

ESTIMATION OF HETEROSIS IN F₁ GENERATIONS OF PAW SAN AND IR24 CROSSES AND THEIR RECIPROCALLS FOR GRAIN SIZE AND AGRONOMIC CHARACTERISTICS IN RICE

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ABSTRACT

The purpose of the present study was to evaluate the grain size and agronomic characteristics, including yield components in F₁ generations compared with the parents. Three Paw San rice varieties, Paw San Hmwe (PSM), Paw San BayKyar (PSBK) and Paw San Yin (PSY) (japonica), and IR24 (indica) were planted in plastic tanks under short day length conditions, and these three Paw San rice varieties were crossed with IR24 at the Tokyo University of Agriculture, greenhouse in April-October 2022. The grain length, grain width and length-width ratio of PSMxIR24, IR24xPSM, PSYxIR24, IR24xPSY, PSBKxIR24 and IR24xPSBK were not different from those of the Paw San parent. All F₁ generations showed grain sizes similar to those of Paw San and might have a grain elongation gene similar to that of Paw San. The heading dates of all F₁ generations had heading dates later than those of the late-heading parents and all were strongly photoperiod-sensitive varieties, possibly due to a photoperiod-sensitivity gene such as *Hdl*. In this study, the yield per plant of all F₁ generations was found to be higher than that of the Paw San parent but lower than that of the IR24 parent. PSMxIR24, PSYxIR24 and IR24xPSY showed positive heterosis values in days to heading and panicle length compared to midparent and better parent values, but showed negative values in most of the examined traits. Positive or negative heterosis values provide significant information on the preferred traits for the development of grain elongation and agronomic characteristics. Future research involves growing F₂ generations to improve the photoperiod-insensitive and grain quality traits in Paw San. Molecular marker analysis can also be carried out to select the desired traits and compare the grain quality of the F₁ generation with that of backcrossed generations.

Key words: F₁ generations, hybridization, short day, natural day length

INTRODUCTION

Rice is a cultivated crop that provides staple food for more than half of the world's population (Fukagawa and Ziska 2019). Rice is the staple food of the people of Myanmar whose population was 59.78 million in 2010 and has been increasing at a rate of 1.15% per year (MOAI 2014). Among Myanmar rice varieties, Paw San was traditionally grown in rainfed lowlands, including water-logged areas and land. Paw San's suitability for a very specific climate and long maturation period have largely

confined its cultivation to the Ayeyarwady and Sagaing regions and small areas in the states of Bago, Mon and Rakhine. Patheingyi, Phyayon and Myaungmya delta are the major areas of Paw San rice production, and Paw San rice varieties, Paw San Hmwe, Paw San Bay Kyar and Paw San Yin are cultivated in different regions in Myanmar. Specifically, Paw San Hmwe (PSM) is cultivated in the Yangon, Pango and Shwebo regions, and Paw San Bay Kyar (PSBK) and Paw San Yin (PSY) in the Ayeyarwady region. Approximately 391,000 ha of Paw San rice were planted, accounting for 6% of the country's total rice production area, in 2012 and 2013 (Nwe et al. 2001). The key characteristics of Paw San rice are a strong aroma, good taste and its elongation during cooking (Tin and Tun 2004). Among Paw San varieties, the PSM cultivar won third prize in the World Rice Conference in 2009 (Philippines) (Tin 2004) and was recognized as the best rice in the World Rice Conference in 2011 (Vietnam) (Glenn et al. 2013). IR24 is photoperiod-insensitive Indica variety. It has low amylose content, a long and slender grain and a short stem. Paw San varieties provide good cooking and eating quality and are famous for elongation characteristics (up to three times longer than the original size after cooking). Grain elongation also correlates with grain quality traits, such as grain appearance, head rice yield, and cooking and eating quality (Nelson et al. 2011; Tan et al. 2000). However, Paw San are photoperiod-sensitive, long duration varieties with poor yields. Hence, there is a need to develop Paw San varieties that are photoperiod-insensitive, with a short heading duration, a dwarf plant with high yield, and maintaining good grain quality. Therefore, crosses between 3 Paw San varieties and IR24 rice were cultivated. Heterosis was first reported in some rice F₁ hybrids that had more culm and higher yields than the parents (Jones 1926). Heterosis in rice grain yield is seen primarily in comparatively more spikelets per panicle, a higher thousand-grain weight, a higher number of panicles per hill, a higher number of filled grains, among others (Kim 1985).

The selection of parent is very important to develop improved generations. Therefore, the value of negative or positive heterosis depended on the degree of selected parental lines (Bhatti et al. 2015; Rahimi et al. 2010) and positive or negative heterosis indicate significant information of desired traits for rice development program (Reddy and Reddy 2012). Improvement in rice grain yield through conventional breeding is limited today in Myanmar. Hence, rice breeders need to seek another genetic mechanism through which to improve the genetic potential of Paw San rice with desired traits. Therefore, studies on heterosis are important in this quest for further genetic improvement of Paw San rice. This study sought to evaluate the grain size and yield component characteristics in F₁ generations PSMxIR24, PSBKxIR24, PSYxIR24, IR24xPSM, IR24xPSBK and IR24xPSY compared to the parents, and to estimate heterosis for grain size and agronomics characteristics in these F₁ generations.

MATERIALS AND METHODS

In the present experiment, 3 Japonica photoperiod-sensitive Paw San varieties (PSM, PSBK and PSY) from the seed of bank Myanmar (Table 1), and an Indica photoperiod-insensitive variety (IR24) that was conserved at the Tropical Crop Science Laboratory of the Tokyo University of Agriculture were used. All 4 varieties were grown in simple 164 cm x 79 cm plastic tanks under short day length conditions (9 h light and 15 h dark). The plant-to-plant distance within rows was 20 cm and the row-to-row distance was 25 cm. At the same time, another IR24 rice variety was also grown in 300 cm x 200 cm concrete tanks under natural day conditions at the Tokyo University of Agriculture synchronized with the three Paw San rice varieties in the greenhouse. When Paw San rice varieties reached 5 days old, additional PSBK and PSY plants were grown in pots to synchronize with IR24 in

the greenhouse. After the Paw San and IR24 rice varieties were synchronized, the three Paw San rice varieties were crossed with IR24 while IR24 was crossed with the three Paw San rice varieties.

Table 1. Varietal characteristics of Paw San rice.

Characteristic	Paw San rice (Accession no.)		
	Paw San Hmwe (1207)	Paw San Bay Kyar (807)	Paw San Yin (930)
Aroma	Aromatic	Aromatic	Aromatic
Amylose content (%)	21	21	24.2
Eating quality	Good	Good	Moderate
Drought resistance	Excellent	Very Good	Very Good
Flood resistance	Moderate	Moderate	Good
Pest resistance	Good	Good	Moderate
Disease resistance	Moderate	Moderate	Resistant
Potential yield (ton/ha)	2.58	3.1	3.61

Hybridization with hot water emasculation. The hybridization of 6 combinations: PSMxIR24, PSBKxIR24, PSYxIR24, IR24xPSM, IR24xPSBK and IR24xPSY, was done by panicle emasculation using hot water method (Matsubayashi et al. 1965) at the Tokyo University of Agriculture greenhouse. The flag leaves and undeveloped grains were removed and panicles were dipped into the water at 43°C for 7 minutes to kill the pollen and create a female Japonica or Indica parent. After hot water emasculation, pollination was accomplished by using several panicles. Hot water emasculation for female parents was carried out between 4:00 and 6:00 pm by dipping the panicles into hot water and pollination was carried out between 10:00 and 11:00 am the morning after emasculation. The pollinated panicles were covered with butter paper bags and labeled.

Growing parent genotypes and F₁ generations. Parent genotypes and F₁ generations were grown in pots under short day length conditions (9 hours light and 15 hours dark) at the Tokyo University of Agriculture greenhouse. The plants in this study were laid out in a randomized complete block design with three replications. Heterosis values were calculated in the following equation as suggested by Nadaranjan and Gunasekaran (2005).

$$\text{Mid parent heterosis} = (F_1 - MP) / MP \times 100$$

$$\text{Better parent heterosis} = (F_1 - BP) / BP \times 100$$

RESULTS AND DISCUSSION

F₁ seed setting. Approximately 7 days after crossing, the F₁ seed set started in the 6 combinations. F₁ seeds produced a light green color in all of these crosses. After 7 weeks, all these crosses had matured fully and were harvested.

The highest F₁ seed set was found in IR24xPSY (80.77%), followed by PSYxIR24 (19.25%), IR24xPSM (15.91%), IR24xPSBK (14.28%), PSBKxIR24 (8.24%) and PSMxIR24 (4.26%). The highest F₁ seed set was found in Indica x Japonica which was IR 24 x PSY (80.77%) and F₁ seed set of Indica/Japonica was higher than those of Japonica/Indica. There was only one combination with high percentage of success.

Evaluation of grain size in parent genotypes and F₁ generations of Paw San and IR24 crosses and their reciprocals. The grain sizes of the parent genotypes and F₁ generations of Paw San and IR24 crosses and their reciprocals are shown in Fig.1. The grain lengths, grain widths and length-width ratios of all crosses were not different from those of the Paw San parent. According to these data, the grain size of all F₁ generations was similar to that of Paw San, suggesting that the crosses might have a grain elongation gene similar to that of the parent, Paw San. The findings in this study suggest that early generation selection for grain size would not be effective.

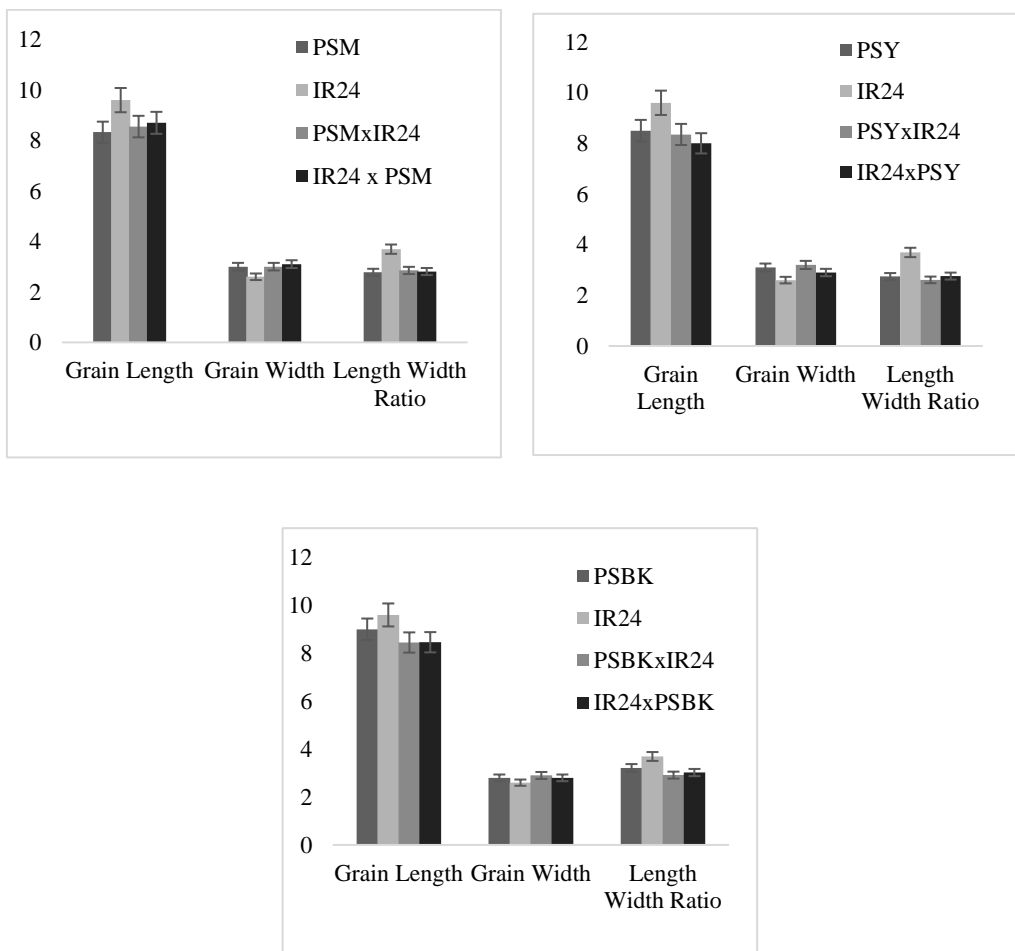


Figure 1. Grain size of parent genotypes and 6 F₁generations.

Evaluation of agronomic characteristics of the parent genotypes and F₁ generations of Paw San and IR24 crosses and their reciprocals. There were significant differences in days to heading between the parent genotypes and all F₁ generations (Fig. 2). The days to heading of PSMxIR24 (143 days) and IR24xPSM (141 days) were significantly longer than those of PSM (103 days) and IR24 (98days). Likewise, the days to heading of PSYxIR24 (160 days) and IR24xPSY (155 days) were significantly longer than those of PSY (125 days) and IR24 (98days), and the days to heading of PSBKxIR24 (154 days) and IR24xPSBK (146 days) were also significantly longer than those of PSBK (110 days) and IR24 (98 days). In this experiment, all F₁ generations were not only late heading but also highly sensitive to photoperiod like the parent, Paw San.

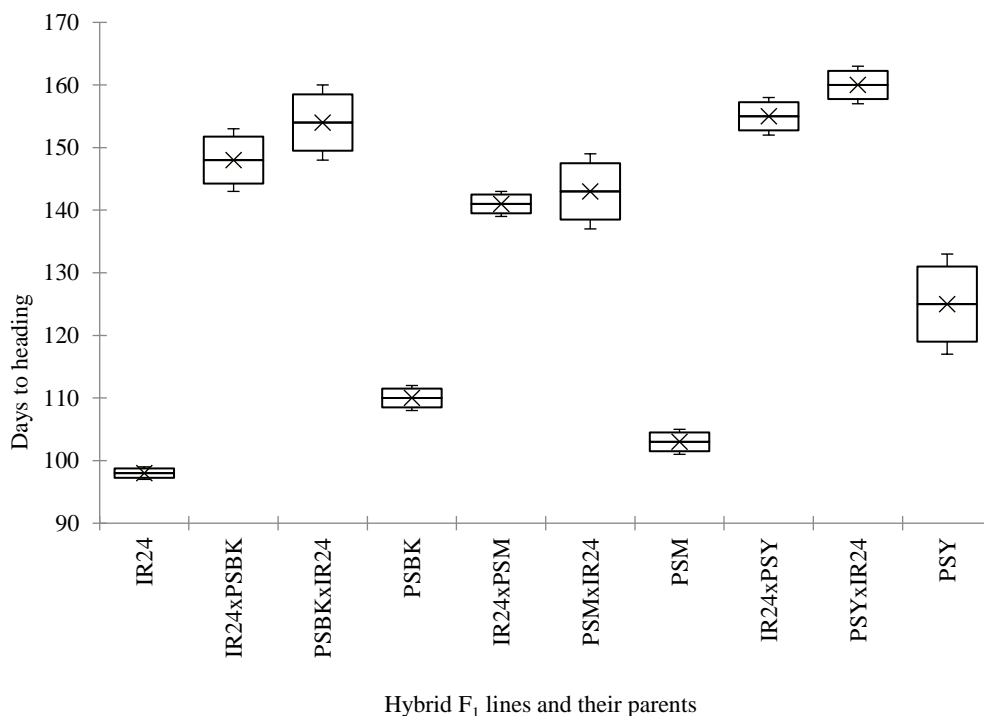


Figure 2. Days to heading of parent genotypes and F₁ generations under short day length conditions.

The induction of early heading and the effects of growing under short days were quantitative in nature (Sircar and Ghose 1954). In the present study, all F₁ generations headed later than the late-heading parents and were strongly photoperiod-sensitive, possibly due to the presence of a photoperiod-sensitivity gene such as *Hdl*. This is consistent with the results obtained with the Bhasamanik variety (Sircar 1948) and with the Rupsail variety (Sircar and Sen 1953).

The agronomic characteristics of the parent genotypes and F₁ generations of the present Paw San and IR24 crosses and their reciprocals are shown in Table 2. PSM displayed significantly taller plant height (107.66 cm) ($P < 0.0001$) than those of its F₁ generations (PSMxIR24) and (IR24xPSM), while IR24 (76.9cm) was significantly shorter than the F₁ generations. On the other hand, PSYxIR24 and

IR24xPSY were both significantly taller than the parent, IR24, though these were significantly shorter than PSY. The mean plant height of PSBKxIR24 and IR24xPSBK was significantly shorter than that of the parent PSBK, but taller than that of IR24.

F₁ hybrids are reported to show a plant height similar to that of the female parent, which was the taller parent in their cross combinations (Khan et al. 1998). In the present study, it was found that the plant height of all F₁ generations was similar to that of Paw San, which is genetically tall. The plant height of the F₁ generations in the present experiment is consistent with the findings of other researchers (Alam et al. 2004; Li et al. 2002). Not listed.

The mean effective numbers of tillers hill⁻¹ of PSMxIR24 and IR24xPSM were not significantly different from that of PSM (3.33). Although the mean effective number of tillers hill⁻¹ of IR24xPSM was significantly lower than that of IR24 (6.66), that of PSMxIR24 was not significantly different from male parent, IR24. The mean numbers of tillers hills⁻¹ in PSYxIR24 and IR24xPSY were not significantly different from those of the parents. In the case of PSBKxIR24 and IR24xPSBK, the mean effective numbers of tillers hill⁻¹ were not significantly different from that of PSBK but these were significantly lower than that of IR24.

Tiller mortality was determined to be not significantly different in F₁ hybrids compared with the parent cultivars (Govindaraj and Siddiq 1986). In another study, short day conditions inhibited tillering in all sowings (Misra and Khan 1973). The results of the present study suggested that mean numbers of tillers hill⁻¹ were not significantly different from parents and F₁ generations.

Although the panicle lengths of PSMxIR24 (18.29cm) and IR24xPSM (20.08cm) were not significantly different from that of PSM (19.73cm), these were significantly longer than that of IR24 (16.05cm). The panicle length of PSYxIR24 (20.52cm) was significantly longer than those of its two parents while that of IR24xPSY (19.26cm) was not significantly different from that of PSY but was significantly longer than that of IR24. Although PSBKxIR24 (18.62cm) and IR24xPSBK (17.92cm) showed significantly longer panicle lengths than IR24, the lengths were not statistically different from that of PSBK (18.67cm). Thus, the panicle lengths of all F₁ generations were significantly longer than that of IR24. Earlier studies reported that average panicle length is slightly increased by short day exposure in some rice varieties (Gadadhar 1953).

The numbers of spikelets per panicle of PSMxIR24 (74) and IR24xPSM (61) were not significantly different from those of the parents. Although the number of spikelets per panicle of PSYxIR24 (87.33) was significantly higher than that of the parents, that of IR24xPSY (66.66) was not significantly different. In PSBKxIR24 (83.33) and IR24xPSBK (79), the numbers of spikelets per panicle were significantly higher than in IR24 (68.33), but were not statistically different from that of PSBK (84). Thus, the number of spikelets panicle⁻¹ was significantly different in the tested lines and this characteristic may be useful in improving grain production (Akinwale et al. 2011).

All F₁ generations showed significantly lower filled grain % values than their parents. Filled grains are an important yield-contributing character in rice (Mehetre et al. 1994). Phenotypic performance selection would be effective to improve 1000 grain weight, floret fertility % and number of grains panicle⁻¹ (Khan et al. 2019).

Table 2. Evaluation of the agronomic characteristics of parent genotypes and F₁ generations of Paw San and IR24 crosses and their reciprocals.

Parent genotypes and F₁ generations	Plant height(cm) (Mean±SE)	Number of effective tillers per plant (Mean±SE)	Panicle length (cm) (Mean±SE)	Number of spikelets per panicle (Mean±SE)	Filled grain (%) (Mean±SE)	1000-Grain weight(g) (Mean±SE)	Yield plant¹(g) (Mean±SE)
PSM	107.66±1.20 b	3.33±0.57cd	19.73±0.29abc	62.00±2.08de	52.15±2.93bc	20.7±0.4c	1.43±0.04f
IR24	76.9±1.08f	6.66±0.33ab	16.05±0.36e	68.33±2.33cde	80.66±1.31a	31.1±0.5ab	15.16±0.76a
PSM×IR24	103.56±0.63bcd	4.66±1.20abcd	18.29±0.03cd	74.00±3.21bcd	42.51±0.46cd	22.5±0.3c	7.05±0.12e
IR24×PSM	104.46±0.98bc	3.00±0.33d	20.08±0.14ab	61.00±1.15e	37.66±0.62d	21.2±0.1c	1.89±0.17f
PSY	116.66±0.88a	6.00±0.57abc	18.36±0.21cd	73.66±3.92bcde	72.70±2.09a	31.0±0.6ab	11.19±0.49bcd
IR24	76.9±1.08f	6.66±0.33ab	16.05±0.36e	68.33±2.33cde	80.66±1.31a	31.1±0.5ab	15.16±0.76a
PSY×IR24	107±0.56bc	7.00±0.57ab	20.52±0.34a	87.33±3.28a	48.80±4.05bc	31.4±0.1a	12.27±0.10b
IR24×PSY	106.40±1.22bc	7.33±0.33a	19.26±0.43abcd	66.66±0.88cde	55.61±1.48b	29.1±0.6b	11.76±0.34bc
PSBK	103±0.57cde	3.66±0.33cd	18.67±0.63bcd	84.00±2.08ab	56.61±2.29b	29.0±0.6b	9.59±0.29d
IR24	76.9±1.08f	6.66±0.33ab	16.05±0.36e	68.33±2.33cde	80.66±1.31a	31.1±0.5ab	15.16±0.76a
PSBK×IR24	99.5±0.48de	4.33±0.33bcd	18.62±0.34bcd	83.33±3.38ab	42.14±1.18cd	30.4±0.3ab	10.34±0.20cd
IR24×PSBK	98.73±0.56e	4.33±0.33bcd	17.92±0.12d	79.00±1.52abc	48.65±0.40bc	29.7±0.2ab	11.37±0.32bc
Pr>F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
CV%	1.45	19.08	3.09	6.03	6.49	2.53	6.52

Means followed by the same letter are not significantly different at 5% level by Tukey's Studentized Range (HSD) test

The 1000-grain weight of IR24 (31.1g) was significantly higher than those of PSMxIR24 and IR24xPSM while those of the other tested parent genotypes and F₁ generations were not significantly different from one another. Previous studies have demonstrated that 1000- grain weight is affected by flag leaf area and other factors such as adaptability, temperature, soil fertility, and transplantation season and time (Bharali and Chandra 1994; Bhatti et al. 1998).

The yield per plant of IR24 (15.16g) was significantly higher than those of PSMxIR24 and IR24xPSM. Although the yield per plant of IR24xPSM (1.89g) was not significantly different from that of PSM (1.43g), that of PSMxIR24 (7.05g) was significantly higher than that of PSM. The yields per plant of PSYxIR24 (12.27g) and IR24xPSY (11.76g) were not significantly different from that of PSY but were significantly lower than that of IR24. Although the yield per plant of PSBKxIR24 (10.34g) was not significantly different from that of PSBK (9.59g), it was significantly lower than that of IR24. The yield per plant of IR24xPSBK (11.37) was significantly higher than that of PSBK and significantly lower than that of IR24. Grain yield is significantly correlated with number of tillers plant⁻¹, panicle weight, number of grains panicle⁻¹ and 1000-grain weight (Akinwale et al. 2011; Liu et al. 2015).

Evaluation of heterosis in F₁ generations of Paw San and IR24 crosses and their reciprocals with respect to agronomic characteristics. Table 3 presents the heterosis values of the agronomic characteristics of all 6 F₁ generations. IR24xPSBK showed negative heterosis values for all agronomic characteristics except days to heading. However, when comparing midparents plant height values, IR24xPSBK showed a positive heterosis value. Although IR24xPSM, PSYxIR24 and IR24xPSY showed positive heterosis values in days to heading and panicle length compared to both midparent and better parent values, these showed negative values for most traits.

Table 3. Estimation of heterosis relative to midparent and better parent values for yield and yield component characteristics under short day length condition.

Characteristics	Heterosis	PSMx	IR24x	PSYx	IR24x	PSBKx	IR24x
		IR24	PSM	IR24	PSY	IR24	PSBK
DH	MP	39.83	42.47	43.88	38.80	47.84	40.16
	BP	36.01	38.58	28.19	23.67	39.57	32.52
PH	MP	10.56	13.20	10.62	12.22	9.94	9.76
	BP	-8.28	-2.97	-3.40	-3.81	-8.79	-4.15
ETH	MP	10.58	-39.94	-16.09	-6.71	15.80	-16.09
	BP	5.10	-54.95	-34.98	-30.03	10,06	-34.98
PL	MP	19.27	12.24	7.26	2.24	11.94	3.23
	BP	11.76	1.77	-0.27	-7.30	4.90	-4.02
SPP	MP	23.01	-6.39	9.41	13.56	-6.11	3.72

Characteristics	Heterosis	PSMx IR24	IR24x PSM	PSYx IR24	IR24x PSY	PSBKx IR24	IR24x PSBK
	BP	18.56	-10.73	-0.80	8.30	-9.50	-5.95
FG (%)	MP	-36.36	-43.29	-38.60	-35.98	-27.48	-29.14
	BP	-39.50	-53.31	-47.76	-47.30	-31.06	-39.69
1000GW	MP	1.13	-18.15	1.16	-13.13	-6.28	-1.16
	BP	0.96	-31.83	-2.25	-27.65	-6.43	-4.50
YPP	MP	-0.07	-0.77	-0.16	-0.15	-0.11	-0.08
	BP	-19.06	-87.53	-31.79	-53.50	-22.43	-25.00
GL	MP	-0.03	-0.05	-0.08	-0.01	-0.06	-0.06
	BP	-9.38	-10.94	-13.02	-6.25	-11.88	-11.46
GW	MP	0.11	0.07	0.12	0.02	0.07	0.04
	BP	19.23	15.38	23.08	11.54	11.54	7.69
L/W	MP	-0.13	-0.12	-0.19	-0.04	-0.13	-0.10
	BP	-23.99	-22.81	-29.33	-15.95	-20.99	-17.78

DH,= Days to heading; PH,= Plant height; ETH,= No. of effective tillers hill⁻¹; PL,=Panicle length; SPP,=No. of spikelets per panicle; FG%,=Filled grain %; 1000 GW,= 1000 Grain weight; YPP,= Yield per plant; GL,= Grain length; GW, =Grain width; L/W, =Length-width ratio; MP, = midparent: $H_{MP} = (F1-MP)/MP$; BP,= better parent: $H_{BP} = (F1-BP)/BP$;

Earlier studies revealed that panicle length in rice showed positive heterosis in some crosses (Julfiquar and Tepora 1994). Positive heterosis values in days to heading, number of effective tillers per hill, panicle length, number of spikelets per panicle and 1000-grain weight were found in PSMxIR24, while this cross showed negative heterosis values in plant height compared with better parent values, and negative filled grain (%) and yield per plant values compared with midparent and better parent values. Reports indicated that negative heterosis value in plant height is desirable for breeding short stature hybrids (Jelodar 2010). PSBKxIR24 showed positive heterosis values in days to heading number of effective tillers per hill, and negative values in most other traits except plant height, panicle length and number of spikelets per panicle compared with midparent values. High heterosis values have been reported for number of spikelets per panicle in rice (Gnanasekaran and Muthuramu 2006).

In addition, panicle length is an important trait in rice breeding as it is associated with number of spikelets, which contributes to high yield (Elixon et al. 2015). Although negative heterosis values in grain length and length-width ratio were observed in all crosses, positive heterosis values in grain width

were found in all crosses compared to midparent and better parent values. Both positive and negative heterosis values provide important information on the preferred traits for the development of grain elongation and agronomic characteristics.

CONCLUSION

A total of 6 F₁ generations (PSM×IR24, PSBK×IR24, PSY×IR24, IR24×PSM, IR24×PSBK and IR24×PSY) were cultivated in a greenhouse at 38°C to evaluate grain size, yield and yield component characteristics in the F₁ generations. This study contributes to present knowledge and understanding of various genetic variations in grain size and agronomic characteristics in parent genotypes and F₁ generations, which can be used for future breeding programs. The present results showed that the grain sizes of all F₁ generations were similar to that of Paw San, and that the crosses might have a grain elongation gene, *GS3* or *SLG7*, similar to that found in Paw San. The heading dates of all F₁ generations were longer than those of their parents and all F₁ generations were strongly photoperiod-sensitive, perhaps due to photoperiod sensitivity gene such as *Hdl*. In the present study, the yields per plant of all F₁ generations were higher than that of Paw San and lower than that of IR24. The PSM×IR24 cross showed high performance in some agronomic characteristics, and information on heterosis values must be further evaluated in the development of rice lines with specific grain size traits and/or agronomic characteristics. In future research, molecular marker analysis could be used in rice production, improvements in rice grain quality, and the evaluation of selected lines to select specific desired traits for genetic improvement. The knowledge of the degrees of heterosis between PSM and IR24 revealed in this study will provide useful information for rice hybridization. Future research must include the F₂ generation in order to improve photoperiod insensitivity and grain quality traits in Paw San crosses. Molecular marker analysis can also be used to select desired traits and evaluate grain quality from the F₁ generation to backcrossed generations.

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