exsertion length plant^{-1}(cm) (e) fertile grain panicle^{-1} vs.1000 grains weight (g) (f) no. of character vs. correlation value.

Discussion

Various metrical characters under different treatments were studied carefully till harvesting. In this experiment it is recorded (Table 2) that plant height was highest (83.78 cm) in treatment-2 (penicillin) and lowest (76.46 cm) in treatment 10 (ammonium molybdate). Highest number of tiller (11.53) was found in case of treatment-2 (penicillin) and lowest was (10.16) in case of treatment-1 (control). An increased number of effective tillers plant^{-1} is closely associated with higher seed yield plant^{-1}, which subsequently contributes to enhanced overall productivity. This trait plays a critical role in determining yield potential in hybrid rice production. The exogenous application of NAA at 90 ml ha^{-1} during the panicle initiation and tillering stages significantly enhanced yield in coarse rice (IR-6), resulting in greater net returns. Thus NAA application increases yield potential and profitability in rice cultivation.[12]

Similarly, total panicle length plant^{-1} was also highest (25.75cm) in case of penicillin treatment. GA_3-T_5 also showed good result in this regard. Among the treatments, penicillin (treatment-2) exhibited the highest panicle exsertion length (22.61 cm), while the control (treatment-1) showed the lowest (19.41 cm). GA_3 treatment resulted in an exserted panicle length of 21.46 cm, and potassium dichromate recorded a panicle exsertion of 21.32 cm. GA_3-T_5 and potassium dichromate-T_7 exhibited good results in case of panicle emergence. The parental lines of hybrid rice KRH 2 (CMS line IR-58025A and restorer line KMR 3) treated with five GA_3 doses and a water control in a randomized block design with three replications.[13] Across both seasons, GA_3 application at 200 ppm was most effective in enhancing spikelet fertility in the CMS line, positively impacting seed yield. The highest seed yield (2.18 t ha^{-1}) was observed with 200 ppm GA_3 during the kharif season, while a slightly lower yet significant yield (2.15 t ha^{-1}) was achieved with 100 ppm GA_3 during the boro season. This suggests that GA_3 concentration may be optimized seasonally for maximizing hybrid rice seed production. Grain yield in case of penicillin treatment was 71.26 g plant^{-1} which was the highest among all the treatments. No. of fertile grain was also highest (81.81g) in case of penicillin treatment and it was lowest (54.90 g) in case of treatment 9 (copper sulphate). Potassium has been shown to promote panicle development and enhance rice yield, particularly at higher application rates.[14] Conversely, higher concentrations of copper sulphate (100-250 mg kg^{-1}) have been found to reduce growth, dry matter production, and nutrient content in greengram.[15] Lead compound also showed decline in productivity.[16] The analysis of variance revealed that F-values for all metrical characters were significant at the 0.01 probability level, with treatment as the source of variation. Heritability (h^2) values ranged from 0.97 to 0.99, with the highest value (0.99) recorded for traits such as the number of tillers plant^{-1}, total grains panicle^{-1}, fertile grains panicle^{-1}, grain yield plant^{-1} (g), and 1000-grain weight (g). High heritability, coupled with substantial genetic advance and significant genotypic and phenotypic coefficients of variation, suggests strong potential for the genetic improvement of this traits.[17]

The component of variances both genotypic and phenotypic gave the significant data. Higher values were found in case of $\delta^2 g$ for the metrical characters like number of grain panicle^{-1} (85.21), number of fertile grains panicle^{-1} (4.40) and grain yield plant^{-1} (27.99) (Table 3). Weekly foliar application of GA_3 during the 10–30% panicle heading stage can significantly enhance grain yield in MR219, a widely cultivated Indica rice variety released by the Malaysian Agricultural Research and Development Institute (MARDI).[18] This finding underscores the potential of GA_3 application timing in optimizing yield outcomes for hybrid rice production.

Similarly, in case of $\delta^2 p$ some metrical traits showed the significant higher values viz. number of grain panicle^{-1} (85.26), number of fertile grain panicle^{-1} (4.40) and grain yield plant^{-1} (28.17). In all the cases the $\delta^2 e$ were found to be very lower values except grain yield plant^{-1} (0.18). In our study, the phenotypic coefficient of variation (PCV) was consistently higher than the genotypic coefficient of variation (GCV) across all assessed traits. Relevant observations were also reported.[19-20] $\delta^2 p$ was higher than that of $\delta^2 g$ in all the characters in our case (Table 3). All the correlation values (Table 4) were found to be positively correlated and significant at 1% which ranges from 0.6108 to 0.9570. A strong positive and significant correlation was observed between the number of fertile grains panicle^{-1} and 1000-grain weight (r = 0.9570). Grain yield plant^{-1} has been reported to be significantly influenced by the number of grains panicle^{-1}, highlighting the importance of these traits in improving overall yield.[21] The above result suggested the possibility of selection of one of the above component character would result in the improvement of other characters. Correlation values are graphically represented in Figure1.

Various treatments have different role in their mode of action. The rice was most sensitive to sulfonamide. Similar to bacteria, plants possess a folate biosynthetic pathway that plays a crucial role in their growth and development. This pathway is essential for various physiological processes, including DNA synthesis, repair, and methylation.

Sulfonamide antibiotics can interfere with this pathway, thereby affecting plant growth and development by inhibiting folate biosynthesis, a key process essential for plant vitality.[22] Gentamycin was utilized as a low-cost alternative to gibberellic acid (GA_3).[23] Its potential to replace GA_3 in enhancing plant growth and hybrid seed production makes it a viable option for more cost-effective agricultural practices. Gentamycin, an aminoglycoside antibiotic, exerts its antibacterial effect by inhibiting protein synthesis through binding to the bacterial ribosome. This mechanism disrupts cellular processes essential for bacterial growth and survival. In addition to its medical applications, gentamycin is also used for various agricultural purposes in countries such as Mexico and the USA, demonstrating its versatility in managing plant health and productivity. Plants uptake nitrogen in the form of nitrates and ammonium and molybdenum play an important role in this process and also work as a catalyst in many enzymatic activities.[24] In case of nickel compound it is evident that nickel is an essential metal and plays an important role in plant metabolism. The potential of penicillin and certain heavy metal compounds as promising alternatives to traditional methods for enhancing panicle emergence in CMS (A-line) rice plants which suggest valuable implications for developing cost-effective agricultural practices and optimizing hybrid rice seed production.[25]

In this experiment, treatment-2 (Penicillin) out -performed other alternatives to GA_3 due to its lower cost, consistently yielding superior results across the cropping season. Penicillin significantly promoted shoot elongation in rice seedlings. Additionally, seedlings treated with penicillin maintained higher levels of nucleic acids and proteins, indicating a positive impact on both growth and metabolic processes.[26] Penicillin has been shown to enhance chloroplast pigment formation in intact rice seedlings,[27] affecting leaf morphology, stem circumference, and internode length.[28] A concentration of 400 mg/L is effective in modifying leaf morphology in *Glycine max* and increasing leaf area in *Chrysanthemum.* There is a scope of future research to standardize the dose of penicillin for panicle emergence in IR58025A (CMS) line rice for maximum hybrid rice seed production. Penicillin also promotes stem circumference and internode length through cell elongation, likely by increasing levels of gibberellin and cytokinin-like substances, as observed in mungbean seedlings.[29] From this result it is evident that the treatment penicillin and GA_3 were given more or less similar though penicillin effect was indicated a little bit better than that of GA_3 effect. Penicillin as the most promising treatment, yielding optimal panicle emergence results at a lower cost while significantly enhancing crop output. GA_3 and gentamycin also demonstrated strong efficacy, supporting their role as effective treatments. Micro-histological analysis of CMS line rice (IR58025A) using SEM provided further insights into treatment impacts on plant structures.[30] Notably, penicillin treatment induced a marked loosening of the endothecium layer in the androecium and strengthened the gynoecium, promoting a more fibrous and gametophytic structure. These micro-structural modifications indicate an enhanced seed set potential, underscoring penicillin's viability in improving F_1 hybrid rice production.

Conclusion

Among the ten alternative treatments, penicillin emerged as the top performer, offering a compelling combination of high yield and cost-effectiveness. Notably, penicillin's performance was on par with GA_3, with a slight advantage. Potassium dichromate also demonstrated promising results in promoting panicle emergence. Thereafter, remaining eight treatments are performing according to their mode of action. The role of penicillin as a growth regulator presents a promising avenue for agricultural applications, brightening the prospects for its broader use in crop enhancement. The characters showing positive and significant correlation will help to improve yield. In this case further advancement of lines or genotypes can be done based on these character combinations will definitely leads to the development of superior variety with higher yielding ability.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Author Contributions

- **Riya Pal :** Monitored field trials and execution, Data collection and analysis, Manuscript preparation.
- **Chand Kumar Santra:** Conceptualization, Field layout, Methodology and Correlation analysis.

References

1. Kumar R.V. Hybrid Rice Seed Production -Post planting operations. In: Ahmed MI, Viraktamath BC, Ramesha MS and Kumar CHMV, EDITORS. Hybrid Rice Technology. Directorate of Rice Research, *Hyderabad*; 1996; 84-86.
2. Virmani S.S., Sharma H.L. Manual for Hybrid Rice Seed Production. IRRI. Manila. Philippines; 1993 ; 72 .
3. Yuan L., Wu X., Liao F., Ma G., Xu Q. Hybrid Rice Technology. *China Agricultural Press. Beijing, China*; 2003;132 .
4. Viraktamath B.C., Ilyas A. “Principal Scientist of Hybrid Rice”. Training of Hybrid Rice Seed ProductionSukamandi - *Indonesia*, 2005.
5. Gavino R.B., Pi Y., Abon Jr. C.C. Application of geberellic acid (GA3) in dosages for three hybrid rice seed production in the Philippines. *Journal of Agricultural Technology*; 2008; 4(1):183-192.
6. ElShameyEssam A. Z., Hamad Hassan Sh., Alshallash Khalid S., AlghuthaymiMousa A., Ghazy Mohamed., SakranRaghda M., Selim Mahmoud E., ElSayed Mahmoud A. A, Abdelmegeed Taher M., Okasha Salah A., Behiry Said I, Boudiar Ridha ., Mansour Elsayed. Growth regulators improve outcrossing rate of diverse rice cytoplasmic male sterile lines through affecting floral traits. *Plants*; 2022 ; 11 :1291.
7. Swain P., Rao R.N. Identification of chemicals to maximize seed setting in hybrid rice. *Oryza*; 2009; 46.(2):145-148.
8. Tiwari D.K., Pandey P., Giri S.P., Dwivedi J.L. Effect of GA3 and Other Plant growth Regulators on Hybrid Rice Seed Production. *Asian Journal of Plant sciences*; 2011; 10(2):133-139.
9. Parihar Akarsh., Mahesuria, Ankul A., Chaurasia Pratibha., Pathak A.R. Effect of GA3 and other chemicals for increased seed yield of cms lines in rice. *Electronic Journal of Plant Breeding*; 2012; 3(4): 952-955.
10. Kumar N., Kumar V., Assessment of genetic diversity in linseed germplasm using morphological traits. *Electronic Journal of Plant Breeding*; 2021; 12(1) :66-73.
11. Sing R.K., Chaudhary B.D. Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi, 1995.
12. Hussain Iqtidar., Khakwani Abdul Aziz, Bakhsh Imam., Khan Ejaz Ahmad., Sheheryar. Effect of Napthalene Acetic Acid (NAA) on Grain yield and Bioeconomic Efficiency of Coarse Rice (*Oryza sativa* L.). *Pak. J. Bot*; 2021; 53(6): 2017-2023.
13. Biswas S., Bordolui S.K., Chattopadhyay P. Influence of GA_3 on hybrid rice seed production in West Bengal. *Journal of Crop and Weed;* 2020;16(3): 136-142.
14. Aide Michael., Picker Jennifer. Potassium and Phosphorus Nutrition in Rice. Information from 1996 Missouri Rice Research Update . 1996.

15. Manivasagaperumal R., Vijayarengan P., .Balamurugan S., Thiyagarajan G. Effect of copper on growth, dry matter, yield and nutrient content of *Vignaradiata* (L.) WILCZEK. *Journal of Phytology*; 2011; 3(3): 53-62.
16. Sharma P., Dubey R.S. Ascorbate peroxidase from rice seedlings: properties of enzyme isoforms, effects of stresses and protective roles of osmolytes. *Plant Sci;* 2004; 167: 541-550 .
17. Sureshbabu G., Mishra S.C., Lavanya G.R., Kumar S., Pravenkumar R.U. and Nagaraju P. Assessment of basmati rice (*Oryza sativa* L.) germplasm for yield and quality traits suited to easter zone of U.P. Extended Summaries : ARRW Golden Jubilee International Symposium, Cuttack, India; 2013; 24-25.
18. Haifaa, Iffah M. D., Moses, Christopher., Effects of Foliar and Soil Application of Gibberellic Acid (GA3) at Different Growth Stages on Agronomic Traits and Yield of Rice (*Oryza sativa* L.). *Journal of Agricultural Science*; 2022; 14(6): 55-67.
19. Buu Bc. and Truong D.X. Path analysis of rice grain yield under saline conditions.Inter. Rice Res. Newsletter; 1988; 13:20-21.
20. Zahid M.A., Akhtar M., Sabir M., Manzoor Z.,and Awan T. Correlation and path analysis studies of yield and economic traits in Basmati rice (*Oryzasativa* L.), *Asian J. Pl. Sci.* 2006; 5 (4): 643-645.
21. Ram T. Character association and path coefficient analysis in rice hybrids and their parents. *J. Andaman Sci. Assoc*; 1992; 8: 26-29.
22. Hanson A.D., Gregory J.F. Folate Biosynthesis, Turnover, and Transport in Plants. Annu. Rev. Plant Biol; 2011; 62:105–125. doi: 10.1146/annurev-arplant-042110-103819.
23. McManus P.S., Stockwell V.O., Sundin G.W. and Jones A.L. Antibiotic use in plant agriculture. Annu. Rev. Phytopathol ; 2002; 40:443-465 .
24. Imran M., Ali Zulfiqar and Gurmani. Role of Macro and Micro Nutrients in the Plant Growth and Development. Science, Technology &Development ; 2011; 30 (3) : 36-40.
25. Pal R., Bhattacharyya Subhendu Sekhar., Santra Chand Kumar. Study the Effect of Different Treatments on Hybrid Rice Seed (F_1) Production and Assessment of Heavy Metals by Using Atomic Absorption Spectrophotometry. *Research Journal of Agricultural Sciences*; 2023;14(5):1476-1479.
26. Mukherjee S. and Biswas A.K. Penicillin action stimulating growth and metabolism in seedlings of rice *(Oryza sativa). Canadian Journal of Botany*; 1985; 63 (6):1150-1156,10.1139/b85-158.
27. Biswas A.K., and Mukherjee S..Penicillin induced regulation of chlorophyll formation and Hill activity of isolated chloroplasts in rice *(Oryza sativa L.) seedlings. Curr. Sci*; 1978; 47: 555-556.
28. Purohit S.S. and Purohit G.R. Penicillin-induced morpho-physiological parameters in soybean leaves. *Comp. Physiol. Ecoi*.; 1983;8(4): 379-380.
29. Mukherjee, S. and Wareing P.F. The effect of penicillin on the levels of endogenous gibberellins and cytokinin sinmungbean (*Vigna radiata* L.) seedlings. Biologia Plantarum (Praha); 1983; 25 (3):161-165.
30. Pal R., Santra Chand Kumar. Scanning Electron Microscopy Investigation of Cytoplasmic Male Sterile Line Rice : Exploring Treatment Effects on Plant Components and their Impact on Hybrid Rice Seed Production. *Eco. Env. & Cons*; 2024; 30 (July Suppl. Issue): S242-S246.