The Improvement of Rice Agronomic Traits Through Hybrid Breeding

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Abstract. Rice breeding is an agricultural practice aimed at developing new rice varieties with improved traits. It could be the solution for the Malaysian rice production that has been suffering from various challenges and yield reduction. This study aimed to improve rice agronomic traits through the hybridization breeding technique. Three varieties were used in this study: PB317 (male/restorer), PB202s, and PB204s (female). The commercial variety, MR297, was used as a control. The Thermosensitive Genic Male Sterility (TGMS) for male sterility technique was used on female parents to prevent self-pollination. The progenies were harvested between 119 and 123 days after sowing. The crossbreeding technique used has produced an average of 378 tillers per crossing. Several morphological parameters have been collected for plant performance comparison. The results indicated that hybrid progeny (PB317 x PF204s) has the highest production with an average of 12.19 mt/ha, followed by PB317 x PF202s (10.01 mt/ha) and MR297 (7.41 mt/ha). The hybrid progenies have significantly higher yield with better morphological characteristics than both parents. The hybrid progenies have shown that the yield can be increased up to seven tonnes compared to the inbred varieties and proven to improve agronomic traits and produce high-performance rice varieties.

1 Introduction

Rice (*Oryza sativa* L.), which belongs to the Poaceae family, has been one of the most consumed foods for more than a thousand years [1]. Rice has a high carbohydrate content and acts as the source of more than 20% of the total calorie intake that serves more than half of the world's population [2]. More than 90% of the rice is cultivated in Asia, including China, India, Bangladesh, Thailand, Malaysia, Indonesia, Vietnam, Japan, and many more [3]. Rice cultivation has been introduced in Malaysia since the 15th century, starting in northern states such as Kedah [2]. Since that era, Kedah has become the biggest rice producer in Malaysia and thrives with the construction of the Wan Mat Saman Aqueduct for water supply. In the early 1960s, rice cultivation kick-started in other states, including Penang, Perak, Selangor, Melaka, and Terengganu, with small-scale farming, which later expanded by leaps and bounds [4]. The Ministry of Agriculture and Food Security (MAFI) has taken the role of

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improving rice breeding and cultivation since rice is emerging as the country's most important food crop.

Over the decades, the production of Malaysian rice has been suffering from various challenges, including extreme weather conditions, pests and diseases, poor soil fertility and nutrient management, reduction of paddy fields, poor deployment of technology, and farmers' lack of awareness and knowledge [5]. Previous reports show Malaysians consumed nearly 2.7 million tonnes of rice, of which only 67% was produced locally, while the balance was imported from neighboring countries in 2016 [3]. Malaysia needs to hit a 100% self-sufficient level (SSL) status in rice supply for food security reasons. Although rice production in Malaysia for 2022 achieved 2.28 million metric tons, it is not sufficient to meet the demand of people in Malaysia [3]. Moreover, the rising cost of rice production has also affected rice production. The decrease in income has made paddy growers switch to other crops [6]. Therefore, more rice needs to be produced to meet the demand. Among the factors that can improve the production of rice in Malaysia is the usage of high-quality seeds. High-quality rice seeds, such as hybrid seeds, can improve rice yield production.

Hybrid rice, derived from crossing between two genetically distinct rice lines, is among the successful technologies with proven methods to increase rice yield. The hybrid rice varieties have an average yield advantage of about 20% more than inbred lines [7]. Previous studies showed that hybrid rice could increase between 2 and 3 tonnes per hectare over the best-inbred varieties [7]. The hybrid rice technique was initially developed in Japan and adopted in China in 2005 [6]. Currently, the majority of rice-producing countries, such as the USA, Brazil, Colombia, Uruguay, and Argentina, have invested in hybrid rice technology with the cooperation of the seed industry [8]. The unique properties of hybrid rice are the heterosis phenomenon. Heterosis is a phenomenon where F1 hybrids derived from two genetically distant parents show superiority over their parents in several characteristics, including vigor, yield, panicle size, number of spikelets per panicle, number of productive tillers, and many more [9]. The exploitation of heterosis in rice can improve grain yield and have advantages for Malaysia in meeting the 100% SSL status.

Hybrid rice technologies involve the use of the male sterility technique. The male sterility technique is used to prevent self-pollination. There are two methods of producing male sterility, including the three-line method and the two-line method. The three-line method is based on cytoplasmic genic male sterility (CMS), which involves the CMS line, maintainer line, and restorer line [10]. To improve hybrid breeding, the two-line method, such as thermosensitive genic male sterile (TGMS), was introduced. The two-line method involves a male sterile line and any inbred variety as a restorer. Specific temperatures control the expression of TGMS to produce male sterile plants [9]. Previous studies showed that TGMS lines have a higher frequency of heterotic combinations than CMS lines [11].

The present study conducted hybrid breeding using the TGMS line technique to assess the changes in agronomic traits of rice varieties and determine the grain yield of F1 hybrid rice. We hypothesize that F1 hybrid rice will show superiority over its parent through heterosis. This study could be used as a reference for the development and cultivation of hybrid rice in Malaysia to increase grain yield in order to achieve 100% SSL.

2 Research method

Two sets of putative TGMS lines possessing stable sterility, PF202s and PF204s, were used in the present study, along with a restorer line, PB317. The parental lines were developed at Green World Genetics Sdn. Bhd., Research Central at Batu Arang, Selangor, Malaysia, by repeated self-pollination. These TGMS lines possessed essential features, including complete and stable male sterility lines, higher out-crossing rates, better grain quality, and good crossing combinations. Moreover, an inbred variety, MR297, was used as a control for plant characteristics comparison. Table 1 shows the list of the hybrid lines and inbred varieties used for the breeding program.

Table 1. The list of hybrid lines and inbred varieties

List	TGMS lines	Restorer/male lines
Hybrid 1	PF202s	PB317
Hybrid 2	PF204s	PB317
Control	**MR297**	

2.1 Parental lines growth and hybridization

The rice seeds were germinated in the tray for seven to ten days before being transplanted to the field. The sowing dates were adjusted according to the flowering date of 50% of parental lines to synchronize the flowering times due to the usage of TGMS lines. The parental lines were pollinated through open cross-pollination with a spacing of 25 x 25 cm for each inbred line. Moreover, the TGMS lines were planted in six rows for each crossing, while the restorer/male line variety was planted in two rows between TGMS lines.

The F1 hybrid seeds were collected and germinated again. The F1 hybrid seeds were grown at three different locations, including Rawang, Cameron Highland, and Arau, with five replications of data collection. The data were pooled together for performance consistency. The data collections were taken during the maturity stage. The control variety (MR297) also grown at the same location.

2.2 Morphological characteristics

2.2.1 Plant height

The height of the main tiller was recorded at maturity from the ground to the top of the spike. The measuring tape was placed flat on the soil surface, and the height was expressed in centimeters. In each cultivar (parental lines) and hybrid, five replicates were used to record these measurements.

2.2.2 Number of tillers

The number of tillers was calculated and recorded at maturity. The 50 cm length of rope was placed randomly in two rows of crops. The number of tillers was recorded along each row on both sides of it. In each cultivar (parental lines) and hybrid, five replicates were used to record these measurements.

2.2.3 Category of Stalk

The category of stalk was identified and recorded at maturity from the ground to the top of the spike. In each cultivar (parental lines) and hybrid, five replicates were used to record these measurements.

2.2.4 Number of Seeds

The number of seeds for each plant was recorded after harvesting. The seeds were harvested and counted on flat paper. In each cultivar (parental lines) and hybrid, five replicates were used to record these measurements.

2.2.5 Maturity Dates

The maturity date was recorded after the plant was harvested. Three replicates were used to record these measurements in each cultivar (parental lines) and hybrid.

2.2.6 Number of Yields

The yields for each variety were recorded after harvesting. The total number of seeds from three different places was calculated and recorded. In each cultivar (parental lines) and hybrid, five replicates were used to record these measurements.

2.3 Experimental design and statistical data analysis

The experimental design was a randomized complete block design (RCBD) with five replications. The data obtained from two successive seasons were pooled and analyzed using SPSS version 30. A one-way ANOVA was applied to evaluate significant differences in the studied parameters in the different treatments. The least significant difference (Fisher's protected LSD) was calculated following a significance F-test (P=0.05).

3 Results and discussion

3.1 Morphological characteristics

The selected parental lines with average characteristics and performance were used in this study to determine the heterosis effect in hybrid breeding. Between the parental lines, PF202s variety showed significantly highest number of tillers with 251, while it has the lowest number of seeds with an average of 113. Another female parent, PF204s, has the highest number of seeds (135) and number of whole seeds (32,540) compared to other parental lines. Table 2 shows the morphological characteristics of parental lines.

Table 2. The morphological characteristics of parental lines

Parental lines	Number of Tillers	Plant Height (cm)	Number of Seeds	Maturity Date
PB317	178 ± 1.32	92 ± 3.45	128 ± 2.93	121 days ± 2.31
PF202s	251 ± 2.58	70 ± 3.29	113 ± 1.87	119 days ± 1.89
PF204s	216 ± 1.76	90 ± 2.88	135 ± 2.11	119 days ± 2.14

The hybrid breeding of genetically distant parental lines has produced a better F1 hybrid with better characteristics and performance. The F1 hybrid characteristics were expressed mainly due to the heterosis, where the progenies could have superiority over both parental lines. Among the morphological characteristics of the F1 hybrid progenies, a number of whole seed characters showed the biggest improvement from the inbred variety. The highest number of whole seeds was PB317 x PF204s (82,842), followed by PB317 x PF202s (50,386). Interestingly, the number of whole seeds is proportional to the number of tillers. The higher the number of tillers, the higher the number of whole seeds in F1 progenies. Table 3 shows the morphological characteristics of F1 progenies.

Apart from that, both F1 hybrid progenies have higher plant heights compared to their parental lines. The parental lines PB317 and PF202s have plant height of 92cm and 70cm, respectively, while the progeny PB317 x PF202s has an average of 98.67 cm. There is an increase of about 6.67cm in the height of the highest parental characteristics. The same

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situation also occurs in another F1 hybrid, PB317 x PF204s. Figures 1 to 3 shows the comparative morphological characteristics analysis between parental lines and the F1 hybrid.

Table 3. The morphological characteristics of F1 progenies.

Progenies	**Number of Tillers**	**Category of Stalk**		**Plant Height (cm)**	**Number of Seeds**	**Maturity Date**
PB317 x PF202s	331 ± 1.43	Long	104 ± 1.02	98 ± 2.34	174 ± 1.25	119 days ± 2.22
		Median	227 ± 1.85	101 ± 1.59	129 ± 1.38	
		Short	0	97 ± 1.07	0	
		Total	331 ± 1.54	98.67 ± 1.88	303 ± 1.33	
PB317 x PF204s	453 ± 2.31	Long	375 ± 1.08	135 ± 2.14	180 ± 1.63	123 days ± 2.35
		Median	78 ± 2.07	130 ± 1.05	126 ± 1.93	
		Short	0	133 ± 1.83	0	
		Total	453 ± 1.84	132.67 ± 1.57	306 ± 1.68	
MR297	350 ± 1.62	Long	140 ± 2.09	90 ± 1.65	151 ± 1.64	119 days ± 1.99
		Median	210 ± 1.72	92 ± 1.72	105 ± 1.79	
		Short	0	95 ± 2.01	0	
		Total	350 ± 1.59	92.3 ± 1.71	256 ± 1.66	

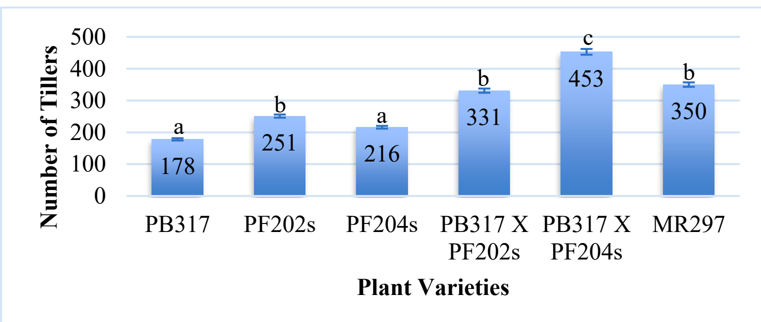

Fig. 1. The comparative analysis of number of tillers characteristics between parental lines and the F1 hybrid. PB317 x PF204s showed significantly highest number of tillers compared to others. Means followed by the different lowercase letters within the same-colored column are significantly different according to the LSD.

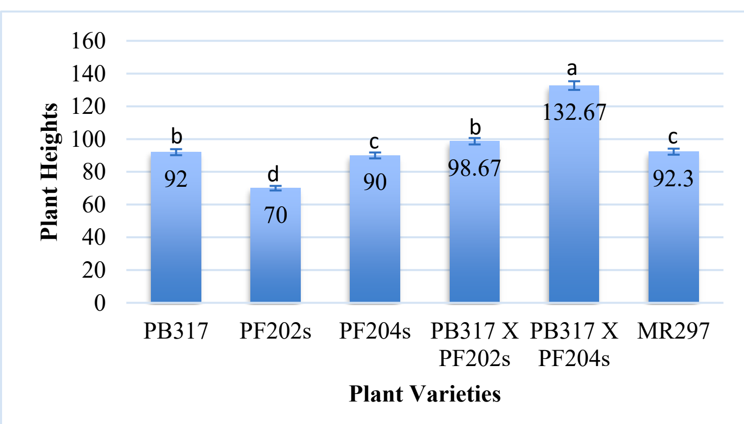

Fig. 2. The comparative analysis of plant heights characteristics between parental lines and the F1 hybrid. PB317 x PF204s showed significantly highest number of tillers compared to others. Means followed by the different lowercase letters within the same-colored column are significantly different according to the LSD.

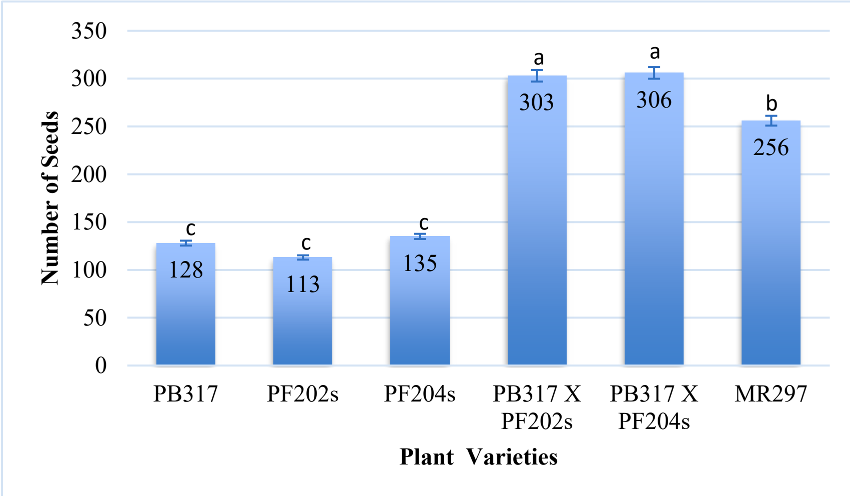

Fig. 3. The comparative analysis of number of seeds characteristics between parental lines and the F1 hybrid. The hybrid progeny showed significantly highest number of tillers compared to others. Means followed by the different lowercase letters within the same-colored column are significantly different according to the LSD.

The improved morphological characteristics in hybrid rice compared with the inbred variety have been reported in previous studies. A study done by [12] shows that the number of spikelet panicles influenced the grain yield, where the hybrid Heera2 produced an average of 130.2 spikelet panicles and produced 8.03 mt/ha, while the inbred BRRI45 produced an average of 101.1 spikelet panicles and produced 6.16 mt/ha. Moreover, the high number of tillers in hybrid AAIOK 6201 (327.3) has a higher grain yield of 5.9 mt/ha than inbred BRRI Dhan28 (285.5) with 5.1 mt/ha [13]. Apart from that, hybrid rice has also been reported to be resistant to diseases. There are two major diseases of rice, including bacterial blight (*Xanthomonas oryzae* pv. oryzae) and fungal blast (*Magnaporthe grisea*). Hybrid Guodao 6, which has multiple tolerances to diseases and environmental stresses and high-yielding characteristics, is cultivated widely in Southern China [14].

3.2 Grain yield production

Hybrid breeding also produced F1 progenies with high-yield characteristics. According to the comparative analysis of grain yield production, the F1 hybrid produces a higher yield than the inbred variety. Although MR297 is one of the high-yielding rice varieties, the heterosis phenomenon in hybrid breeding showed significant grain yield production of 12.19 mt/ha ± 2.98 for PB317 x PF204s progeny, which is almost 5 tons higher than the former. Another hybrid progeny, PB317 x PF202s, also recorded higher than the parent. The parental lines have an average grain yield of 5.15 mt/ha ± 1.64 (PB317), 4.98 mt/ha ± 1.83 (PF202s), and 6.30 mt/ha ± 2.07 (PF204s). The grain yield produced by F1 hybrid varieties was almost double that of their parental lines. The results showed that the hybridization of two genetically distant inbred varieties could produce progenies with high-yielding characteristics. Figure 4 shows the average grain yield produced by parental and progenies rice varieties.

The high grain yield from hybrid rice is also consistent with the previous studies. Several studies have shown a comparison between hybrid and inbred rice varieties. According to [15], hybrid Heera2 produced a higher grain yield of 8.03 mt/ha than inbred IRRI (6.16 mt/ha). Moreover, the Indica hybrid has been reported to have 7.62 mt/ha, while the Indica inbred has 6.64 mt/ha of grain yield [14]. Furthermore, [15] reported that the cultivation of hybrid rice has produced 10% more yield in 672 experiments compared with inbred varieties. The results produced are parallel with the previous studies, where the F1 hybrid progenies have higher performance than the parental lines in terms of yield production.

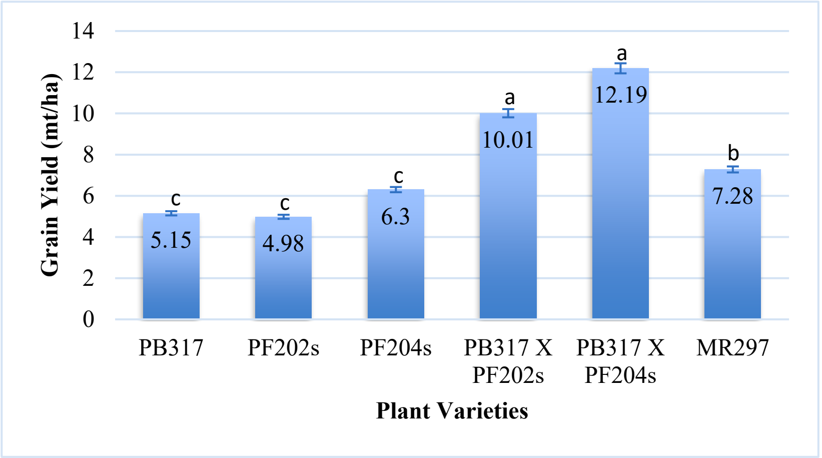

Fig. 4. The average grain yield is produced by parental lines and progenies of rice varieties. Means followed by the different lowercase letters within the same-colored column are significantly different according to the LSD test.

4 Conclusion and recommendation

The high grain yield provided by hybrid rice can increase rice production in Malaysia. Based on the results, the hybrid progenies have shown that the yield can be increased up to seven tonnes compared to the inbred varieties. PB317 X PF204s has been shown to be the best variety for morphological characteristics and performance analysis. Therefore, hybrid rice breeding can provide an alternative for improving grain yield, although paddy fields are being reduced in Malaysia. The high yield of hybrid rice can be introduced to the paddy grower to increase their yield, which can also increase their salary. Through the data, Malaysia could achieve 100% SSL and meet the consumption demand of Malaysians if most of the growers used hybrid seeds. The food security agenda could be achieved when Malaysia reduces imports of essential food crops from other countries.

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