can be purified genetically and then used in developing hybrids.

V2O A/ADT39 recorded 4.3% spikelet fertility, so pollen parent ADT39 was identified as a potential maintainer for CMS line V2O A. This prospective maintainer line will be used in a back-crossing program to develop new CMS lines.

CMS lines V2O A, IR58025 A, and IR62829 A, which all have the same wild abortive cytoplasm but different nuclear genotypes, differed in maintainer and restorer frequencies. Genetic background of CMS lines seems to influence their maintaining and restoring abilities.

Restoration-maintenance behavior sometimes differed among pollen parents for CMS lines with the same cytoplasmic source, possibly because of nuclear and cytoplasmic interactions between pollen parents and CMS lines or the heterozygosity of pollen parents. ■

## Identifying restorers and maintainers for cytoplasmic male sterile (CMS) lines IR62829 A and IR58025 A in Myanmar

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CMS lines IR62829 A and IR58025 A were crossed with seven high-yielding rice varieties in 1991 wet season at Hmawbi Research Station and CARI at Yezin. $F_1$s and their parents were grown in 3-m rows at 20- × 20-cm spacing in 1992 dry season. Florets from each plant were collected before flowering and stained with KI solution to determine pollen sterility. Pollen sterility percentage was estimated by counting filled and unfilled grains from harvested $F_1$ panicles.

Varieties were classified as effective maintainers of male parents if pollen fertility of the hybrid was less than 5%. Male parents were identified as restorers if pollen fertility of the hybrid was more than 80%. Partial maintainers were those with 6-30% pollen fertility in the hybrid and partial restorers were those with 31-79% pollen fertility.

Varieties Sinekeri 3, BG120 3, Hmawbi 2, and Kyawzeya were found to be effective maintainers for both CMS lines and Sintheingyi, Theedatyin, and IR50, effective restorers. We will use effective maintainer lines as male parents for backcrossing to develop new CMS lines. Effective restorer lines will be used in hybrid seed production next season.

## Identifying restorers and maintainers for cytoplasmic male sterile (CMS) lines IR58025 A and IR62829 A in Vietnam

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We identified restorers and maintainers for promising CMS lines IR58025 A and IR62829 A in Cuu Long Delta of Vietnam. Improved lines and varieties adapted to Cuu Long Delta were crossed with IR58025 A or IR62829 A. $F_1$ hybrids were grown in the field during 1992 wet season and 1993 dry season. Hybrids and their male parents were transplanted in rows of 30 plants spaced at 20 × 20 cm.

To observe pollen sterility, florets from the upper part of the panicles were collected before flowering and pollen grains were stained with KI. Spikelet sterility was recorded on bagged panicles. When a hybrid showed 99-100% pollen sterility, its male parent was designated as a maintainer for the corresponding female parent (CMS line). When a hybrid showed more than 90% pollen and spikelet fertility, its male parent was identified as a restorer. Other male parents were considered to be partial restorers (see table). The frequency of maintainers for IR58025 A was higher than that for IR62829 A.

Most of the maintainers and restorers identified were adapted to Cuu Long Delta and are being used in a backcross program or to develop new $F_1$ hybrids. ■

**Maintalner and restorer lines identified for CMS lines IR58025 A and IR62829 A in Cuu Long Delta, Vietnam, 1992 WS and 1993 DS.**

| IR58025 A | | | IR62829 A | | |
|---|---|---|---|---|---|
| Maintainer | Restorer | Partial restorer | Maintainer | Restorer | Partial restorer |
| OM43-26 | OM53-71 | IR72 | IR44595-70 | OM1037 | OM987-1 |
| OM59-7 | OM80 | OM547 | OMCS6 | IR42068 | OMCS9 |
| OM576 | OM725 | IR31917 | | IR35546 | OM90-2 |
| MTL 58 | OM269 | IR43158 | | IR35311 | OM43-26 |
| MTL 61 | IR52287-15 | IR42068 | | | IR49517 |
| IR44595-70 | IR9729 | OM987-1 | | | IR64 |
| IR50404 | IR64 | 16B | | | OM547 |
| IR52280 | IR42068 | | | | |
| IR19725 | S818B | | | | |

## Identifying and evaluating photoperiod-sensitive genic male sterile (PGMS) lines in China

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Both photoperiod and temperature regulate fertility alteration of PGMS rice, including the original line Nongken 58 S. Photoperiod sensitivity in fertility alteration of PGMS occurs only within a specific temperature range. The higher point (HP) of the range affects multiplication of the sterile line. The lower point (LP) affects sterility fluctuation of the PGMS line when occasionally exposed to low temperature under long-day conditions.

We studied the percentages of fertile pollen and spikelet fertility of PGMS lines in China under different temperature conditions during 1992 long-day and short-day seasons in Wuhan (30°30' N, 114° 04' E, 23 m altitude). Plants were grown in phytotrons for about 10 d, from the differentiation of stamen and pistil primordia stage to the meiotic division of pollen mother cell stage. We recorded percentage of fertile pollen during heading and percentage fertility 30 d after heading of selfed-PGMS lines (Table 1).

Results show that HPs and LPs of PGMS lines are different. Lines can be divided into four groups based on combinations of HPs and LPs (Table 2).

The high HP-low LP group sterile lines (Nongken 58 S, 5088 S, 7001 S, 31111 S, and 1541 S) have stable sterilities under long-day conditions and can be easily multiplied under short-day conditions regardless of temperature changes. They can be used in the two-line hybrid production system for subtropical rice-growing areas.

**Table 1. Fertility of PGMS lines under various conditions. Wuhan, China, 1992.**

| Line | Daylength[a] | Fertility[b] | Fertility (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 22°C | 24°C | 26°C | 28°C | 30°C | 32°C |
| Nongken 58 S | LD | 1 | 9.5 ± 15.6 | 2.3 ± 3.5 | 2.7 ± 9.9 | 0.6 ± 2.0 | | |
| | | 2 | 0.3 ± 0.8 | 0.2 ± 0.9 | 0.0 ± 0.0 | 0.0 ± 0.0 | | |
| | SD | 1 | | | 47.3 ± 17.3 | 38.4 ± 24.6 | 16.4 ± 17.7 | 2.2 ± 5.1 |
| | | 2 | | | 27.7 ± 15.2 | 24.1 ± 29.1 | 7.4 ± 12.6 | 0.0 ± 0.0 |
| 5088 S | LD | 1 | 3.7 ± 5.6 | 0.2 ± 1.0 | 1.2 ± 2.0 | 0.0 ± 0.0 | | |
| | | 2 | 0.5 ± 1.7 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | | |
| | SD | 1 | | | 18.5 ± 20.4 | 6.6 ± 10.6 | 9.7 ± 17.9 | 0.9 ± 1.3 |
| | | 2 | | | 4.0 ± 5.4 | 1.7 ± 0.3 | 1.8 ± 3.6 | 0.0 ± 0.0 |
| 7001 S | LD | 1 | 0.3 ± 1.5 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | | |
| | | 2 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | | |
| | SD | 1 | | | 17.7 ± 20.8 | 6.2 ± 16.9 | 4.9 ± 13.6 | 4.8 ± 9.2 |
| | | 2 | | | 2.5 ± 4.6 | 2.8 ± 6.2 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 31111 S | LD | 1 | 0.9 ± 0.4 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | | |
| | | 2 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | | |
| | SD | 1 | | | 26.1 ± 26.7 | 13.1 ± 21.0 | 3.2 ± 5.6 | 0.0 ± 0.0 |
| | | 2 | | | | 1.1 ± 3.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 1541 S | LD | 1 | 0.5 ± 1.5 | 0.1 ± 0.5 | 0.0 ± 0.0 | 0.0 ± 0.0 | | |
| | | 2 | 0.0 ± 0.0 | 0.2 ± 0.2 | 0.0 ± 0.0 | 0.0 ± 0.0 | | |
| Pei>ai 64 S | LD | 1 | 2.7 ± 4.1 | 12.0 ± 14.5 | 8.2 ± 14.4 | 1.3 ± 0.9 | | |
| | | 2 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | | |
| | SD | 1 | | | 39.2 ± 32.6 | 2.3 ± 10.6 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | | 2 | | | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| M901 S | LD | 1 | 10.8 ± 25.4 | 0.1 ± 0.9 | 0.0 ± 0.0 | 0.0 ± 0.0 | | |
| | | 2 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | | |
| HN5-2 S | LD | 1 | 4.6 ± 8.8 | 2.3 ± 5.9 | 0.0 ± 0.0 | 2.1 ± 8.6 | | |
| | | 2 | 1.3 ± 0.5 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | | |
| | SD | 1 | | | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | | 2 | | | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 8906 S | LD | 1 | 8.9 ± 9.2 | 0.0 ± 0.0 | 0.1 ± 0.1 | 1.0 ± 1.0 | | |
| | | 2 | 4.5 ± 9.8 | 0.1 ± 0.1 | 0.3 ± 0.2 | 0.2 ± 0.1 | | |
| | SD | 1 | | | 6.9 ± 9.8 | 0.2 ± 0.4 | 1.7 ± 8.0 | 1.8 ± 4.3 |
| | | 2 | | | 1.9 ± 3.8 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 8902 S | LD | 1 | 25.3 ± 17.2 | 27.6 ± 25.9 | 35.4 ± 23.4 | 0.8 ± 3.2 | | |
| | | 2 | 17.3 ± 26.6 | 13.3 ± 12.2 | 18.4 ± 16.9 | 0.0 ± 0.0 | | |
| | SD | 1 | | | 47.0 ± 22.1 | 9.1 ± 13.2 | – | 0.0 ± 0.0 |
| | | 2 | | | 15.5 ± 16.5 | 4.6 ± 1.1 | 5.8 | 0.0 ± 0.0 |
| 8912 S | LD | 1 | 40.7 ± 19.2 | | 8.9 ± 14.5 | – | | |
| | | 2 | 15.73 | | 0.0 ± 0.0 | – | | |
| 5047 S | LD | 1 | 16.0 ± 16.1 | 31.8 ± 26.8 | 18.9 ± 13.4 | 7.6 ± 7.2 | | |
| | | 2 | 15.9 ± 23.6 | 2.6 ± 2.7 | 0.0 ± 0.0 | 0.0 ± 0.0 | | |
| Shuangguang S | LD | 1 | 31.5 ± 4.1 | 58.2 ± 3.8 | 48.5 ± 6.8 | 2.9 ± 1.1 | | |
| | | 2 | 11.4 ± 13.4 | 22.9 ± 9.9 | 17.5 ± 13.2 | 1.1 ± 1.5 | | |
| | SD | 1 | | | 43.0 ± 18.9 | 20.3 ± 23.1 | 16.2 ± 23.7 | 4.0 ± 2.5 |
| | | 2 | | | 37.4 ± 25.6 | 9.6 ± 2.4 | 1.3 ± 2.5 | 1.5 ± 2.9 |
| 9044 S | LD | 1 | 19.9 ± 17.4 | 8.5 ± 14.8 | 1.4 ± 4.6 | 0.6 ± 1.9 | | |
| | | 2 | 2.0 ± 2.8 | 2.9 ± 2.7 | 2.4 ± 2.1 | 0.0 ± 0.0 | | |
| W6154 S | LD | 1 | 17.2 ± 26.4 | 10.2 ± 20.0 | 17.1 ± 14.9 | 2.5 ± 6.0 | | |
| | | 2 | 0.7 ± 1.6 | 3.4 ± 7.8 | 3.8 ± 6.0 | 0.5 ± 0.9 | | |

[a]LD = long-day season. Heading occurred 1-10 Aug 1992. SD = short-day season. Heading occurred 11-20 Sep 1992. [b]1 =fertile pollen % ± S, 2 = spikelet fertility % ± S.

**Table 2. Temperature range of photoperiod sensitivity in fertility alteration in PGMS.**

| Line | Low point (°C) | High point (°C) |
|---|---|---|
| Nongken 58 S | 24 | 30 |
| 5088 S | 22 | 30 |
| 7001 S | 22 | 28 |
| 31111 S | 22 | 28 |
| 1541 S | 22 | (28)[a] |
| Pei>ai 64 S | 22 | (24) |
| M901 S | 24 | (26) |
| HN5-2 S | 24 | (24) |
| 8906 S | 24 | (26) |
| 8902 S | 28 | 30 |
| 8912 S | 26 | 30 |
| 5047 S | 26 | (30) |
| 9044 S | 28 | (32) |
| Shuangguang S | 28 | 32 |
| W6154 S | 28 | (28) |

[a]Numbers in parentheses were in other experiments.

The low HP-low LP group sterile lines (Pei'ai 64 S, M901 S, HN5-2 S, and 8906 S) have stable sterilities under long-day conditions (without impact of temperature) and under high temperature conditions (regardless of daylength). Their multiplications, however, are easily lost under short-day conditions when temperature rises occasionally. These PGMS lines can be used for producing hybrid seed in most rice-growing areas. Sterile lines can only be multiplied during certain seasons or at higher altitudes in tropical areas.

The high HP-high LP group sterile lines (8902 S, 8912 S, 5047 S, 9044 S, and Shuangguang S) can be multiplied easily under short-day conditions, but their sterilities are unstable under long-day conditions as temperature decreases. Because their sterilities are unstable, they are difficult to use in the two-line system. They can possibly be used in subtropical rice-growing areas of China.

The low HP-high LP group sterile lines (W6154 S and others) have stable sterilities under higher temperature without impact of photoperiod. Their sterilities, however, are unstable under long-day conditions when exposed to lower temperature. Their multiplication under short-day conditions can be affected when temperature rises occasionally. These lines can be used for hybrid seed production and multiplied during a specific season or at higher altitudes in tropical rice-growing areas. We do not recommend their use in the subtropics. ■

## Inflorescence culture in rice

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We successfully induced calli and regenerated plants in salt-tolerant indica line IR3 1662-47-2-1 for exploitation of somaclonal variation. Young tillers were cut about 7-9 cm from the base of vigorous plants in pots. Tillers were thoroughly scrubbed with and dipped in 75% alcohol for 10 min. Leaf sheaths were stripped off under aseptic conditions.

Young inflorescences were divided into groups based on their length: $DS_1$ (0.5 cm), $DS_2$ (1 cm), $DS_3$ (2 cm), $DS_4$ (3 cm), $DS_5$ (4 cm), and $DS_6$ (5 cm). Inflorescences were cleaned with the commercial detergent Teepol (5% vol/vol) for 5 min, rinsed in water, sterilized with 0.1 % aqueous $HgCl_2$ for 10 min, and then washed three times with sterile double-distilled water. Sterilized explants were placed on slant cultures with basal LS medium supplemented with 2,4-D and kinetin at rates of 0.5 ($LS_1$), 1 ($LS_2$), 1.5 ($LS_3$), and 2 ($LS_4$) mg/liter each, and on MS basal medium supplemented with naphthalene acetic acid and kinetin at rates of 0.5 ($MS_1$), 1 ($MS_2$), 1.5 ($MS_3$), and 2 ($MS_4$) mg/liter each.

Cultures were kept in total darkness at 25±2 °C. After 28 d, portions of calli were subcultured on the same medium without hormone. Remaining calli were directly placed on regeneration medium (LS basal + 2 mg kinetin/iter + 0.5 mg indole acetic acid/liter). For subculturing, culture tubes were kept in total darkness; for regeneration, cultures were maintained under 16:8 h 1ight:dark cycle. Light intensity was 2,000 lux.

$LS_1$, $MS_1$, $MS_2$, $MS_3$, and $MS_4$ failed to induce any calli, but direct plant regeneration without a visible callus stage was obtained in $MS_2$, $MS_3$, and $MS_4$ with 2-cm-long young inflorescences. $LS_2$, $LS_3$, and $LS_4$ induced callus formation. During peak callus formation, callus induction percentage and callus health varied significantly according to developmental stage of inflorescences (Table 1). Calli formed earliest in young inflorescences (0.5 and 1.0 cm), which grew vigorously and had high induction frequencies of healthy calli. Induction percentage was inversely proportional to developmental stage of the inflorescence. $LS_3$ was the best hormonal level for inducing calli.

In the subculturing experiment, all calli died within 16-22 d, suggesting

**Table 1. Degree of callus-forming ability and callus health in IR31662-47-2-1.**

| Treatment | Inflorescence length (cm) | Callus induction (%) | Peak callus-forming period (d) | Callus health (1-6 scale)[a] |
|---|---|---|---|---|
| $LS_2$ | 0.5 | 82 | - | 2 |
| | 1 | 78 | - | 1 |
| | 2 | 70 | - | 3 |
| | 3 | 68 | - | 3 |
| | 4 | 50 | - | 5 |
| | 5 | 44 | - | 6 |
| $LS_3$ | 0.5 | 100 | 12 - 15 | 1 |
| | 1 | 100 | 12 - 15 | 1 |
| | 2 | 90 | 16 - 18 | 2 |
| | 3 | 84 | 16 - 19 | 4 |
| | 4 | 62 | 25 - 26 | 6 |
| | 5 | 52 | 40 - 42 | 6 |
| $LS_4$ | 0.5 | 90 | - | 1 |
| | 1 | 86 | - | 1 |
| | 2 | 82 | - | 4 |
| | 3 | 74 | - | 5 |
| | 4 | 60 | - | 3 |
| | 5 | 47 | - | 3 |

[a] 1 = excellent, 2 - 3 =good, 4 - 5 = moderate, and 6 = poor.