Dry Matter Accumulation and Heterosis in Photosynthesis in F₁ Hybrids of Rice (Oryza sativa L.)

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Abstract Photosynthetic rate, dry matter accumulation, leaf area and number of tillers for F₁ hybrids of sixteen cross-combinations and the parental cultivars of rice (Oryza sativa L.) were studied at the vegetative growth stage. Most of the F₁ hybrids showed a positive heterosis value for these characters. Average heterosis value for the CO₂ exchange rate in sixteen F₁ hybrids was 1.08. The magnitude of heterosis for the CO₂ exchange rate depended on the F₁ cross-combinations. The resulting F₁ hybrids from cultivars with a similar number of tillers per plant, showed a positive heterosis value while the F₁ hybrids derived from cultivars with wide differences in the tillering ability did not show heterosis for the number of tillers per plant. Heterosis for dry weight accumulation was correlated with heterosis for leaf area.

Key words Dry matter, F₁ hybrid, Heterosis, Photosynthesis, Rice

Introduction

Heterosis is the biological phenomenon whereby an F₁ hybrid of two genetically dissimilar parents shows an increased vigour at least over the performance of the mid-parents. The economic significance of heterosis in crop plants was widely appreciated after the successful introduction of hybrid maize varieties. In rice, heterosis was first observed by JONES 6) but its commercial exploitation was not explored until the 1960s. Research on the commercial exploitation of heterosis in rice was carried out in different countries.17,18) MURAYAMA et al.14) discussed the advantages of utilizing hybrid vigour under field conditions. F₁ hybrids in rice attracted an increasing attention from rice breeders and agronomists after its successful commercial introduction in China during the 1970s. Grain yield increase of 20-30% over the best check varieties in replicated yield trials was reported in China8).

Production of hybrid seeds in this strictly self pollinated crop was difficult. However, with the development of cytoplasmic male sterile lines it became easier to produce hybrid seeds in bulk and large-scale adoption was promoted. During the period 1976-1991, hybrid rice varieties helped China to increase its production by nearly 200 mt21). In 1991, hybrid rices were grown over about 17 mha in China, accounting for approximately 55% of the total rice area, and con-
tributing to 66% of the total Chinese rice production and 20% of the total rice production of the world.

Although heterosis for grain yield in rice has been studied relatively extensively from the viewpoint of genetics, the physiological and morphological characters responsible for the superiority of F1 hybrids are poorly documented. Biological productivity of crop plants depends on photosynthesis and the factors limiting the process. There are discrepancies regarding the photosynthetic ability of F1 hybrids in terms of CO₂ exchange rate, as some authors⁹,¹³ reported higher values unlike other authors⁷. AKITA¹⁰ stated that heterosis for the photosynthetic rate and respiration in F₁ hybrid rice was not fully understood. Therefore, it seemed important to study the heterosis for the CO₂ exchange rate (CER) and its relation to dry matter production along with other physio-morphological characters of the F₁ hybrids. The present study was undertaken to investigate the photosynthetic ability of the F₁ hybrids in terms of CER and some of the important physiological and morphological differences between the F₁ hybrids and their parent cultivars in relation to dry matter production.

Materials and Methods

The experiment was conducted in a glasshouse of the Faculty of Agriculture, University of the Ryukyus, Okinawa, Japan (26°10’ N and 127°45’ E). Parental cultivars Akebono, Chiyonishiki, Suzunari, Murasaki-ine, Zhenshan 97A, Zhenshan 97B and Zenith were taken from the seed stock of the Faculty of Agriculture, University of the Ryukyus to produce F₁ seeds during the first season of 1995. Zhenshan 97A and Zhenshan 97B are cytoplasmic male sterile (cms) line and maintainer, respectively, developed in China, the others are semidwarf Japonica cultivars except for Zenith, which is a tall American variety. Chiyonishiki is commercially cultivated in Okinawa. Akebono and Zenith showed a high general combining ability (gca) for different morphological characters, while Murasaki-ine was found to have a high gca for the CO₂ exchange rate in previous observations at the Faculty of Agriculture, University of the Ryukyus. F₁ cross combinations, shown in Table 1 and used in this study, were selected on the basis of their performance in previous years at the Faculty of Agriculture, University of the Ryukyus.

Seeds of parent cultivars and F₁ hybrids were treated with systemic fungicide ‘Benlate Τ’ and were incubated for 24 hours at 30 °C. Soil on the seed bed was mixed with inorganic fertilizers at the following rates: N, 2.8 g/m²; P₂O₅, 2.1 g/m² and K₂O, 2.4 g/m². Pregermminated seeds were sown on nursery boxes (60×35×8 cm) on 12 August, 1995. After three weeks, seedlings of each parental cultivar and F₁ hybrid were individually transplanted into 0.02 m² Wagner pots containing Shimajiri Mahji (Dark Reddish) soil distributed in the Okinawa region. In order to improve its structure and moisture holding properties, organic matter (3 kg/m²) was added and the resulting mixture in the pots was puddled in order to simulate wetland paddy field conditions. A basal inorganic fertilizer was applied at the following rates: N, 7.5 g/m²; P₂O₅, 6 g/m²; K₂O, 6.7 g/m². An additional application was also made as top-dressing 25 days after transplanting (at the active tillering stage) at the following rates: N 4.5 g/m²; P₂O₅, 3.6 g/m²; and K₂O, 4.2 g/
m². The pots were watered daily so that at least five cm of standing water remained throughout the trial. The glasshouse was well ventilated in order to maintain natural temperature fluctuations; no artificial lighting was used.

To minimize border effects, guard rows of the variety Suzunari were used. Although the crop was free from insect and disease infestation, as a precautionary measure, Diazinon (granular form) and Kelthen EC 40 were applied two weeks after transplanting. The experiment was laid out in a completely randomized design.

Carbon dioxide exchange rate (CER) was measured fifty-five days after sowing using a portable infra red CO₂ gas analyzer and Parkinson leaf chamber specifically designed for physiological studies on grass leaves. Three halogen lamps of 1000 W were used to maintain a light intensity of 1000 μ moles m⁻²sec⁻¹ during the evaluation period. An atmospheric air flow rate of 400 ml min⁻¹ inside the chamber was achieved using an Asum-2 air supply unit.

Two plants from the parent cultivars and F₁ hybrids, each were randomly selected for CER estimation. Two topmost fully expanded leaves were used, and three readings were taken from each leaf. Means of these readings were used to compare the different F₁ hybrids and inbred parent cultivars.

Six plants were sampled at the age of ninety days so that leaf area, dry matter accumulation and the number of tillers per plant could be estimated. Leaf area measurements were carried out using an automatic area meter (AAM Hayashi Denko, Japan). To avoid shrinkage and leaf curling, leaf area measurement was taken immediately after the leaf removal. Dry matter accumulation was estimated after drying the plants at 80°C for 48 hours.

Heterosis was expressed as the ratio between the performance of the F₁ hybrid and the mid-parent

\[
\text{heterosis} = \frac{F₁}{\text{Mid-parental value}}
\]

The mid-parental value was calculated as the average performance of the female parent and the male parent

<table>
<thead>
<tr>
<th>Parental combinations</th>
<th>Female parent (P₁)</th>
<th>F₁ hybrid (F₁)</th>
<th>Male parent (P₂)</th>
<th>Sig.</th>
<th>LSD (5% level)</th>
<th>Heterosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suzunari × Zenith</td>
<td>21.4±1.22</td>
<td>21.9±1.24</td>
<td>16.9±0.63</td>
<td>ns</td>
<td>3.42</td>
<td>1.14</td>
</tr>
<tr>
<td>Suzunari × Chiyonishiki</td>
<td>21.4±1.22</td>
<td>21.4±1.30</td>
<td>23.7±0.75</td>
<td>ns</td>
<td>3.48</td>
<td>0.94</td>
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<tr>
<td>Suzunari × Murasaki-ine</td>
<td>21.4±1.22</td>
<td>23.8±1.05</td>
<td>19.6±1.06</td>
<td>ns</td>
<td>4.50</td>
<td>1.16</td>
</tr>
<tr>
<td>Suzunari × Akebono</td>
<td>21.4±1.22</td>
<td>22.1±2.84</td>
<td>23.2±1.01</td>
<td>ns</td>
<td>6.01</td>
<td>0.99</td>
</tr>
<tr>
<td>Suzunari × Zhenshan 97 B</td>
<td>21.4±1.22</td>
<td>20.2±1.57</td>
<td>18.3±0.84</td>
<td>ns</td>
<td>3.88</td>
<td>1.08</td>
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<td>Akebono × Chiyonishiki</td>
<td>23.2±1.01</td>
<td>24.7±1.03</td>
<td>23.7±0.75</td>
<td>ns</td>
<td>3.08</td>
<td>1.04</td>
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<td>Akebono × Zhenshan 97 B</td>
<td>23.2±1.01</td>
<td>19.2±1.21</td>
<td>18.3±0.84</td>
<td>*</td>
<td>3.30</td>
<td>0.92</td>
</tr>
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<td>23.2±1.01</td>
<td>23.0±1.05</td>
<td>21.4±1.22</td>
<td>ns</td>
<td>3.51</td>
<td>1.03</td>
</tr>
<tr>
<td>Zhenshan 97A × Murasaki-ine</td>
<td>17.5±0.47</td>
<td>23.8±1.08</td>
<td>19.6±1.06</td>
<td>*</td>
<td>2.92</td>
<td>1.28</td>
</tr>
<tr>
<td>Zhenshan 97 A × Akebono</td>
<td>17.5±0.47</td>
<td>24.2±1.42</td>
<td>23.2±1.01</td>
<td>*</td>
<td>3.34</td>
<td>1.18</td>
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<td>Zhenshan 97 B × Murasaki-ine</td>
<td>18.3±0.84</td>
<td>23.6±1.56</td>
<td>19.6±1.06</td>
<td>*</td>
<td>3.81</td>
<td>1.24</td>
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<td>19.4±1.34</td>
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<td>*</td>
<td>3.46</td>
<td>0.93</td>
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<td>Zhenshan 97 B × Chiyonishiki</td>
<td>18.3±0.84</td>
<td>22.1±1.23</td>
<td>23.7±0.75</td>
<td>**</td>
<td>3.08</td>
<td>1.05</td>
</tr>
<tr>
<td>Chiyonishiki × Zhenshan 97 B</td>
<td>23.7±0.75</td>
<td>23.7±1.23</td>
<td>18.3±0.84</td>
<td>**</td>
<td>3.20</td>
<td>1.13</td>
</tr>
<tr>
<td>Murasaki-ine × Akebono</td>
<td>19.6±1.06</td>
<td>23.1±2.43</td>
<td>23.2±1.01</td>
<td>ns</td>
<td>5.24</td>
<td>1.08</td>
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<tr>
<td>Zenith × Akebono</td>
<td>16.9±0.63</td>
<td>20.2±1.45</td>
<td>23.2±1.01</td>
<td>**</td>
<td>3.47</td>
<td>1.01</td>
</tr>
</tbody>
</table>

Average heterosis = 1.08

Number represents mean ± standard error; ns, * and ** indicate statistically nonsignificant and significant at the 5% and 1% levels respectively; Heterosis = Mid-parental value/F₁; Mid-parental value = (P₁ + P₂)/2
mid-parental value = \frac{\text{Female parent} + \text{Male parent}}{2}

Data were analysed using ANOVA techniques described by Clewer and Scarisbrick\(^2\). Relationships between heterosis for different characters were determined by linear regression analysis.

**Results**

The CO\(_2\) exchange rate differed among the parent cultivars and between the F\(_1\) hybrids and parent cultivars (Table 2). Out of sixteen F\(_1\) cross-combinations twelve F\(_1\) hybrids had a CER higher than the mid-parental value. Six F\(_1\) hybrids, namely Suzunari × Zenith, Suzunari × Murasaki-ine, Akebono × Chiyonishiki, Zhenshan 97A × Murasaki-ine, Zhenshan 97A × Akebono and Zhenshan 97B × Murasaki-ine showed a higher CO\(_2\) exchange rate than higher parents of the respective crosses. By comparison, only four F\(_1\) hybrids Suzunari × Chiyonishiki, Suzunari × Akebono, Akebono × Zhenshan 97B and Zhenshan 97B × Akebono were found to display a lower CER than the mid-parent. Average value of heterosis of the sixteen F\(_1\) hybrids was 1.08.

Number of tillers was different between F\(_1\) hybrids and parent cultivars (Fig. 1). In thirteen F\(_1\) cross-combinations the difference in tillering between the F\(_1\) hybrids and their parents was significant. Ten F\(_1\) hybrids produced higher numbers of tillers per plant than the mid-parent, and in seven cross combinations the number of tillers per plant exceeded that of the higher parent. In contrast, six F\(_1\) hybrids produced fewer tillers per plant than the mid-parent. F\(_1\) hybrids of the cross-combinations Suzunari × Zenith, Suzunari × Murasaki-ine, Zhenshan 97A × Murasaki-ine, Zhenshan 97B × Murasaki-ine, Murasaki-ine × Akebono, Zenith × Akebono had fewer tillers per plant than the mid-parent, while F\(_1\) hybrids of the cross-combinations Suzunari × Chiyonishiki, Zhenshan 97B × Chiyonishiki, and Chiyonishiki × Zhenshan 97B showed a slightly higher number of tillers than the respective mid-parents. In these F\(_1\) cross-combinations, the differences between female and male parents were relatively more pronounced (Fig. 1). On the contrary, the magnitude of heterosis from the F\(_1\) hybrids of the cross-combinations of Suzunari × Akebono, Suzunari × Zhenshan 97B, Akebono × Zhenshan 97B, Akebono × Suzunari, Zhenshan 97A × Akebono, Zhenshan 97B × Akebono was higher than in the previous cross-combinations. In these combinations the parental cultivars had a higher number of tillers per plant and their tillering ability did not differ appreciably.

Leaf area per plant varied between F\(_1\) hybrids and parent cultivars and among the parent cultivars (Fig. 2) and significant differences were found for all the cross-combinations. In fourteen F\(_1\) hybrids the leaf area was larger than that of the respective mid-parent, and in eleven F\(_1\) hybrids the leaf area was larger than that of the higher parent of the respective cross-combinations.

Dry matter accumulation in the F\(_1\) hybrids differed from that of their parents and there was also a difference among the parents (Fig. 3). In fourteen F\(_1\) cross-combinations the difference in dry matter accumulation was significant. In fifteen F\(_1\) hybrids dry matter accumulation was larger than that of the respective mid-parents and in eleven F\(_1\) cross-combinations dry matter accumulation exceeded that of the highest parents of the respective crosses. A slight difference between the F\(_1\) hybrids from the reciprocal cross-combinations of Suzunari × Akebono, Akebono × Zhenshan 97B and Chiyonishiki × Zhenshan 97B in the CO\(_2\) exchange rate (Table 2), number of tillers per plant (Fig. 1), leaf area (Fig. 2) and dry matter accumulation (Fig. 3) was observed.

Linear regression analysis indicated that the expression of heterosis for dry matter accumulation depended \((r=0.801)\) on heterosis for leaf area (Fig. 4). Correlations between heterosis for the number of tillers per plant and dry matter accumulation \((r=0.514, \text{Fig. 5})\) and between leaf area and number of tillers \((r=0.644, \text{Fig. 6})\) were also significant.

**Discussion**

The small variations in CER (Table 2) among the cultivars used in this experiment were in agreement with the findings of Nakamine\(^3\) who demonstrated the existence of genetic variations in the photosynthetic capacity of indigenous Asian rice varieties. In the present glasshouse experiment, an aver-
Fig. 1. Comparison of tiller production in the F1 hybrids and parent cultivars.

Ake = Akebono; Chiyo = Chiyonishiki; Mura = Murasaki-ine; Suzu = Suzunari; 97A = Zhenshan 97A; 97B = Zhenshan 97B; Zen = Zenith. Bars represent LSD at the 5% level.

A female parent; ■ male parent; ■ F1 hybrid; □ mid-parental value (Mid-parental value was not included in the statistical analysis).

Averagage heterosis value of 1.08 was observed for this trait. Murayama et al.13) using seven F1 hybrids recorded an average heterosis value of 1.17. By comparison, in earlier studies, Kabaki et al.7) did not find any heterosis for leaf photosynthesis of F1 hybrids in rice. They argued that the lower photosynthetic rate in the F1 hybrids was due to the decrease in the nitrogen content in the leaf caused by higher heterosis for leaf area. Murayama et al.13) observed a significant positive correlation between the leaf nitrogen
content and photosynthesis. In the present experiment, the plants were well fertilized, each Wagner pot receiving more than 1.5 times the concentration of fertilizer normally applied in the Okinawa region and the CER measurement was carried out ten days after the second application of the fertilizer. Thus it is unlikely that nitrogen had been in short supply when the CER measurements were taken. Consequently, some expression of heterosis was evident in most of the F1 rice hybrids although a higher degree of heterosis
for leaf area was also observed in the present experiment. It was observed in parental combinations having one of the parents as Murasaki-ine, that heterosis was always present and its magnitude was relatively larger than in other F1 hybrids (Table 2). Genetic analysis of single leaf photosynthesis by NAGAMINE and HAYASHI et al. revealed that...
single leaf photosynthesis in F₁ rice hybrids was closer to that of the lower parent and that there was no heterosis for this character. They suggested that single leaf photosynthesis in rice was mainly controlled by a single major gene and that low photosynthetic rate was dominant. Results of the present experiment suggest that the photosynthetic rate depended on the cross-combinations and that under adequate nitrogen supply, heterosis was more likely to be detected. Further studies on the photosynthetic rate of rice F₁ hybrids should be carried out in a range of nitrogen levels and environmental conditions.

High heterosis value for tillering was observed in some F₁ hybrids in the present experiment (Fig. 1). Higher tillering ability in rice F₁ hybrids was also reported by other authors. However, the results of the present study are not consistent with those of Virmani et al. who observed a lower number of tillers per plant in the F₁ hybrids compared with the mid-parents. Results for tillering behaviour suggested that the magnitude of heterosis in the F₁ hybrids became lower when the parents differed markedly in their tillering ability. By comparison, the F₁ hybrids of parents which had a higher tillering ability but did not differ widely in the number of tillers per plant had a higher heterosis value for tillering. In cereal crops at the time...
Fig. 6. Relationship between heterosis for number of tillers and heterosis for leaf area in F₁ hybrids of rice.

\[ y = 0.546x + 0.566 \quad r = 0.644^{**} \]

Heterosis for leaf area

Heterosis for number of tillers

of breeding F₁ hybrids for a higher number of tillers, this fact should be considered as an important factor. In most cereal crops enhanced tillering promotes early canopy cover which is often important for natural weed control and the interception of photosynthetically active radiation.

The correlation coefficients between heterosis in leaf area and number of tillers (Fig. 6) were higher than the values calculated for the relationship between heterosis for the number of tillers and dry matter accumulation (Fig. 5). Correlation between heterosis for dry matter accumulation and heterosis for leaf area (Fig. 4) was higher than that between heterosis for dry matter accumulation and number of tillers. The order of magnitude of the correlation coefficients suggests that the expression of heterosis for dry matter accumulation primarily depended on heterosis for leaf area, and heterosis for leaf area depended on heterosis for the number of tillers per plant. In rice, since tillers and leaves emerge from the same node on the culm⁻ a larger leaf area may be due to a higher number of leaves per plant. However, in some cross-combinations, for example Suzunari × Zenith, Suzunari × Murasaki-ine and Zhenshan 97A × Murasaki-ine and Zhenshan 97A × Murasaki-ine the heterosis value for tillering was negative (Fig. 1) while the heterosis value for the leaf area was positive (Fig. 2). Thus it appears that the number of leaves as well as individual leaf size contribute to the increase of the leaf area in the F₁ rice hybrids. Cytoplasmic effect was manifested by the differences in characters among the reciprocal crosses such as Suzunari × Akebono, Akebono × Zhenshan 97B and Chiyonishiki × Zhenshan 97B. Since Zhenshan 97B is the maintainer of the cms line Zhenshan 97A, the genetic make up of the F₁ cross-combinations Zhenshan 97A × Murasaki-ine and Zhenshan 97B × Murasaki-ine is identical. Similarly, the genetic make up for Zhenshan 97A × Akebono and Zhenshan 97B × Akebono is also the same. However, the difference in the expression of different characters between them may be attributed to the cytoplasmic effect.

It was observed that F₁ hybrids with a higher heterosis value for dry matter accumulation did not always show a higher heterosis value for the CO₂ exchange rate. However, it appears that the expression of heterosis for dry matter accumulation did not depend on heterosis for the CO₂ exchange rate. MURAMOTO et al.¹²) observed that in cotton and some grass species leaf area development was much more important than the CO₂ exchange rate. MILTHORPE ¹⁰) also stressed the importance of the rate of leaf area development as a factor affecting the growth rate in crops and minimized the importance of the leaf photosynthetic rate.

The main objective of this study was to investigate some of the important physiological and morphological differences between
F1 hybrids and their parent cultivars. Earlier studies using a range of species had shown that during vegetative development and under non-limiting environmental conditions the rate of dry matter accumulation by crops was proportional to the amount of radiation intercepted, e.g., cereals\textsuperscript{3}), apples and potatoes\textsuperscript{11}). In the present study it appears that a larger leaf area enabled the F1 hybrids to improve radiation interception and may have led to a larger dry matter accumulation. Differences in leaf area resulting from hybridization are probably more important for larger dry matter accumulation than other effects on photosynthetic capability like CER.

References