segregant analysis. TGMS line SA2, selected from mutagenified populations, was nonallelic to other sources of TGMS genes and was designated as tms4. TGMS and fertile bulks were made from the F_2 population of the cross SA2 (TGMS)/N22 (fertile). Seventy-five operon primers were used to examine polymorphism in SA2, N22, and bulks. Seventeen polymorphic products were specific to the fertile parent N22 and 19 were specific to the TGMS parent SA2. Of these, the 0.7-kb amplicon of OPA12 and 1.9-kb amplicon of OPS1 were specific to the TGMS trait.

TGMS line ID24 was allelic to Norin PL 12 (tms2) and was nonallelic to SA2. Earlier studies on tagging the Chinese TGMS source reported that the 1.2-kb amplicon was closely linked. A bulked segregant analysis of the F_2 of the cross ID24/N22 revealed that a 1.2-kb amplicon of OPB19 was specific to TGMS. Further studies related to mapping are in progress. ■

Breeding methods

Heterosis over environments in crosses involving indica and tropical japonica rice cultivars

D.K. Dwivedi, M.P. Pandey, S.K. Pandey, and Li Rongbai, Genetics and Plant Breeding Department, G.B. Pant University of Agriculture and Technology, Pantnagar 263145, India

The discovery of wide compatibility genes in rice recently shifted the emphasis on enhancing heterosis from intervarietal hybrids to intersubspecific hybrids. We therefore studied the nature and magnitude of heterosis in inter- and intrasubspecific crosses involving improved tropical japonica (J) parents (BSI 10 [P1], BSI 16 [P2], B4116 [P3], and B4122 [P4]) and indica (I) parents (Govind [P5], Manhar [P6], Pant Dhan 4 [P7], Sarjoo 52 [P8], Pant Dhan 12 [P9], and Narendra 359 [P10]). We evaluated all 45 hybrids and parents in a randomized complete block design under environments with optimum sowing and high fertility (E1), optimum sowing and optimum fertility (E2), and late sowing and high fertility (E3). We studied 10 agronomic traits, including harvest index and grain yield, for heterosis, heterobeltiosis, and standard heterosis using Pant Dhan 4 as the standard variety.

Analysis of variance indicated a large variation among hybrids and parents. Moderate to high heterosis for yield and agronomic traits across environments was recorded. Trends in the magnitude of heterosis were I/J > I/I > J/J for grain yield and plant height and I/J > J/J > I/I for days to flowering. Standard heterosis for grain yield percentage in the respective E1, E2, and E3 environments ranged from -64.5 to 146.1, -70.4 to 82.2, and -67.2 to 63.8. E1 (optimum sowing and high fertility) gave a better response for heterosis expression.

Higher heterosis in grain yield accompanied heterosis in panicle number, total dry matter and/or spikelet number, and grain number panicle^{-1}. Almost 95% of the hybrids recorded negative heterosis for flowering and some were earlier by 11-27 d across environments. Hybrids P3/P8 in E1, P4/P6 in E2, and P1/P5 in E3 recorded 19.1, 20.1 and 20.4% standard heterosis for earliness and 146.1, 66.8, and 60.2% for grain yield, respectively. Standard heterosis for height was 2.0-13.7% across environments. Both parents having *Sd1* genes caused F_1 height to increase only slightly. Promising hybrids for direct exploitation were P3/P8, P1/P9, and P1/P10 in E1; P4/P7, P4/P6, and P4/P10 in E2; P4/P7, P7/P9, and P7/P8 in E3; and P3/P8, P4/P7, and P4/P10 over environments. These hybrids showed good prospects for increasing potential yields and per-day productivity in the subtropics. Studies are in progress to improve parents for the thermosensitive genic male sterility trait and resistance against biotic stresses. ■

Plant regeneration from protoplasts of wild rice, *Oryza meyeriana* Baill.

Lahong Sheng, Guangcun He, Lihui Shu, and Lanjie Liao, Department of Biochemistry and Biophysics, College of Life Sciences, Wuhan University, Wuhan 430072, China

Oryza meyeriana is a wild rice species distributed in southern China and Southeast Asia. An accession found in China's Yunan Province has a strong resistance to bacterial leaf blight. It is difficult, however, to transfer the disease resistance character of *O. meyeriana* to cultivated rice (*O. sativa*) by sexual hybridization because of sexual incompatibility between the two species.

Plant regeneration from protoplasts is important for rice breeding and is a prerequisite for genetic manipulation via somatic hybridization. Progress has been made in protoplast culture and regeneration of cultivated rice and other *Oryza* species. Plants have been recovered from cryopreserved calli in *O. meyeriana*. Here, we report on successful plant regeneration from protoplasts of this wild rice.

The immature panicles in the stamen and pistil differentiation stage were taken from cryopreserved callus-regenerated plants and used as explants. Calli were induced on N6 medium containing 2 mg L^{-1} 2,4-dichlorophenoxyacetic acid, 45 g L^{-1} sucrose, and 5 g L^{-1} agar at pH 5.8. Pale yellow primary calli with a dry appearance were transferred to liquid AA2 medium. Fine dispersed calli were

selectively subcultured every 3 d and maintained in the dark at 25 °C on a gyratory shaker at 120 rpm. The suspension cultures with a high regeneration frequency were established within 1 mo and were suitable for protoplast isolation. The suspension cells were precultured for 3 d on MS liquid medium before they were isolated by enzyme solution.

The isolated protoplasts had dense cytoplasm and many starch grains were seen in them. The highest yield of protoplasts was 2×10^7 g^{-1} fresh weight cells. Protoplasts were cultured in KPR medium with the membrane nurse culture method. An aliquot of about 0.4 mL liquid KPR medium containing protoplasts (4×10^4) was plated onto a filter (Whatman, 0.2 µm) placed over a layer of nurse cells. Nurse cells were embedded in KPR medium with 1% agarose. After 20-30 d of culture, the cell clusters formed could be seen by the naked eye. The rate of colony formation was about 0.0075%.

When protocalli grew to 1-2 mm in diameter, they were transferred to N6 medium for proliferation and then to MS regeneration medium for plant regeneration. Green plantlets were regenerated from protocalli after 2-3 wk. The average plant regeneration frequency was 3 plants per 1×10^7 protoplasts. The total time from callus initiation to plant regeneration of protoplasts was 4-6 mo.

Our results showed that the presence of nurse cells was required to induce division of protoplasts. In contrast to suspension cultures, it was difficult to isolate pure populations of intact protoplasts directly from the primary calli. Suspension cells with a good growth status, dense cytoplasm, and embryogenic characters played a critical role in successful culture. Protoplast division and colony formation also depended on the age and status of the suspension cells used for preparation of the layer of nurse cells. Establishment of a protoplast regeneration system makes it possible to transfer resistance genes from *O. meyeriana* into cultivated rice. ■

Chinoor: a promising spontaneous mutant and high-quality rice variety of Madhya Pradesh, India

D. Sharma, Department of Plant Breeding and Genetics, I. G. Agricultural University, Raipur; and M.P. Janoria, J.N. Agricultural University, Jabalpur, Madhya Pradesh, India

Chinoor is a low-yielding, late, tall, and lodging-prone indigenous rice variety of Madhya Pradesh, India. It continues to be grown in spite of its low yield because of its high-quality aromatic slender grains that sell for more than twice the price of Mahsuri. Chinoor is also exported to Middle East countries to a limited extent.

We began efforts to reduce duration and plant height of Chinoor in 1995 with a survey in farmers' fields to collect possible spontaneous mutants of interest. Progenies of the 25 single-plant selections made were transplanted along with Chinoor in non-replicated plots for observation. Each progeny plot consisted of 50 plants grown in 2 rows 20 cm apart. Plants in a row were spaced 15 cm apart. Fertilizer was applied at the rate of 60-40-20 kg NPK ha^{-1}. Twenty plants from each plot were selected randomly and tagged before flowering for observation.

One of the progenies flowered 31 d earlier, matured 38 d earlier, and had 28 cm less height than Chinoor. A comparison of the mutant with Chinoor for grain yield, its components, and some physical quality characters was made (see table).

Although kernel aroma and translucence apparently stayed the same, the mutant recorded 80% higher grain yield $plant^{-1}$ than Chinoor. This high yield advantage of the mutant resulted largely from its 30% more panicles $plant^{-1}$ and 20% higher grain weight $panicle^{-1}$.

The Chinoor mutant, with its higher yield potential and reduced duration and plant height, seems to have potential to not only replace Chinoor but also to be extended to new rice areas where it may enhance productivity and thus profitability of rice cultivation. ■

Comparison of Chinoor mutant with Chinoor, Waraseoni, India, 1996 wet season.

Character	Chinoor mutant (mean ± SD)	Chinoor[a] (mean ± SD)
Days to 50% flowering	89.6 ± 2.4	120.7 ± 2.5
Days to maturity	114.7 ± 1.6	153.0 ± 1.3
Plant height (cm)	113.2 ± 1.9	141.0 ± 2.5
Panicles $plant^{-1}$ (no.)	12.8 ± 1.7	8.8 ± 1.5
Grains $panicle^{-1}$ (no.)	211.3 ± 4.1	184.1 ± 7.6
1,000-grain weight (g)	15.3 ± 0.2	16.8 ± 0.2
Panicle weight (g)	2.4 ± 0.3	2.0 ± 0.2
Grain length (mm)	8.1 ± 0.1	7.5 ± 0.1
Grain width (mm)	2.1 ± 0.1	1.9 ± 0.1
Kernel length (mm)	6.0 ± 0.1	5.8 ± 0.1
Kernel width (mm)	1.9 ± 0.1	1.7 ± 0.1
L/B (grain)	3.9 ± 0.2	4.0 ± 0.1
L/B (kernel)	3.2 ± 0.2	3.4 ± 0.2
Plant yield (g $plant^{-1}$)	31.6 ± 2.0	17.5 ± 2.7

[a]Av of 20 individuals.

Multiple submissions. Normally, only one report for a single experiment will be accepted. Two or more items about the same work submitted at the same time will be returned for merging. Submitting at different times multiple notes from the same experiment is highly inappropriate. Detection will result in the rejection of all submissions on that research.