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Research Article

Agronomic traits of rice hybrids (*Oryza sativa* L.) derived from new plant type and male sterile parents

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ABSTRACT

Hybrid rice is derived from a cross between the cytoplasmic male sterile (CMS) as the female parent and the restorer line as the male parent. This study aimed to evaluate the ability of several new plant type (NPT) rice lines as restorers of the CMS female parent and to elucidate the performance of the hybrid combinations. The research was carried out from December 2019 to November 2020 at Babakan Sawah Baru Experimental Station, Department of Agronomy and Horticulture, IPB University, Bogor, Indonesia. The hybrids were evaluated in the field together with the CMS female parent, male parents, and two check varieties, namely Ciherang and hybrid P05 Prima Seed. The experimental design used was a randomized complete block design (RCBD) with two replications. The results showed that two male parental lines, IPB189-F-10-3-2 (T6) and IPB189-14-1-1 (T9), had a hybrid seed yield per hill of 66.04 g and 75.27 g, respectively. These two parents have the restorer gene (R) which is substantiated by the good characteristics of the number of filled grains and total grain per panicle of the two hybrids. The potential hybrids CMS x IPB189-F-10-3-2 and CMS x IPB189-14-1-1 had a positive heterosis for panicle length and 1,000 grain weight, and could be evaluated in future research.

Keywords: CMS; heterosis; heterobeltiosis; rice hybrid

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INTRODUCTION

Rice (*Oryza sativa* L.) is Indonesia's most important cereal crop. The increasing demand for rice affects food availability. Some factors may decrease rice production including pests and disease attacks, paddy field conversion, and unfavorable climatic conditions. New approaches and innovations are needed to increase rice production, and one is the development of hybrid varieties in breeding programs. The plant breeding program is one of the activities that support the success of the agricultural system by producing superior varieties that contribute to increasing food availability (Shelton & Tracy, 2016).

Hybrid rice is a cross between different two parents, namely the male sterile (MS) line and the fertility restorer (R) line. The MS line cannot produce functional pollen and the R line is needed to restore fertility (Satoto & Rumanti, 2011). Rice is a self-pollinated crop, with stamen and pistil positioned together in each floret. Therefore, the MS parent plays a vital role in the production of F_1 hybrid seeds.

Characterization is carried out to determine the differences and superiority of the hybrids over their parents and existing varieties. Regarding the parents, at least there are two considerations: (1) the female and male parents should have a good combining ability so that a superior hybrid could be produced, and (2) the male parent should serve as a good restorer for efficient hybrid seed production. Phenotypic performance is affected by genotype, environment, and genotype by environment interactions. This is in line with Kumar et al. (2010) that genotype by environment interactions influence the variability of a plant trait. Phenotypic characters are divided into two categories, namely qualitative characters, which can be observed visually, and quantitative characters, which are measured in units. Aryana et al. (2017) indicated that the distinguishing traits of rice genotypes could support the determination of good hybrids. Traits that are commonly measured in a rice genotype trial are plant height, number of tillers, days to harvest, number of grains per panicle, 1,000-grain weight, and yield potential (Mara et al., 2015).

The superiority of an F_1 hybrid over its parents is called heterosis. Heterosis could be differentiated into mid-parent heterosis and high-parent heterosis or heterobeltiosis. Mid-parent heterosis is the advantage of F_1 hybrids over the average of the parents, whereas heterobeltiosis is the superiority of an F_1 hybrid over the best parent. While heterosis could be considered in determining good hybrids, the actual F_1 performance should be one of the topmost criteria for selection. A preliminary yield trial could be useful for identifying potential hybrids which may be evaluated further in an advanced yield trial. This study aimed to evaluate the ability of several new plant type (NPT) rice lines as restorers of the CMS female parent and to elucidate the performance of the hybrid combinations.

MATERIALS AND METHODS

This research was conducted from December 2019 to November 2020 at Babakan Sawah Baru Experimental Station, Department of Agronomy and Horticulture, IPB University, Bogor, Indonesia. The genetic materials for F_1 hybrid seed production were 9 new plant type (NPT) rice lines from the Department of Agronomy and Horticulture, IPB University, namely: IPB187-F-42-2-2 (T2), IPB187-F-49-1-2 (T3), IPB187-F-62-1-2 (T4), IPB193-F-25-2-1 (T5), IPB189-F-10-3-2 (T6), IPB189-F-11-2-2 (T7), IPB189-F-13-1-3 (T8), 1PB189-F-14-1-1 (T9), and IPB194-F-91-2-1 (T11) as male parents and a CMS line A05 Prima Seed as the female parent. Then, a field experiment was conducted using a total of 21 genotypes consisting of 9 F_1 hybrids, 9 NPT lines (the female parents), one CMS line (the male parent), and two check varieties, namely Ciherang (inbred) and P05 Prima Seed (hybrid).

Hybrid seeds were formed through a cross between the CMS and the restorer lines. This process is carried out by preparing the parents' seeds, providing planting media, sowing seeds, planting, fertilizing, maintaining, and harvesting. The planting of the parents was carried out at three different times. Sixty-six buckets were prepared for the male parents, and 10 buckets for the female parent (CMS), each measuring 5 kg. The fertilizer used was urea at a dose of 45 kg ha^{-1}, applied at the age of seven days and four weeks after planting. Crosses were made during the flowering phase. After pollination is complete, the plants are covered and labeled. Plant maintenance was continued until harvest. The criteria for harvesting were that the grains were dark brown and hard. Harvesting was done manually and then air-dried. Seeds resulting from crosses are hybrid seeds. Hybrid seeds are continued at the field testing stage.

The field test was carried out using a randomized complete block design (RCBD) with two replications. The seedlings were planted with 25 cm x 25 cm spacing with 1 seedling per planting hole. Each tested genotype was planted in 1-3 rows depending on the adequacy of the seeds, and observations were made on 6 plant sample families. The dosage of fertilizer used was 300 kg ha^{-1} for Phonska (15% N, 10% P_2O_5, 12% K, 10% S) and 200 kg ha^{-1} for urea (46% N). Both fertilizers were applied on days 7 and 42 after planting. Cultivation management included irrigation according to the plant growth phase, weeding, and pest and disease control as necessary.

Agronomic traits measured were plant height (cm), stem length (cm), days to flowering, days to harvest, number of productive tillers per hill, panicle length (cm), number of filled grains per panicle, total number of grains per panicle, 1,000-grain weight (g), percentage of filled grain (%), and grain weight per hill (g). Data were analyzed using analysis of variance (ANOVA) followed by the least significant difference (LSD) at 5% level if the genotype effect was significant. The SAS software was used for these analyses. The mid-parent heterosis (MPH) and high-parent heterosis (HPH) values were calculated using the formulas by Virmani et al. (1997) as follows: $MPH = \frac{F_1 - Mid\ parent}{Mid\ parent} x\ 100\%$, and $HPH = \frac{F_1 - High\ parent}{High\ parent} x\ 100\%$.

RESULTS AND DISCUSSION

Performance of NPT lines as male parents

The nine NPT male parental lines evaluated have a good agronomic performance in general. These characteristics include decent plant height and number of productive tillers, early maturity (< 120 days to harvest), relatively long panicle length, and a high number of filled grains per panicle (Table 1).

Table 1. Average agronomic traits of 9 new plant types and one cytoplasmic male sterile parental lines.

Code	Genotype	PH (cm)	PTN	DTF (d)	DTH (d)	PL (cm)	NFG
T2	IPB187-F-42-2-2	114.0	13.0	74	117	27.5	145.5
T3	IPB187-F-49-1-2	125.5	22.0	74	117	29.5	120.5
T4	IPB187-F-62-1-2	126.5	19.5	72	115	28.0	137.0
T5	IPB193-F-25-2-1	136.5	20.0	74	117	30.0	114.0
T6	IPB189-F-10-3-2	125.5	17.0	74	117	30.0	161.0
T7	IPB189-F-11-2-2	122.0	19.5	72	115	30.0	115.5
T8	IPB189-F-13-1-3	115.5	18.5	74	117	27.5	192.0
T9	IPB189-F-14-1-1	126.0	23.0	74	117	28.0	146.5
T11	IPB194-F-91-2-1	130.0	23.0	74	117	29.0	130.0
CMS	A05 Prima Seed	102.5	25.0	76	119	25.5	36.5
	F-test	**	*	- [a)]	- [a)]	**	**
	LSD 0.05	10.5	6.0	-	-	2.0	33.9
	CV (%)	3.8	13.2	-	-	3.1	11.6

Note: CMS = cytoplasmic male sterile; PH = plant height; PTN = number of productive tillers; DTF = days to flowering; DTH = days to harvest; PL = panicle length; NFG = number of filled grains. [a)] the trait was observed on the genotype basis and therefore ANOVA and LSD test were not performed.

Plant height is an important agronomic trait that should be considered for hybrid seed production, as indicated by Gavino et al. (2008). The male parent should be taller than the CMS female parent to facilitate an effective pollination process in hybrid seed production. The analysis showed the variation in the average plant height between the male parental lines, but all male parents were taller than the CMS female parent (Table 1). This difference in plant height facilitates the pollination process between the male parent and female parent so that the chances of receiving pollen from the male parent are higher. NPT lines T2 and T5 having average plant heights of 114.0 and 136.5 cm, respectively, may therefore be used as male parents for the CMS female parent (102.5 cm) (Table 1). However, it is important to note that the higher the plant statures, the more chance the plant to lodge. Diptaningsari (2013) suggested considering stem firmness as an important trait to prevent tall plants from lodging.

The 9 male parental lines had an average days to flowering of 72-74 days, while CMS had 76 days (Table 1). The difference in flowering time of the male and female (CMS) parent lines affects the interest of farmers and seed companies in producing hybrid seeds.

The flowering time difference between CMS and male parents is still within tolerance because the difference in flowering age is only around 2-4 days. If there is a substantial difference in flowering time, planting time management for the two parents is required. In hybrid rice production, a relatively small difference in flowering time can be solved by adjusting the sowing time between the two parents. Genetic factors (Zheng et al., 2020) and environmental factors, such as heat stress (Jagadish, 2020) and high temperatures (Sage et al., 2015), influence the success of hybridization.

Days to harvest were calculated from sowing days to until 85% of the grains in the panicles are ripe (Silitonga et al., 2003). The harvesting age characteristics of the 9 male parental lines and CMS as the female parent were classified as early maturity (harvesting time less than 125 days). Data analysis showed that there was a difference in days to harvest between the female parent and the 9 male parents. The days to harvest of the 9 new plant type lines (male parent) ranged from 115-117 days, while for the female parent was 119 days (Table 1).

Important traits in rice include the number of filled grains which affects grain yield per hill, the number of productive tillers, and 1,000-grain weight (Kim et al., 2014; Bai et al., 2017). Panicle traits could affect the success of seed production. Male parents with long and thick panicles may be suitable for use as a source of pollen in hybrid seed production. Meanwhile, a CMS female parent with long or thick panicles may produce a high yield of hybrid F_1 seeds if the grain filling is effective. There were three male parental lines, T5, T6, and T7, with an average panicle length of 30 cm, significantly longer than the CMS line (25.5 cm) (Table 1).

The male parental lines had a different number of filled grains. The average number of filled grains in lines T2 to T11 ranged from 114-192 grains per panicle (Table 1). The T6 and T9 lines had an average of 161 and 147 grains per panicle. The increasing percentage of filled grain could lead to an increase in grain yield (Anggraeni et al., 2021).

Variability of F_1 hybrids

The average plant height of the hybrids ranged from 105.5 cm (CMS x T5) to 120.5 (CMS x T4 and CMS x T8), but the difference is not significant ($p>0.05$) (Table 2). The CMS x T3 hybrid had the highest average number of productive tillers (34.5) and CMS x T7 had the lowest (12.5), but the difference is not significant. The capacity to produce tillers is affected by genetic and environmental factors (Harmansis et al., 2019; Yulina et al., 2021). The flowering and harvest times of all F_1 hybrids were significantly earlier than both check varieties. All test hybrids had a significantly greater average panicle length than Ciherang, whereas CMS x T2 had also a greater average panicle length than P05 (Table 2).

The 1,000-grain weight of the test hybrids ranged from 22.5 g (CMS x T8) to 28.0 g (CMS x T7), while that of the check varieties Ciherang was 29.5 g and P05 Prima Seed was 30.0 g (Table 2). These differences are not significant ($p>0.05$). The 1,000 grains weight is a yield-related trait that is associated with grain size. The yield in this experiment is represented by grain weight per hill, and the genotype effect on it is significant ($p<0.05$) (Table 2). Seven test hybrids had a similar average grain weight to the checks, and two were lower. Differences in grain weight per hill are affected by genetics (Hu et al., 2021), and are associated with the number of productive tillers and grain size (Zuo and Li, 2014). The grain yield may also be affected by panicle size and is influenced by environmental conditions including climate (Huang et al., 2017).

Table 2. Average agronomic traits of 9 rice hybrids and two check varieties.

Hybrid	PH (cm)	PTN	DTF (d)	DTH (d)	PL (cm)	NFG	TNG	TGW (g)	GWH (g)
CMS x T2	116.0	17.0	74cd	117cd	29.5ab	112.0	255.5ab	27.5	44.71
CMS x T3	111.0	34.5	76cd	119cd	27.0a	51.5c	277.0ab	26.0	46.46
CMS x T4	120.5	18.5	74cd	117cd	28.0a	95.0	222.0a	27.5	48.13
CMS x T5	105.5	15.0	76cd	119cd	28.0a	84.0	232.5a	27.5	37.97
CMS x T6	116.5	20.5	76cd	119cd	28.0a	117.5	276.0ab	27.5	66.04
CMS x T7	111.0	12.5	74cd	117cd	26.5a	74.0	225.0a	28.0	29.13d
CMS x T8	120.5	15.0	74cd	117cd	28.5a	32.5cd	250.0ab	22.5	12.65cd
CMS x T9	118.0	22.0	74cd	117cd	27.5a	124.0	256.0ab	27.5	75.27
CMS x T11	111.5	15.5	76cd	119cd	28.0a	99.5	253.0ab	27.5	42.65
Ciherang	119.0	21.5	79	122	23.5	93.5	135.0	29.5	58.99
P05	109.5	23.0	79	122	26.5	91.5	183.0	30.0	63.29
F-test	ns	ns	*	*	*	*	**	ns	*
LSD 0.05	-	-	2.9	2.9	2.7	40.9	53.3	-	31.0
CV (%)	4.6	27.5	1.7	1.1	4.4	20.7	10.3	9.8	29.1

Note: CMS = cytoplasmic male sterile; PH = plant height; PTN = number of productive tillers per hill; DTF = days to flowering; DTH = days to harvest; PL = panicle length; NFG = number of filled grains; TNG = total number of grains; TGW = 1,000 grains weight; GWH = grain weight per hill. a, b = signficantly higher than Ciherang and P05; c, d = significantly lower than Ciherang and P05, respectively, based on LSD at α=5%.

The percentage of filled grain of the male parents ranged from 49.7% (T7) to 79.8% (T8) and for the hybrids ranged from 13.7% (CMS x T8) to 48.4% (CMS x T9) (Figure 1). Variations in the restoring ability of the male parents, as shown by the ratio of the number of filled grains of the hybrid to its male parent, were evident. Relatively high ratios were observed in CMS x T4 (84.4%) and CMS x T9 (80.8%), and conversely, a small ratio was observed in CMS x T8 (17.2%) (Figure 1). These results indicate that the T4 and T9 parents have good restoring abilities and have the potential to be used in developing hybrid rice varieties. The percentage of filled grain is one of the determinants of rice yield potential. Increasing rice productivity can be obtained from improvements in the characters of grain weight and number of grains per panicle. The percentage of filled grain of the hybrid are affected by the genetics of the hybrid itself as well as the fertility-restoring ability of the male parent (R) line (Chen and Liu 2014). The selection of male parents is therefore important to obtain hybrids with desired performance.

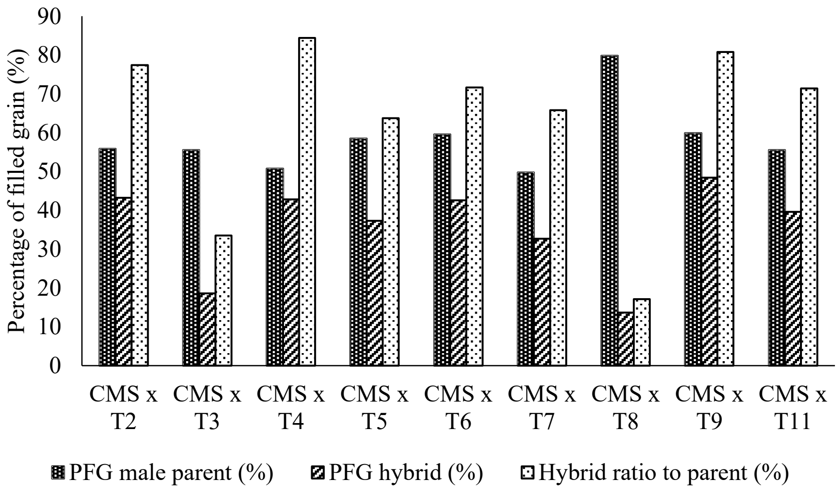

Figure 1. Percentage of filled grain (PFG) of male parents, hybrids, and their ratio. CMS = cytoplasmic male sterile. See Table 1 for genotype names of T2-T11.

Figure 1 shows that the 9 hybrids have variations in the percentage filled grains. The high or low percentage of hybrid produced is affected by genetic factors (Kobata et al., 2013) and environmental factors (Kobata et al., 2017). The ability of an NPT line as a restorer in hybrid formation can be seen from the success of seed formation on the CMS female parent.

The formation of a rice hybrid uses the A (CMS) line, which has the *rfrf* genotype (i.e., recessive homozygous at the fertility-restorer locus), and the R (restorer) line, which has the *RfRf* genotype. Hence, crossing the A and R lines produced a male fertile hybrid (*Rfrf*). Using the A (CMS) line in hybrid formation can avoid the need for emasculation which is technically impossible for rice commercial hybrid seed production. The seeds of the A (CMS) line could be produced by crossing the A line and the B (maintainer) line. Nevertheless, an obstacle in the formation of hybrids is the sterility level of CMS less than 100%. It causes the formation of fertile seeds in the female parent and caused a decrease in the purity of the F_1 hybrid seeds produced. The sterility level of CMS is mostly determined by genetic (Liu et al., 2015; Mishra and Bohra 2018) and environmental factors (Matsui et al., 2014; Koumoto et al., 2016).

Heterosis and heterobiltiosis

Mid-parent heterosis (MPH) is the superiority of a hybrid over the average of both parents, whereas high-parent heterosis (HPH) or heterobeltiosis is the advantage of a hybrid over the best parent. Positive heterosis values are usually desired for yield-related traits. On the contrary, negative heterosis values for plant height, days to flowering, and days to harvest imply that the hybrids had a shorter plant stature and earlier maturity than the parents, which may be an advantage.

The CMS x T9 hybrid with the highest grain weight per hill is better than the mid-parent for 1,000-grain weight (13.4%), panicle length (2.8%), days to flowering (-1.3%), days to harvest (-0.8%) (Table 3). Compared with the new plant type parent, this hybrid had a lower plant height (-6.3%) and a greater total number of grains (4.7%). The second highest-yielding hybrid, CMS x T2, had a similar heterosis pattern with CMS x T9, except that it has slightly later days to flowering (1.3%) and days to harvest (0.8%) than the mid-parent (Table 3).

The heterosis in some traits is thought to be related to the overdominant gene action that regulates their expression (Zhou et al., 2012; Chen et al., 2018). Desired heterosis values could be positive or negative, depending on the traits. For the number of productive tillers, panicle length, number of filled grains, number of total grains, and total grain weight, positive heterosis values are desired, implying that the hybrid has a higher value than the parents. On the contrary, a negative heterosis value may be desired for plant height, days to flowering, and days to harvest, indicating that the hybrids had lower values than the parents.

There are four hybrids with negative MPH for plant height, namely CMS x T3, CMS x T5, CMS x T7, and CMS x T11, while for HPH there were 7 hybrids with negative values (Table 3). The differences in plant height among genotypes may be affected by dominant and epistasis gene actions (Shen et al., 2014). For the number of productive tillers, CMS x T3 had the highest MPH (46.8%) and HPH (38.0%) (Table 3). Meanwhile, for flowering age, three hybrids had the lowest mid-parent heterosis of -1.3%, and five hybrids with the lowest heterobeltiosis of -2.6%. The CMS x T9 hybrid showed both negative mid-parent and heterobeltiosis, indicating the opportunity to produce high-yielding hybrids with a relatively early maturity than their parents in a rice breeding program. Early flowering age opens the possibility for early harvest and obtaining selection results sooner (Aryana et al., 2017).

The 1,000-grain weight is one of the yield-related traits in rice. The analysis showed that one hybrid, CMS x T2, had the highest mid-parent heterosis (23.6%) and best-parent heterosis (14.6%) (Table 3). This indicates the opportunity to obtain hybrids with greater seed sizes than both CMS and new plant type rice parents. There are six hybrids with similar or greater total number of grains than the best parent; however, none of the hybrids are better than the best parent for number of filled grains (Table 3).

Table 3. Estimates of mid-parent heterosis (MPH) and heterobeltiosis (HPH) of rice hybrids

Hybrid	PH		PTN		DTF		DTH	
	MPH	HPH	MPH	HPH	MPH	HPH	MPH	HPH
CMS x T2	7.2	1.8	-10.5	-32.0	-1.3	-2.6	-0.8	-1.7
CMS x T3	-2.6	-11.6	46.8	38.0	1.3	0.0	0.8	0.0
CMS x T4	5.2	-4.7	-16.9	-26.0	0.0	-2.6	0.0	-1.7
CMS x T5	-11.7	-22.7	-33.3	-40.0	1.3	0.0	0.8	0.0
CMS x T6	2.2	-7.2	-2.4	-18.0	1.3	0.0	0.8	0.0
CMS x T7	-1.1	-9.0	-43.8	-50.0	0.0	-2.6	0.0	-1.7
CMS x T8	10.6	4.3	-31.0	-40.0	-1.3	-2.6	-0.8	-1.7
CMS x T9	3.3	-6.3	-8.3	-12.0	-1.3	-2.6	-0.8	-1.7
CMS x T11	-4.1	-14.2	-35.4	-38.0	1.3	0.0	0.8	0.0
Hybrid	PL		NFG		TNG		TGW	
	MPH	HPH	MPH	HPH	MPH	HPH	MPH	HPH
CMS x T2	11.3	7.3	23.1	-23.0	13.9	-1.7	23.6	14.6
CMS x T3	-1.8	-8.5	-34.4	-57.3	36.5	27.4	4.0	-11.9
CMS x T4	4.7	0.0	9.5	-30.7	-3.8	-18.7	12.2	-3.5
CMS x T5	0.9	-6.7	11.6	-26.3	21.1	18.9	7.8	-9.8
CMS x T6	0.9	-6.7	19.0	-27.0	19.5	0.9	14.6	0.0
CMS x T7	-4.5	-11.7	-2.6	-35.9	6.6	-3.6	19.1	5.7
CMS x T8	7.5	3.6	-71.6	-83.1	16.7	4.2	-1.1	-10.0
CMS x T9	2.8	-1.8	35.5	-15.4	18.2	4.7	13.4	-1.8
CMS x T11	2.8	-3.4	19.5	-23.5	19.6	7.9	11.1	-5.2

Note: MPH = mid-parent heterosis; HPH = high-parent heterosis; CMS = cytoplasmic male sterile; PH = plant height; PL = panicle length; PTN = number of productive tillers; DTH = days to harvest; DTF = days to flowering; FG = number of filled grains; TNG = total number of grains; TGW = 1,000-grain weight.

CONCLUSIONS

Two male parental lines, IPB189-F-10-3-2 (T6) and IPB189-14-1-1 (T9), had a relatively large number of filled grains and produced hybrids with relatively high grain weight per hill. Thus, these two NPT lines have the potential to be used as male parents in the production of hybrid rice seeds. These two parents have the restorer (R) gene which is substantiated by the good characteristics of the number of filled grains and total grain per panicle of the two hybrids. The potential hybrids CMS x IPB189-F-10-3-2 and CMS x IPB189-14-1-1 had a positive heterosis for panicle length and 1,000 grain weight, and could be evaluated in future research.

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