Jurnal Agronomi Indonesia
(Indonesian Journal of Agronomy)

ISSN 2085-2916 | e-ISSN 2337-3652
April 2024, *52*(1), 10-16
DOI: https://dx.doi.org/10.24831/jai.v52i1.51380

Research Article

Effects of row ratio and plant spacing for enhancing hybrid rice seed production in the tropics

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ABSTRACT

The seed yield production determines the success of the development of hybrid rice. Here, an effective and efficient cultivation technology for producing hybrid rice seeds is optimized through spacing and row ratio management. This study aimed to evaluate the effects of spacing and row ratio of restorer (R) : CMS (A) on F_1 hybrid rice seed production. The experiment was conducted at the Indonesian Center of Rice Research's experimental field in the dry season of 2016, using parental lines HIPA18 hybrid rice, i.e., CMS: IR79156A line and Restorer: IR53942 line. The experiment was laid out in a strip-plot design replicated three times. Two factors were studied, i.e., plant spacing between CMS lines and row ratio R by A-lines. The results indicated that the highest HIPA18 seed production was obtained from 20 cm x 20 cm spacing of CMS with row ratio 2R:14A, i.e., 3.62 tons ha^{-1}. Plant spacing and row ratio interactions were improved tillers number per plant of restorer and F_1 seed yields.

Keywords: A-lines; CMS; F_1 hybrid rice; R-lines

Edited by:
Okti Syah Isyani Permatasari
IPB University

Received:
10 November 2023
Accepted:
22 April 2024
Published online:
30 April 2024

Citation:
Wibowo, B. P., Widyastuti, Y., Prabowo, R. N., Suparman, C., Satoto, & Sitaresmi, T. (2024). Effects of row ratio and plant spacing for enhancing hybrid rice seed production in the tropics. *Jurnal Agronomi Indonesia (Indonesian Journal of Agronomy)*, *52*(1), 10-16

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the food crops proven to be able to increase yield potential through heterosis exploitation. The phenomenon of heterosis only appears in the first generation (F_1) of offspring from genetically different parents. Therefore, exploitation of heterosis can be applied through the use of hybrid technology in its cultivation system using F_1 seeds. Hybrid rice is capable of producing 1.0-1.5 tons ha^{-1} or 20-30% higher than conventional varieties (Ma & Yuan, 2015; Qian et al., 2021), so that hybrid rice technology is expected to reduce hunger problems in Asia, Africa, and Latin America (Spielman et al., 2013).

In the tropics like Indonesia, hybrid rice production uses the three-line system consisting of a sterile male line (GMJ=A=CMS) as a female parent, a fertility restoring line (R), and a maintainer line (B) as a male parent (Chen & Liu, 2014). Cytoplasmic male sterility (CMS) combined with a fertility restoration system was reported effective as a genetic source for developed hybrid rice. The cytoplasmic male sterility (CMS) system can exploit heterosis in grain yields when combined with effective restorer lines (Sattari et al., 2008; Xu et al., 2023).

The success of hybrid rice technology is determined by the superiority of heterosis and the efficiency and effectiveness of hybrid rice seed production (He et al., 2022). Economical seed production depends, among other things, on seed purity, timely availability, and affordability at the farm level. So far, the high price of hybrid rice seeds at the farmer level is due to the complexity of seed production, which is not supported by high seed yields, causing high production costs. Hybrid seed production technology is different from inbred seed production. F_1 seeds should be used by farmers every growing season (He et al., 2020a). Strict isolation of the surrounding rice fields and the roguing process or selection of intercropping plants can help achieve the required seed purity. Therefore, the seed production of hybrid rice requires fertile land, appropriate climatic conditions as well as a proficient and well-trained workforce.

To successfully produce hybrid seeds on a large-scale commercial basis, male and female parents planted side by side in a fixed row ratio should flower simultaneously, a process known as synchronization. Finding the best time for pollination is essential to guarantee a synchronous flowering time of the pollen and female parents. This can be done by quickly and accurately identifying the male and female parents (Chakrabarty et al., 2023). It is possible to achieve this through the implementation of a staggered planting technique (Gaballah et al., 2022; Yanning et al., 2021; Casco et al., 2021). The CMS parent and restorer lines were planted using a certain row ratio and spacing to maximize seed output in producing hybrid rice seeds (Hamad et al., 2022). Considering the previously mentioned aspects, the current study has main goal to standardize the appropriate row ratio for enhancing their outcrossing rate, which could result in higher seed yield, as well as to investigate the optimal plant spacing to increase the population of the female parent for enhancing HIPA18 hybrid rice seed production. This study aimed to evaluate the effects of spacing and row ratio of R:A lines on F_1 hybrid rice seed production.

MATERIALS AND METHODS

The field experiment using hybrid rice seed production of HIPA18 was conducted at Sukamandi field station of the Indonesian Center for Rice Research (-6°20'48", 107°39'6", 24.0 m asl, 325°) during the dry season of 2016. HIPA18 production used a pair of parental lines, i.e., IR79156A (CMS lines) and IR53942 (restorer line) corresponding to A and R lines. The experiment was arranged in a strip-plot design with three replications. Strip-plot designs are beneficial when the treatments have a factorial structure and the factor levels are difficult to adjust, requiring them to be applied to larger clusters of experimental units (Alqallaf et al., 2019). In each replication, the plant spacing between CMS plants (20 cm x 20 cm, 20 cm x 15 cm, and 15 cm x 15 cm) was assigned as horizontal strip-plot, and the row ratio R:A (2:8, 2:10. 2:12 and 2:14) were allocated as vertical strip-plot. The female parent (CMS) plant population was 240, 280, 320, and 360, respectively.

The pre-germinated seed was uniformly broadcast in the nursery bed three times for R line and to provide adequate pollen load to the female (male sterile) A line, then sown in May 2016. The sowing variations were followed for complete flowering synchronization based on the previous season's growth duration as 81±3 for the A line and 87±3 days for the R line. The distance between the restorer lines was 30 cm. The spacing between the A and R lines was 30 cm. The recommended fertilizer dose of 200 kg ha^{-1} of Urea, 200 kg ha^{-1} of NPK, and 10 kg ha^{-1} of $ZnSO_4$ were applied. Intercultural operations like rouging, GA_3 application, and supplementary pollination were performed as and when required. The male parent restorer lines were harvested first and removed from the field before harvesting the seed parent (CMS line).

The crop was harvested, and the grains were sun-dried and adjusted at 14% moisture to calculate the seed yield. The data were recorded per plot of randomly selected hills excluding border rows: plant height (cm) of CMS and R lines, number of productive tillers per plant of CMS and R lines, panicle exertion rate (%), filled grains per panicle, number of un-filled grains per panicle, outcrossing rate (%), and seed yield (tons ha^{-1}). The data collection reference was the standard rice (SES) (IRRI) evaluation system. Panicle exertion rate (PER) was determined by dividing the length of the un-exerted panicle by the total length of the panicle. The outcrossing rate (OCR) was measured by dividing the total number of filled grains by the number of spikelets with filled and unfilled grains.

All obtained data were statistically analyzed according to the analysis of variance (ANOVA) for the strip-plot design by using the STAR computer software package from IRRI. The least significant difference (LSD) method was used to test the differences between treatment means ($p<0.05$) probability level.

RESULTS AND DISCUSSION

Effects of plant spacing

Statistical analysis showed that the treatment of plant spacing A lines 20 cm x 20 cm gave higher results than the plant spacing of 20 cm x 15 cm and 15 cm x 15 cm (Table 1). Restorer plants support a good pollination process in hybrid rice seed production with higher plant heights than CMS plants. Plant height is one of the parameters that can determine seed yield (Gaballah et al., 2021). Restorer plants are expected to have a height of >10-20 cm higher than CMS plants (Awad et al., 2022). Plant height of R lines and tiller number per plant of R showed higher yields than CMS plants.

The panicle exertion rate, number of filled grains per spikelet, number of unfilled grains per spikelet, and outcrossing rate of each treatment with plant spacing A gave their respective advantages. Plant spacing A 15 cm x 15 cm has less than optimal results. Plant density will reduce the number of seeds produced, affecting seed yields (Duan et al., 2019). Yield characters and seed yields did not show linear results; these results were caused by the individual sampling of yield components and yields obtained from seed yields per plot.

Table 1. Effects of plant spacing between A on the seed yield and its components.

Plant spacing A line	PHA (cm)	PHR (cm)	TNA	TNR	PER (%)	FG	UFG	OCR (%)	SY (tons ha^{-1})
20 x 20 cm	97.80	122.27	12.60	16.62	0.77	68.95	119.67	36.41	2.19a
20 x 15 cm	97.07	120.15	12.28	15.39	0.79	65.78	98.62	41.59	1.40ab
15 x 15 cm	96.63	122.12	11.33	17.53	0.77	67.92	102.58	39.56	0.97a
Mean	97.17	121.51	12.07	16.51	0.78	67.55	106.96	39.19	1.52
CV (%)	3.59	3.40	22.40	14.02	5.42	35.71	22.76	17.57	41.87

Note: PHA: Plant height of CMS lines; PHR: Plant height of R lines; TNA: Tillers number per plant of CMS; TNR: Tillers number per plant of R; PER: Panicle of exertion rate; FG: Number of filled grains per spikelet; UFG: Number of unfilled grains per spikelet; OCR: Out-crossing rate; SY: Seed yield of HIPA18. CV: Coefficient of variation. Means with the same letter are not significantly different based on LSD.

Effects of row ratio

The influence of the row ratio of R and A lines indicated enhancing hybrid seed production by improving the outcrossing capacity of A-lines. Statistical analysis showed a significant difference in the panicle of exertion rate and seed yield in the treatment row ratio R:A (Table 2). Production of hybrid seeds is significantly increased when outcrossing rates in CMS lines are improved (Hashim et al., 2021). Increased outcrossing is possible if the row of plants is perpendicular to the wind direction (Hamad et al., 2015; Hamad & Bassiouni, 2020). Panicle of exertion rate female parent of HIPA18 at row ratio 2R:8A and 2R:14A gave higher and more significant results than row ratio 2R:10A. The row ratio in the seed production plot relates to the number of rows of the male parent (R line) to the female parent (CMS or A line).

The seed yield of HIPA18 at a ratio of 2R:14A showed the highest and significantly higher yield than a row ratio of 2R:8A, 2R:10A, and 2R:12A (Table 2). Huang et al. (2018) revealed that there was a significant rise in the number of panicles per area of rice as hill density increased. The effective panicle number per area of rice might be significantly increased by increasing planting density (He et al., 2020b). This shows that a higher number of A-lines plants supported by a good percentage of exertion rate will give higher HIPA18 seed yields. This increase may be due to higher panicle outcrossing and insertion (Hamad et al., 2015).

Table 2. Effects of row ratio R:A on the seed yield and its components.

Row ratio R:A	PHA (cm)	PHR (cm)	TNA	TNR	PER (%)	FG	UFG	OCR (%)	SY (tons ha^{-1})
2R:8A	97.49	119.33	11.89	15.07	0.80a	68.49	120.64	38.10	1.04b
2R:10A	97.56	124.09	12.36	16.20	0.75b	67.96	92.78	41.03	1.38b
2R:12A	97.42	120.36	11.96	17.47	0.78ab	64.53	103.11	38.85	1.46b
2R:14A	96.20	122.27	12.09	17.31	0.79a	69.22	111.29	38.77	2.20a
Mean	97.17	121.51	12.08	16.51	0.78	67.55	106.96	39.19	1.52
CV (%)	3.63	2.71	23.23	14.02	3.18	29.63	22.63	18.58	46.99

Note: PHA: Plant height of CMS lines; PHR: Plant height of R lines; TNA: Tillers number per plant of CMS; TNR: Tillers number per plant of R; PER: Panicle of exertion rate; FG: Number of filled grains per spikelet; UFG: Number of un-filled grains per spikelet; OCR: Out-crossing rate; SY: Seed yield of HIPA18. CV: Coefficient of variation. Means with the same letter are not significantly different based on LSD.

Effects of interaction

The interaction between plant spacing and row ratio combination is given in Table 3. Research on seed production of hybrid rice varieties HIPA18 was relatively good despite the brown planthopper attack in the generative phase which caused the number of productive tillers of CMS plants to be less than the restorer (Table 3). It related to research (Fadzli et al., 2022) that the row ratio and spacing of the pollen parent and seed parent have a distinct effects on the hybrid seed yield. Wibowo et al. (2022) reported that differences in the cytoplasmic type in CMS also influenced seed yield.

Table 3. Effects of interaction between A plant spacing and row ratio R:A on the seed yield and its components

Plant spacing A	Row ratio R: A	PHA (cm)	PHR (cm)	TNA	TNR	PER (%)	FG	UFG	OCR (%)	SY (tons ha^{-1})
20 x 20 cm (P1)	2R:8A (R1)	97.33	119.13	12.67	13.26b	0.79	83.07	147.27	38.54	1.54bc
	2R:10A (R2)	97.40	124.60	11.73	15.80b	0.72	65.73	97.40	38.62	1.95abc
	2R:12A (R3)	101.53	122.27	14.27	20.07a	0.79	58.13	117.53	33.45	2.11ab
	2R:14A (R4)	94.93	123.07	11.73	17.33ab	0.79	68.87	116.47	35.03	3.17a
20 x 15 cm (P2)	2R:8A (R1)	97.27	117.33	11.47	14.27b	0.83	59.20	100.60	39.21	0.98bc
	2R:10A (R2)	99.20	122.67	13.27	15.60b	0.76	62.33	103.93	38.06	1.21bc
	2R:12A (R3)	94.60	117.07	10.07	14.40b	0.79	72.07	89.60	45.01	1.25bc
	2R:14A (R4)	97.20	123.53	14.33	17.27ab	0.78	69.53	100.33	44.08	2.15ab
15 x 15 cm (P3)	2R:8A (R1)	97.87	121.53	11.53	17.67ab	0.77	63.20	114.07	36.55	0.59c
	2R:10A (R2)	96.07	125.00	12.07	17.20ab	0.78	75.80	77.00	46.40	0.97bc
	2R:12A (R3)	96.13	121.73	11.53	17.93ab	0.75	63.40	102.20	38.07	1.03bc
	2R:14A (R4)	96.47	120.20	10.20	17.33ab	0.79	69.27	117.07	37.20	1.28bc
Mean		97.17	121.58	12.07	16.58	0.78	67.55	106.96	39.19	1.52
CV (%)		3.73	2.86	23.43	10.58	5.70	15.95	18.52	18.13	23.26

Note: PHA: Plant height of CMS lines; PHR: Plant height of R lines; TNA: Tillers number per plant of CMS; TNR: Tillers number per plant of R; PER: Panicle of exertion rate; FG: Number of filled grains per spikelet; UFG: Number of un-filled grains per spikelet; OCR: Out-crossing rate; SY: Seed yield of HIPA18. Means with the same letter are not significantly different based on LSD.

In this study, the effects of differences in row ratio and plant spacing combinations were insignificant on plant height (A and R lines), tillers number of A-line, panicle of exertion rate, filled and unfilled grains per panicle, and outcrossing rate (Table 3). Plant spacing 20 cm x 20 cm with a 2R:12A row ratio took a maximum number of tillers on the R line in all spacing combinations used in this experiment (20.07 tiller per plant). Statistically, a similar trend was primarily revealed in combinations. Plant spacing 20 cm x 20 cm with a row ratio of 2:8 showed low productive tillers per plant. One of the critical components for obtaining high seed yields is panicle exertion (Huang et al., 2020). The highest panicle exertion on female sterile parental lines HIPA18 was achieved at row ratio 2R:8A with a spacing of 20 cm x 15 cm. Seed yield is calculated from grain yield per plot, while the calculation of yield components is calculated from yield components per plant, this can provide insignificant seed yield when correlated with yield components per plant. Research by Vennela et al. (2021) revealed that grain length is the main contributor to grain yield, followed by spikelet fertility, number of grains per panicle, and 1000-grain weight. These characters showed a direct positive influence on grain yield per plant.

The highest seed yield of HIPA18 was observed under 20 cm x 20 cm spacing with a 2:14 row ratio combination (3.17 t ha^{-1}) (Table 3). It is followed by 20 cm x 15 cm plant spacing with a 2:14 row ratio (2.15 t ha^{-1}) and 20 cm x 20 cm plant spacing with a 2:12 row ratio (2.11 t ha^{-1}). Plant spacing 15 cm x 15 cm with a row ratio of 2:8 produced the lowest seed yield with all combinations, indicating it was unsuitable for HIPA18 hybrid seed production.

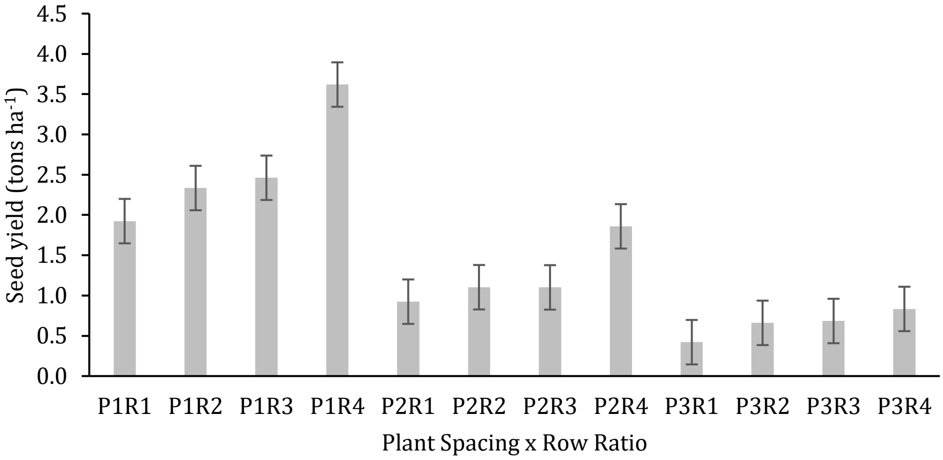

Figure 1. Bar chart comparing seed yield across different plant spacing and row ratio on HIPA18 seed production.

The interaction effects of plant spacing and row ratio on HIPA18 seed production is shown in Figure 1. The results of HIPA18 seeds in this study gave optimum results in combining P1R4 treatments. Combining a plant ratio of 20 cm x 20 cm (P1R4) with a row ratio of 2R:14A gave the highest seed yield compared to other treatment combinations, followed by P2R4 combination which gave a good SY with no significant difference with that of P1R4. These results are in line with (Abo-Youssef et al., 2017) reported that the highest F_1 seed yield was achieved at a higher ratio of A-lines, while the lowest yields of the P3R1 treatment combination was a combination of 15 cm x 15 cm plant ratio treatment with a row ratio of 2R:8A (Figure 1). This study provides information that planting density combined with spacing and row ratio of CMS provides different seed yields.

CONCLUSIONS

The plant spacing and row ratio interaction improved tiller number per plant of restorer and hybrid seed yields. The study found that combining a plant ratio of 20 cm x 20 cm with a row ratio of 2 Restorer:14 CMS gave the highest seed yield compared to other treatment combinations. These findings suggest that this combination could be adopted to increase the seed yield of the HIPA18 hybrid rice variety in Indonesia.

ACKNOWLEDGEMENTS

This research was funded by the Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Republic of Indonesia for the fiscal year of 2015. The authors thank Dede Casim and Firman M. Akbar for their technical assistance in the field.

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