Sterility of Thermo-Sensitive Genic Male Sterile Line, Heterosis for Grain Yield and Related Characters in F1 Hybrid Rice (Oryza sativa L.)

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Abstract : The thermo-sensitive genic male sterile (TGMS) T29s line was sterile when exposed to daily mean temperatures of 24.1°C or above during the critical stage (from 15 to 11 days before heading). It was completely sterile for both pollen and spikelet when the plant headed from June 16 to November 7 in Okinawa, because the temperature exceeded 24.1°C. The heterosis of the F1 hybrids between the T29s line and seven indica cultivars was examined. Most of the F1 hybrids showed positive heterosis over the male parent for grain yield per plant and the number of spikelets per panicle, and one of them manifested heterosis for grain yield over a F1 hybrid from a cytoplasmic male sterile (CMS) line. Dry matter accumulation per plant at the panicle initiation stage in most F1 hybrids was higher than that in the respective male parent or mid-parent, and it was correlated with a larger number of tillers and leaf area per plant. All F1 hybrids produced a larger number of panicles per plant than their respective male parent. Positive heterosis over the male parent for the number of filled grains per panicle, 1000-grain weight and harvest index was obtained in several F1 hybrids. A positive correlation was found between grain yield and the dry matter accumulation per plant in F1 hybrids. Both the larger number of panicles per plant and the larger number of spikelets per panicle were more important for the positive heterosis for grain yield in F1 hybrids rather than the higher 1000-grain weight. Among the yield attributes, a larger number of filled grains per panicle mainly contributed to a higher grain yield of F1 hybrids.

Key words : Dry matter, F1 hybrid, Grain yield, Heterosis, Sterility.

Hybrid rice shows 15-20% higher grain yield than inbred varieties in China and other countries (Virmani, 1994; Yuan, 1997). The cytoplasmic male sterile (CMS) system, which involves three lines (CMS, maintainer and restorer), is the most widely used for producing F1 hybrid seeds. However, this system is cumbersome because CMS lines require specific maintainer and restorer lines, thereby restricting the choice of parent, and its use for F1 seed production is costly. On the other hand, utilization of a CMS system in the long run would make the hybrid crops vulnerable to destructive disease or insect damage. The thermo-sensitive genic male sterile (TGMS) system is considered to be more efficient than the CMS system in the tropics (Yuan, 1987; Lu et al., 1994). Male sterility expression in TGMS lines is controlled by single recessive nuclear gene interacting with temperature (Borkakati and Virmani, 1996). Seeds of TGMS lines are multiplied by self-pollination when they are exposed to a certain temperature range during their critical stage from 15 to 11 days before heading (Wu, 1997). Because commercial F1 hybrid seeds are produced by pollination of a TGMS line with any fertile line, the TGMS system is called a two-line method (Yuan, 1987). Several TGMS lines have been developed and applied in F1 hybrid seed production. Among them, the T29 line is an indica cultivar developed in Vietnam (Tram, 1998). The F1 hybrids, produced from the TGMS system, showed a grain yield advantage of 5-10% over those from the CMS system (Lu et al., 1994; Lopez and Virmani, 2000). There are many reports on positive heterosis for grain yield in F1 hybrids from CMS lines (Virmani et al., 1981; Govinda and Siddiq, 1986; Yao et al., 2000). However, there are very few reports on heterosis in F1 hybrids from TGMS lines. Therefore, we examined the sterility of the TGMS T29 line and heterosis for grain yield and related characters in F1 hybrids from the T29 line.
Materials and Methods

Pot experiments were conducted in a glasshouse at the Faculty of Agriculture, University of the Ryukyus, Okinawa, Japan (26° 10’ N and 127° 45’ E) in 2001.

1. Sterility experiment

Seeds of the TGMS T29s line were treated with a systemic fungicide "Benlate" for 24 hours and were incubated at 30°C for 48 hours for germination. Germinated seeds were sown on seedling trays (60 × 35 × 8 cm) on March 8, 18 and 28 in the first cropping season, and on August 28, and September 8 and 18 in the second season. Organic matter " Minori" at the rate of 3 kg m⁻² and a basal dose of chemical fertilizers N, P₂O₅ and K₂O at the rate of 7.5, 15.0 and 12.5 g m⁻², respectively, were applied and mixed properly with Shimajiri Mahji (dark reddish soil in Okinawa) in 0.02 m² Wagner pot one day before transplanting. In each sowing time, 15 seedlings each with three to four leaves were transplanted singly into the pots. N was top-dressed at 10 and 20 DAT at the same rate of 5.0 g m⁻², and after the panicle initiation was observed N and K₂O were applied at the same rate of 4.5 g m⁻². The pots were watered daily and the glasshouse was well ventilated to maintain natural temperature fluctuation. Ten plants with panicles emerged were randomly selected at the beginning of heading and four days later, and used to estimate sterility. These plants were also used for measuring agronomical characters. Leaf area was measured with an automatic area meter (Li-3100, Li-Cor, USA) immediately after sampling. DM accumulation was measured after oven drying at 80°C to a constant weight.

2. Heterosis experiment

Seven F₁ hybrids from the crosses between the T29s line and indica cultivars (D101, R68, Que99, CR203, Takanari, Dular and Dhaka) were used to estimate heterosis for grain yield and related characters. Shanyou 63, a high yielding F₁ hybrid released in China from CMS line (Zhenshan 97A/Minghui 63), was used as a check variety. Seeds of the F₁ hybrids and parent cultivars were sown on August 5. Fifteen seedlings of each parent cultivar and F₁ hybrids were planted. The procedures for planting and fertilization were the same as those in the sterility experiment. The experiment was laid out in a completely randomized design in which one plant of each F₁ hybrid and parent cultivars was considered as a replication (Gomez and Gomez, 1984).

Four plants of each parent cultivar and F₁ hybrids were randomly sampled for measuring several morphological characters after the panicle initiation was observed. The panicle initiation in the main culms was observed following the method of Yuan (1985). Leaf area was measured with an automatic area meter (Li-3100, Li-Cor, USA) immediately after sampling. DM accumulation was measured after oven drying at 80°C to a constant weight.

At ripening, grain yield and several characters contributing to yield were assessed on four randomly selected plants of each F₁ hybrid and parent cultivars. Plant height was measured from the base to the tip of the highest panicle. Grain yield was measured after threshing, cleaning and drying. The number of spikelets and number of filled grains per panicle were determined for three randomly selected panicles per plant.

Data were analyzed using ANOVA with Duncan test at a 5% significant level using SAS program (SAS,
The percentage and ratio data were subjected to arc sine transformation prior to statistical analysis.

Heterosis over the male parent (Hm), the mid-parent (Ht), the better parent (Hb) and check variety (Hs) was expressed as the ratio of the performance of the F1 hybrid to that of the male parent, mid-parent, better parent and Shanyou 63 (SY63), respectively.

Because the T29' line was completely sterile at ripening, Hb and Ht were not calculated for the number of filled grains per panicle, 1000-grain weight, harvest index, spikelet fertility and grain yield per plant.

Results

1. Sterility experiment

   (1) Sterility and agronomical characters of the TGMS T29' line (Table 1)

   The percentage of fertile pollen grains and filled grains in the T29' line was 4.9-38.1 and 2.9-42.8, respectively, when the plant headed on May 27, 31 and June 8, 12 in the first cropping season, and was 11.9-23.5 and 2.2-35.8, respectively, when the plant headed on Nov 11, 19 and 23 in the second cropping season. Both pollen and spikelet of the T29' line were completely sterile when the plant headed on June 16 in the first cropping season and on Nov 3, 7 and 15 in the second season. The T29' line had 5.7% of fertile pollen grains but no filled grains when the plant headed on June 4.

   The growth period from sowing to heading of the T29' line was 74-84 and 62-68 days in the first and second cropping season, respectively (Table 1). The T29' line possessed 12-13 leaves per main stem and was 65.2-71.1 cm in height in the first cropping season, whereas the value of these traits was 11 and 59.1-62.8, respectively, in the second season. The number of spikelets per panicle of T29' line was 145.6-185.3 and 134.3-141.7 in the first and second cropping season, respectively. These results indicate that the values of all the agronomical characters in the T29' line were lower in the second season.

2. Heterosis experiment

   (1) Dry matter accumulation at the panicle initiation stage (Table 2)

   In this study, the panicle initiated earlier in F1 hybrids than in their respective male parent, but later than in the TGMS line. Dry matter (DM) accumulation

![Table 2. Dry matter accumulation per plant of F1 hybrids and their parent cultivars at the panicle initiation stage.

| F1 hybrid/Parent | DM (g plant⁻¹) | Number of tillers plant⁻¹ | Leaf area (cm² plant⁻¹) |
|------------------|----------------|--------------------------|------------------------|
|                  | Hm  | Ht  | Hb  | Hs  | Hm  | Ht  | Hb  | Hs  | Hm  | Ht  | Hb  | Hs  |
| T29'/D101        | 12.4| 1.65| 1.51| 1.40| 1.08| 15.3| 1.34| 1.22| 1.13| 1.26| 1183.0| a | 1.43| 1.41| 1.40| 1.06|
| T29'/R68        | 10.7| 1.54| 1.35| 1.20| 0.92| 13.3| 1.26| 1.08| 0.95| 1.06| 1000.9| bc | 1.39| 1.28| 1.18| 0.89|
| T29'/Que99      | 9.1 | 1.45| 1.20| 1.02| 0.79| 13.8| 1.20| 1.08| 0.98| 1.10| 903.3| cd | 1.19| 1.12| 1.07| 0.81|
| T29'/CR203      | 9.2 | 1.25| 1.13| 1.04| 0.80| 14.3| 1.33| 1.15| 1.02| 1.14| 984.2| b | 1.19| 1.17| 1.16| 0.88|
| T29'/Takanari   | 9.0 | 0.93| 0.97| 0.93| 0.78| 13.3| 1.06| 1.00| 0.95| 1.06| 979.8| bc | 1.00| 1.08| 1.00| 0.88|
| T29'/Dular      | 8.4 | 0.89| 1.16| 0.94| 0.73| 11.5| 1.53| 1.07| 0.82| 0.92| 819.7| def| 1.37| 1.13| 0.97| 0.73|
| T29'/Dhaka     | 8.9 | 1.07| 1.08| 1.00| 0.77| 14.3| 1.14| 1.08| 1.02| 1.14| 919.7| cd | 1.22| 1.15| 1.08| 0.82|
| D101            | 7.6 | 0.86| 0.86| 0.77| 0.77| 11.8| 0.95| 0.95| 0.95| 0.95| 826.1| de | 1.00| 1.00| 1.00| 1.00|
| R68             | 6.9 | 0.67| 0.67| 0.71| 0.71| 11.8| 0.95| 0.95| 0.95| 0.95| 817.8| f | 1.00| 1.00| 1.00| 1.00|
| Que99           | 6.3 | 0.63| 0.63| 0.63| 0.63| 11.8| 0.95| 0.95| 0.95| 0.95| 806.4| ef | 1.00| 1.00| 1.00| 1.00|
| CR203           | 7.4 | 0.68| 0.68| 0.68| 0.68| 11.8| 0.95| 0.95| 0.95| 0.95| 818.4| de | 1.00| 1.00| 1.00| 1.00|
| Takanari        | 9.7 | 0.75| 0.75| 0.75| 0.75| 11.8| 0.95| 0.95| 0.95| 0.95| 810.4| ef | 1.00| 1.00| 1.00| 1.00|
| Dular           | 5.6 | 0.56| 0.56| 0.56| 0.56| 11.8| 0.95| 0.95| 0.95| 0.95| 810.4| ef | 1.00| 1.00| 1.00| 1.00|
| Dhaka           | 7.7 | 0.67| 0.67| 0.67| 0.67| 11.8| 0.95| 0.95| 0.95| 0.95| 810.4| ef | 1.00| 1.00| 1.00| 1.00|
| T29'            | 8.9 | 0.75| 0.75| 0.75| 0.75| 11.8| 0.95| 0.95| 0.95| 0.95| 810.4| ef | 1.00| 1.00| 1.00| 1.00|
| Shanyou63       | 11.5| 0.68| 0.68| 0.68| 0.68| 11.8| 0.95| 0.95| 0.95| 0.95| 810.4| ef | 1.00| 1.00| 1.00| 1.00|

Mean of F1 hybrids: 9.5; mean of male parents: 7.5; mean of heterosis: 1.26. Mean within a column followed by the same letter (s) are not significantly different at 5% level by DMRT.

DM, dry matter; Hm, heterosis over the male parent; Ht, heterosis over the mid-parent; Hb, heterosis over the better parent; Hs, heterosis over check variety; *: Significant and positive heterosis at 5% level by DMRT.
was measured in each parent cultivar and F1 hybrid at different times depending on the panicle initiation. Six F1 hybrids showed significantly positive heterosis over the male parent (Hm) for DM accumulation per plant (1.17-1.65), number of tillers per plant (1.14-1.53) and leaf area per plant (1.19-1.43). None of the F1 hybrids significantly exceeded the DM accumulation of SY63. The positive heterosis over mid-parent (Ht) values for DM accumulation per plant and number of tillers per plant in six of F1 hybrids were 1.08-1.51 and 1.07-1.22, respectively. All F1 hybrids showed a positive Ht (1.08-1.41) for leaf area per plant. A significantly positive correlation was found between Ht for DM accumulation per plant and Ht for number of tillers per plant (p = 0.0234), and between Ht for DM accumulation per plant and Ht for leaf area per plant, (p = 0.013) (Fig. 1).

(2) Days to heading, number of leaves per main stem and plant height (Table 3)

All F1 hybrids showed negative Hm for days to heading (0.79-0.98), number of leaves per main stem (0.90-0.93) and plant height (0.87-0.97) (Table 3). The negative Hs for days to heading and number of leaves per main stem were obtained in five F1 hybrids. Three
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F1 hybrids showed negative Hs (0.92-0.97) for plant height.

3. Grain yield and yield attributes (Table 4, 5)

The significant and positive Hm for number of panicles per plant (1.06-1.61), number of spikelets per panicle (1.09-1.26), number of filled grains per panicle (1.12-1.18), 1000-grain weight (1.07-1.11), harvest index (1.11-1.16) and grain yield per plant (1.12-1.67) was obtained in 7, 5, 3, 2, 3 and 5 of F1 hybrids, respectively, among 7 F1 hybrids estimated. None of F1 hybrids showed significantly positive Hm for spikelet fertility.

The positive Ht for number of panicles per plant was observed in five F1 hybrids and one of them showed significantly positive Hb (1.13). All F1 hybrids showed positive Ht (1.03-1.22) for number of spikelets per panicle.

The significant and positive Hs values for number of panicles per plant (1.08-1.18) and number of spikelets per panicle (1.15-1.18) were found in 3 and 2 F1 hybrids, respectively. None of the F1 hybrids showed significantly positive Hs for 1000-grain weight, harvest index and spikelet fertility. One of F1 hybrids (T29'/D101) showed significantly positive Hs for grain yield per plant (1.17) and number of filled grains per panicle (1.13).

The number of spikelets per panicle in the F1 hybrids was significantly and positively correlated with the number of spikelets per panicle in their respective male parent (p < 0.001) (Fig. 2).

A significantly positive correlation was found between grain yield per plant and DM accumulation per plant at the panicle initiation stage in F1 hybrids (p < 0.001) (Fig. 3A). A significantly positive correlation was observed between grain yield per plant and the number of panicles per plant (p < 0.001) (Fig. 3B), and between grain yield per plant and 1000-grain weight (p = 0.023) (Fig. 3E) when the values for the F1 hybrids and male parent cultivars were computed together, but no significant correlation when the values for the F1 hybrids were computed separately. A significantly positive correlation was observed between grain yield per plant and the number of spikelets per panicle (p < 0.001), and between grain yield per plant and the number of filled grains per panicle (p < 0.001) in both F1 hybrids and male parent cultivars (Fig. 3C, D). Grain yield per plant was significantly and positively correlated with harvest index (p = 0.0021) and spikelet fertility (p = 0.0497) in the F1 hybrids (Fig. 3F, G).

Discussion

1. Sterility of TGMS line

No filled grains were produced in the T29’ line when the plant headed on June 4 when the daily mean temperature during the critical stage (from 15 to 11 days before heading) was 24.1°C. Both pollen grains and spikelets of T29’ line showed more than 2.2% fertility when the plant headed on the days (May 27, 31; June 8, 12 and Nov 11, 19, 23) with temperatures below 24.1°C during the critical stage. On the contrary, the T29’ line was completely sterile for both pollen and spikelet when the plant headed on the days (June 16 and Nov 3, 7, 15) with mean temperatures of 24.4°C or above during the critical stage (Table

![Fig. 2. Correlation between the number of spikelets per panicle in F1 hybrids and the number of spikelets per panicle in their respective male parent. ***: Significant at 0.1% level.](image-url)
1). These results indicate that the T29' line changed from fertile to sterile when the plant exposed to daily mean temperatures of 24.1°C or above during the critical stage, which is similar to the result obtained by Tram (1998). The critical stage of four days with mean temperatures below 24.1°C rarely appears during the period from June 16 to Nov 7 in Okinawa. Therefore, the T29' line may be completely sterile when the plant flowers any time during this period.

2. **Heterosis in F1 hybrids from TGMS line**

Both positive Hm and Ht for dry matter accumulation per plant were obtained in most F1 hybrids at the panicle initiation stage (Table 2). This is in agreement with the previous reports on different F1 hybrids (Khan et al., 1998b; Sarker et al., 2001). Ht for DM accumulation per plant was correlated with both Ht for number of tillers per plant and Ht for leaf area per plant (Fig. 1). This suggested that the higher DM accumulation per plant of F1 hybrids was due to both the larger number of tillers and the larger leaf area per plant.

All F1 hybrids showed a negative Hm for days to heading (Table 3), which was reported in different F1 hybrids (Singh et al., 1980; Virmani et al., 1981; Murayama and Sarker, 2002). Five of the F1 hybrids showed negative Hs for days to heading and three of them showed negative Hs for plant height (Table 3), suggesting that one of the advantages of using the TGMS line is easier of producing F1 hybrids with both the desirable characteristics of early maturity and short plant.

In the present experiment, all F1 hybrids from the TGMS line showed positive Hm and most of them showed positive Ht for number of panicles per plant. This was not found in the previous reports using F1 hybrids from CMS lines or inbred varieties (Virmani et al., 1981; Ponnuthurai et al., 1984; Khan et al., 1998a). This might be due to the fact that the F1 hybrids inherited a high tillering potential from the TGMS line.

Most F1 hybrids showed positive Hm and Ht for the number of spikelets per panicle and some of them showed positive Hm for number of filled grains per panicle (Table 5). A similar result has been reported by others (Murayama et al., 1974; Khan et al., 1998b; Yao et al., 2000). It was noted that the F1 hybrid T29'/D101 and T29'/R68 showed a greater yielding potential by means of the larger number of spikelets per panicle than SY63 (Table 4, 5). The number of spikelets per panicle in F1 hybrids positively correlated with the value in their respective male parent (Fig. 2). Therefore, the number of spikelets per panicle in the male parent must be increased to produce F1 hybrids with a higher value of this trait.

The present experimental results indicate that most F1 hybrids showed positive heterosis over the male parent for grain yield per plant (Table 5). A
positive correlation was found between grain yield per plant and DM accumulation per plant at the panicle initiation stage in F1 hybrids (Fig. 3A), suggesting that the higher DM accumulation per plant contributed to heterosis for grain yield per plant (Virmani, 1994; Murayama and Sarker, 2002).

A positive Hm for grain yield per plant was found together with both positive Hm for number of spikelets per plant and positive Hm for number of spikelets per panicle in most F1 hybrids (Table 5). Several F1 hybrids showed a positive Hm for 1000-grain weight, but the value was low (Table 5). Although the F1 hybrid T29'/Takanari showed the highest Hm value for 1000-grain weight, it failed to attain heterosis for grain yield per plant due to non-significant heterosis for number of spikelets per panicle. These results indicated that both the larger number of spikelets per plant and the larger number of spikelets per panicle more powerfully contributed to heterosis for grain yield in F1 hybrids rather than the heavier 1000-grain weight. This finding using F1 hybrids from TGMS line is in agreement with the previous reports on F1 hybrids from CMS lines (Govinda and Siddiq, 1986) or inbred varieties (Singh et al., 1980; Murayama and Sarker, 2002).

In the F1 hybrids, grain yield per plant did not significantly correlate with the number of spikelets per plant or 1000-grain weight (Fig. 3B, E). However, both the correlations of grain yield per plant with the number of spikelets per panicle and the number of filled grains per panicle were highly significant (Fig. 3C, D). The F1 hybrid T29'/D101 showed positive Hs for grain yield per plant because it manifested positive Hs for the number of spikelets per plant, number of spikelets per panicle and especially for the number of filled grains per panicle. These results indicate that the larger number of filled grains per panicle, regarded as a component of the number of spikelets per panicle, mainly contributed to the higher grain yield per plant of F1 hybrids (Virmani et al., 1981; Kabaki, 1993; Murayama and Sarker, 2002).

The F1 hybrid T29'/Que99 and T29'/Takanari failed to attain heterosis for grain yield because of the lower spikelet fertility. A positive correlation between grain yield per plant and spikelet fertility was found in the F1 hybrids (Fig. 3G). Therefore, it is important to increase spikelet fertility for improving the grain yield of F1 hybrids.

Our results indicate that the TGMS T29' line changed from fertile to sterile when the plant was exposed to daily mean temperatures of 24.1°C or above during the critical stage. The T29' line can be used for F1 hybrid seed production during the period from June 16 to Nov 7 in Okinawa, because the plants that headed during this period were completely sterile for both pollen and spikelet. Most of the F1 hybrids from the T29' line showed positive heterosis over the male parent for grain yield per plant and one of them (T29'/D101) exceeded the value of the CMS F1 hybrid. The heavier DM accumulation per plant in most F1 hybrids at the panicle initiation stage due to both the larger number of tillers per plant and the larger leaf area per plant contributed to heterosis grain yield per plant of the F1 hybrids. The positive heterosis for grain yield in the F1 hybrids was also due to both the larger number of panicles per plant and the larger number of spikelets per panicle. F1 hybrid rice has been reported to show a similar trend of heterosis for morphological characters under different cultural conditions (Murayama et al., 1974; Murayama, 1976). Therefore, the positive heterosis for grain yield and related characters in the F1 hybrids from the TGMS T29' line may be attained under the field condition.

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