

Rice genetics from Mendel to functional genomics

G.S. Khush and D.S. Brar

From being a poor cousin to maize, wheat, and tomato for genetic knowledge as recently as the 1980s, rice has become a model plant for molecular genetic research. Numerous scientists in laboratories worldwide have helped make rice a favored higher plant for molecular and cellular genetic studies. Below are some of the major advances in this rapid progress in rice genetics:

- Van der Stok in 1908 for the first time reported Mendelian segregation in rice.
- Kuwada established the basic chromosome number of rice to be 24 in 1910.
- The first linkage in rice was reported by Parnell et al in 1917.
- Dr. K. Ramiah advocated the standardization of gene symbols in rice.
- Kadam and Ramiah published a review of gene symbols for the first time in 1943.
- Gene symbolization was discussed by the International Rice Commission working party on rice breeding at its Sixth Session in Penang, Malaysia, in 1955.
- Shastri et al numbered the chromosomes in decreasing order of length at the pachytene stage of meiosis in 1960.
- Rules for gene symbolization were reviewed during the symposium on rice genetics and cytogenetics held at IRRI in 1963.
- Nagao and Takahashi proposed the 12 linkage groups of rice in 1963.
- Regeneration of haploids from anther culture was reported by Niizeki and Oono in 1968.
- Independence of linkage groups was tested by Iwata and Omura in Japan and by G.S. Khush et al at IRRI in 1984 through trisomic analysis.
- Publication of the *Rice Genetics Newsletter* began in 1984 under the editorship of H.I. Oka and G.S. Khush. The First International Rice Genetics Symposium was held at IRRI in 1985 and the Rice Genetics Cooperative was established for international collaboration in rice genetics.

- The Rockefeller Foundation established an International Program on Rice Biotechnology in 1985.
- Yamada et al obtained regeneration from protoplasts in 1985.
- McCouch et al constructed the first molecular genetic linkage map in 1988.
- Transgenic rice plants were produced first by three groups: Toriyama et al, Zhang and Wu, and Zhang et al in 1988.
- The Second International Rice Genetics Symposium was held at IRRI in 1990 and a uniform chromosome numbering system was established.
- The Rice Genome Research Program (RGRP) began at Tsukuba in 1991.
- Ahn and Tanksley constructed comparative linkage maps of the rice and maize genomes in 1993.
- The yeast artificial chromosome (YAC) library in rice was established by the RGRP and a bacterial artificial chromosome (BAC) library by Wang et al in 1995.
- The first agronomically important gene in rice, *Xa21*, was cloned by Song et al through map-based cloning in 1995.
- The Third International Rice Genetics Symposium was held in Manila in 1995 and the correct orientation of morphological and molecular genetic maps was established.
- An international network on rice genome sequencing was established in 1998 under the leadership of the RGRP.
- Projects on functional genomics began in 1999.

Rice is now the model plant for genetic research among crop plants. However, this was not the case till about 15 years ago. In fact, the status of rice genetics was far behind that of maize, wheat, tomato, and barley. An agreed system of chromosome numbering in rice did not exist. Linkage groups were poorly known and their independence had not been tested. Nomenclature for gene symbolization was not uniformly followed and rice geneticists had no platform to discuss and enhance international collaboration in rice genetics. However, a series of events starting in 1985 have contributed much to elevate rice to its present state of preeminence. Here we review the major milestones in rice genetics during the 20th century.

Rice karyotype

Kuwada (1910) first reported the basic chromosome number ($n=12$) in rice from the study of microsporogenesis, megasporogenesis, and mitosis. Since then, many workers have confirmed this number. The somatic chromosomes of rice, however, are very small and difficult to distinguish from each other. The individual chromosomes are easy to identify at the pachytene stage of meiosis and Shastry et al (1960) described the pachytene chromosome complement for the first time. They numbered the chromosomes in decreasing order of length, with the longest as 1 and the shortest as 12. Kurata et al (1981) also analyzed the pachytene chromosome complement of Japa-

nese cultivar Nipponbare. The chromosome designations in two studies agreed remarkably well except for chromosomes 11 and 12, which were interchanged. The Rice Genetics Cooperative adopted the pachytene numbering system of Shastry et al (1960) in 1985.

Genome analysis

Various approaches involving morphological differentiation, meiotic chromosome pairing in F_1 hybrids, molecular divergence analysis, and fraction I protein have been used in genome analysis and in determining species relationships in *Oryza*. Morinaga (1939), based on chromosome pairing in F_1 hybrids, concluded that *O. glaberrima*, *O. breviligulata*, *O. perennis*, *O. cubensis*, *O. sativa* var. *fatua*, and *O. sativa* var. *spontanea* have the same genomic constitution designated as the AA genome. Nezu et al (1960) studied chromosome pairing in F_1 hybrids of *O. sativa* with related diploid species such as *O. perennis*, *O. glaberrima*, *O. stapfii*, and *O. breviligulata*. The hybrids invariably showed 12 bivalents at meiosis, indicating that these species also have the AA genome. Lu et al (1998) observed normal chromosome pairing in hybrids of four A genome species, *O. rufipogon*, *O. nivara*, *O. glumaepatula*, and *O. meridionalis*.

On the basis of meiotic chromosome pairing in F_1 hybrids, *O. sativa*, *O. officinalis*, *O. minuta*, and *O. latifolia* genomes were designated as AA (Morinaga 1942), CC (Morinaga and Kuriyama 1959), BBCC, and CCDD (Morinaga 1943), respectively. Li et al (1963) studied F_1 hybrids of *O. sativa* \times *O. australiensis* and *O. minuta* \times *O. australiensis* and suggested the E genome for *O. australiensis*. Katayama (1967) reported that diploid *O. punctata* has the BB genome. Li et al (1961) and Wu et al (1963) designated the F genome for *O. brachyantha*. On the basis of chromosome pairing in F_1 hybrids, various authors have assigned the genome symbol AA for the *Sativa* complex; BB, CC, BBCC, CCDD, and EE for the *Officinalis* complex; and FF for *O. brachyantha*.

Because of strong crossability barriers between *Meyeriana* and *Ridleyi* complexes, hybrids are difficult to produce and assigning genomes based on meiotic pairing could not be carried out. Under such situations, an alternate approach based on total genomic DNA hybridization and molecular divergence analysis has been used; the genome GG has been proposed for the diploids of the *O. meyeriana* complex and HHJJ for the allotetraploids of the *O. ridleyi* complex (Aggarwal et al 1997). Ge et al (1999) based on sequence analysis of nuclear genes (*Adh1*, *Adh2*) and a chloroplast gene (*matK*) proposed the HHKK genome for *O. schlechteri* and *Porteresia coarctata*, further suggesting that *P. coarctata* should be treated as an *Oryza* species.

Results of random fragment length polymorphism (RFLP) analysis (Wang et al 1992), amplified fragment length polymorphism (AFLP) analysis (Aggarwal et al 1999), sequence analysis of genes (Ge et al 1999), and seed protein analysis (Sarkar and Raina 1992) support the genomic classification based on morphological and cytological data. Table 1 shows the genomic constitution of different species of *Oryza*.

Table 1. Chromosome number, genomic composition, and geographical distribution of *Oryza* species. Modified from Khush and Brar (2001).

Species	2n	Genome	Distribution
<i>O. sativa</i> complex			
<i>O. sativa</i> L.	24	AA	Worldwide
<i>O. nivara</i> Sharma et Shastry	24	AA	Tropical and subtropical Asia
<i>O. rufipogon</i> Griff.	24	AA	Tropical and subtropical Asia, tropical Australia
<i>O. breviligulata</i> A. Chev. et Roehr.	24	AA	Africa
<i>O. glaberrima</i> Steud.	24	AA	West Africa
<i>O. longistaminata</i> A. Chev. et Roehr.	24	AA	Africa
<i>O. meridionalis</i> Ng	24	AA	Tropical Australia
<i>O. glumaepatula</i> Steud.	24	AA	South and Central America
<i>O. officinalis</i> complex			
<i>O. punctata</i> Kotschy ex Steud.	24, 48	BB, BBCC	Africa
<i>O. minuta</i> J.S. Presl. ex C.B. Presl.	48	BBCC	Philippines and Papua New Guinea
<i>O. officinalis</i> Wall ex Watt	24	CC	Tropical and subtropical Asia, tropical Australia
<i>O. rhizomatis</i> Vaughan	24	CC	Sri Lanka
<i>O. eichingeri</i> A. Peter	24	CC	South Asia and East Africa
<i>O. latifolia</i> Desv.	48	CCDD	South and Central America
<i>O. alta</i> Swallen	48	CCDD	South and Central America
<i>O. grandiglumis</i> (Doell) Prod.	48	CCDD	South and Central America
<i>O. australiensis</i> Domin.	24	EE	Tropical Australia
<i>O. meyeriana</i> complex			
<i>O. granulata</i> Nees et Arn. ex Watt	24	GG	South and Southeast Asia
<i>O. meyeriana</i> (Zoll. et Mor. ex Steud.) Baill.	24	GG	Southeast Asia
<i>O. ridleyi</i> complex			
<i>O. longiglumis</i> Jansen	48	HHJJ	Irian Jaya (Indonesia) and Papua New Guinea
<i>O. ridleyi</i> Hook. F.	48	HHJJ	South Asia
Unclassified			
<i>O. brachyantha</i> A. Chev. et Roehr.	24	FF	Africa
<i>O. schlechteri</i> Pilger	48	HHKK	Papua New Guinea

Linkage groups

The first report of Mendelian segregation in rice was by a Dutch botanist, Van der Stok (1908), in Indonesia. The first report of linkage in rice was by Parnell et al (1917), who reported linkage between black hull and colored internode. Yamaguchi (1921, 1926) reported linkage between apiculus color and waxy endosperm and set up the so-called waxy linkage group. Nagamatsu (1942) found linkage among purple leaf, liguleless, and phenol-staining reaction and established the purple leaf linkage group. Jodon (1956) proposed seven linkage groups on the basis of information available up to that time. Nagao and Takahashi (1963) proposed 12 linkage groups (I–XII) for the first time. Iwata and Omura (1975, 1976) and Khush et al (1984) tested the independence of linkage groups through trisomic tests. Three linkage groups of Nagao and Takahashi (1963) were found to belong to one chromosome and the number of linkage groups was reduced to nine. Markers for the three remaining chromosomes were identified and 12 groups were established (Khush et al 1984).

Gene symbolization in rice

In the absence of any rules for assigning gene symbols, different symbols were assigned to the same genes. As an example, the gene symbols *m*, *U*, *am*, *gl*, *g*, and *wx* were assigned to the gene for glutinous endosperm (Kihara 1964). Likewise, the same gene symbols were assigned to designate entirely different genes. The gene symbol *gl* has been used to designate genes for glutinous endosperm and glabrous leaves. Dr. K. Ramiah of India was the first geneticist to advocate the standardization of gene symbols in rice. Kadam and Ramiah (1943) published a review of the existing positions on the use of gene symbols. They suggested rules for gene symbolization wherein many conventions used in maize, cotton, and *Drosophila* were adopted. Later, Nagao (1951) also proposed a partly modified system of gene symbolization.

Gene symbols were discussed by the International Rice Commission (IRC) Working Party on Rice Breeding at its Sixth Session in Penang, Malaysia, in 1955. The Working Party suggested that a unified system of gene nomenclature be evolved and appointed a committee for that purpose with N.E. Jodon of the United States as convenor. The report of that committee was accepted by the IRC Working Party on Rice Production and Protection in 1959 and published in the IRC Newsletter (IRC 1959). The rules for gene nomenclature and gene symbols were reviewed during the symposium on Rice Genetics and Cytogenetics held at the International Rice Research Institute (IRRI) in 1963 and were accepted by the participants. Unfortunately, however, no mechanism existed for monitoring the gene symbols till the establishment of the Rice Genetics Cooperative in 1985.

Associating linkage groups with chromosomes

Primary trisomics, monosomics, translocations, and chromosomal deficiencies are useful for associating linkage groups with respective chromosomes. Primary trisomics of rice were first produced by Ramanujam (1937). Since then, several workers have produced primary trisomics (Khush and Kinoshita 1991). However, only the primary trisomics produced by Iwata et al (1970, 1984) and Khush et al (1984) were used for associating linkage groups with the respective chromosomes through modified segregation ratios. The extra chromosomes of trisomics of Iwata et al (1984) were identified at somatic metaphase and those of Khush et al (1984) at the pachytene stage of meiosis. When the chromosome-linkage group associations determined by the two groups were compared, the results did not agree for some of the chromosome-linkage group associations. These differences were resolved through mutual discussions between two groups and a unified system of numbering chromosome and linkage groups was agreed upon and accepted by rice geneticists during the Second International Rice Genetics Symposium held at IRRI in 1990. The orientation of linkage groups and position of centromeres were determined by Singh et al (1996b) through the use of secondary and telotrisomics. Figure 1 shows the latest linkage map of rice.

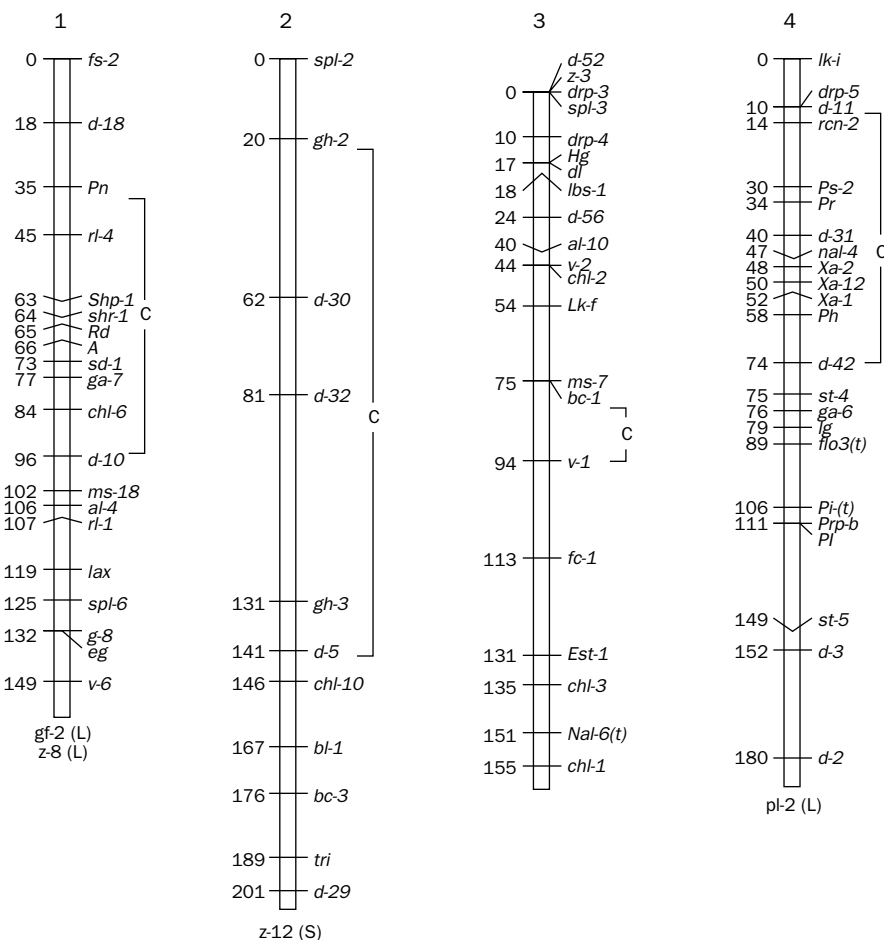
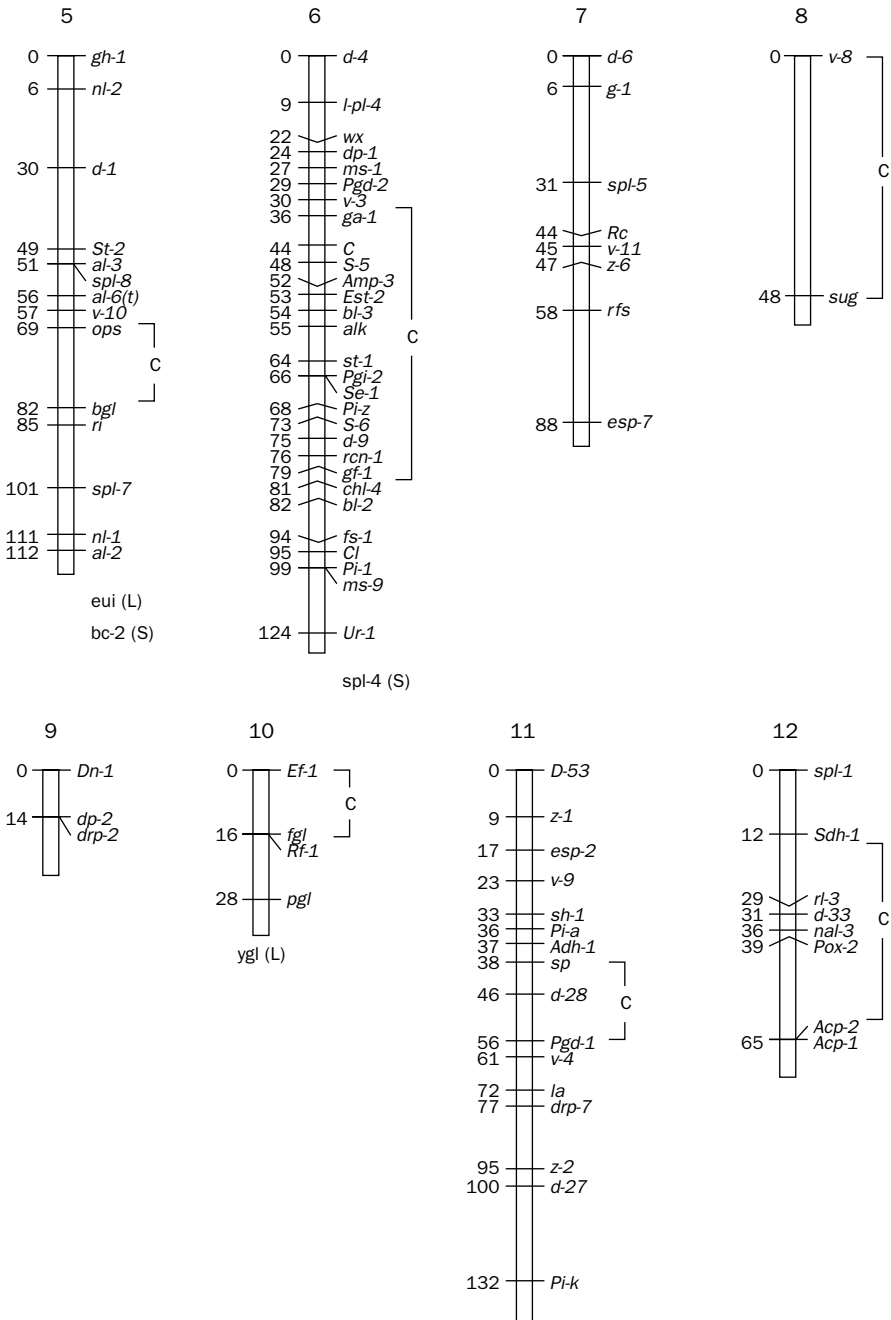


Fig. 1. Chromosome map of rice showing correct orientation of linkage groups. Markers below the map are located on respective chromosome but are not yet mapped. S and L in parentheses indicate their location on short and long arms, respectively. The centromere regions are indicated by a vertical line on the right side of each linkage group. (Adapted from Singh et al 1996b.)

International collaboration on rice research

As mentioned earlier, an international mechanism for coordination and collaboration in rice did not exist. On the basis of correspondence among IRRI scientists (Dr. M.S. Swaminathan and G.S. Khush) and officials of the Japanese Rice Genetics Information Committee (H.I. Oka, T. Kinoshita, and Y. Futsuhara), it was decided to publish an annual *Rice Genetics Newsletter (RGN)* to exchange information among rice scientists and to hold an international rice genetics symposium. The first volume of *RGN* was published in 1984 under the editorship of Drs. H.I. Oka and G.S. Khush. It contained the proposed rules for gene nomenclature. The First International Rice



Genetics Symposium was held at IRRI in 1985. Participants decided to organize the Rice Genetics Cooperative (RGC) to promote international cooperation in rice genetics. One of the standing committees of the RGC monitors and coordinates gene symbols and another publishes the *RGN* once a year. The RGC also holds international rice genetics symposia at five-year intervals to discuss the latest advances in rice genetics. Four international rice genetics symposia have been held to date: in 1985, 1990, 1995, and 2000.

Another event that led to international collaboration and advances in rice genetics was the establishment of the International Program on Rice Biotechnology by the Rockefeller Foundation in 1985. The Foundation invested \$100 million in research grants and human resource development over a 15-year period (1985-2000). Major advances were made in the molecular and cellular biology of rice by the Foundation grantees and several hundred scientists from developing countries received training. Biennial meetings of the rice scientists organized by the Foundation did much to develop camaraderie among rice geneticists.

The third development was the establishment of the Rice Genome Research Program (RGRP) at Tsukuba, Japan, in 1991. Advances in molecular marker research led to the construction of a densely populated molecular genetic map, preparation of BAC, YAC, and P1-derived artificial chromosome (PAC) libraries, physical map-facilitated gene cloning, and molecular marker-aided selection. In 1997, the Tsukuba program began the rice genome sequencing project, which eventually became the International Rice Genome Sequencing Project (IRGSP).

Genetics of disease and insect resistance

One of the major advances in rice genetics has been the identification of genes for major diseases and insects that have been employed for developing disease- and insect-resistant varieties. Sakaguchi (1967) identified *Xa1* and *Xa2* for resistance to bacterial blight. Twenty-two genes (*Xa1*, *Xa2*, *Xa3*, *Xa4*, *xa5*, *Xa7*, *xa8*, *Xa10*, *Xa11*, *Xa12*, *xa13*, *Xa14*, *xa15*, *Xa16*, *Xa17*, *Xa18*, *xa19*, *xa20*, *Xa21*, *Xa22*, *Xa23*, *Xa24*) for resistance to bacterial blight are now known. The first gene for resistance to blast, *Pia*, was identified by Shinoda et al (1971). Since then, 25 genes for resistance to blast have been identified. Athwal et al (1971) identified *Bph1* and *bph2* for resistance to brown planthopper and *Glh1*, *Glh2*, and *Glh3* for resistance to green leafhopper. Eleven genes (*Bph1*, *bph2*, *Bph3*, *bph4*, *bph5*, *Bph6*, *bph7*, *bph8*, *Bph9*, *Bph10*, *bph11*) for resistance to brown planthopper, nine (*Glh1*, *Glh2*, *Glh3*, *glh4*, *Glh5*, *Glh6*, *Glh7*, *glh8*, *Glh9*) for resistance to green leafhopper, six (*Wbph1*, *Wbph2*, *Wbph3*, *wbph4*, *Wbph5*, *Wbph6*) for resistance to whitebacked planthopper, three (*Zlh1*, *Zlh2*, *Zlh3*) for resistance to zigzag leafhopper, two (*Grh1*, *Grh2*) for green rice leafhopper, and six (*Gm1*, *Gm2*, *gm3*, *Gm4*, *Gm5*, *Gm6t*) for resistance to gall midge have been identified.

Tissue culture

As early as 1968, Niizeki and Oono reported the production of haploids from anther culture of rice. Since then, the anther culture technique has been greatly refined. It is now possible to produce haploids from the anther culture of many japonica and indica rice varieties although the frequency of regenerated plants is relatively lower in indicas. Several varieties and improved breeding lines have been developed through anther culture in China. The Republic of Korea has also released several rice varieties through anther culture. One anther culture-derived line, IR51500-AC-11-1, has been named as a variety (PSBRc50) in the Philippines. Heszky and Simon-Kiss (1992) released rice variety DAMA in Hungary through gametoclonal variation. Most of the anther culture-derived varieties are japonicas; however, indica varieties are generally recalcitrant. The doubled-haploid (DH) lines produced from indica \times japonica (IR64 \times Azucena, CT9993 \times IR62226) are being used in molecular mapping of genes and quantitative trait loci (QTLs) governing agronomic traits.

Major progress has been made in plant regeneration from protoplasts of indica and japonica rice. Yamada et al (1985) were the first to regenerate plants from rice protoplasts. Since then, many laboratories have regenerated plants of several japonica and indica cultivars. Yang et al (1989) produced cybrid plants through the donor-recipient protoplast fusion method by electrofusing the gamma-irradiated protoplast of A-58 CMS and the iodoacetamine-treated protoplasts of the fertile cultivar Fujiminori. The donor-recipient method has been successfully used to transfer cytoplasmic male sterility (CMS) from indica variety Chinsurah Boro II into japonica cultivars. Hayashi et al (1988) produced somatic hybrids through protoplast fusion between rice and four wild species of *Oryza*. Ogura and Shimamoto (1991) identified useful somaclonal variants from protoplast-regenerated progenies of japonica variety Koshihikari, and a new cultivar, Hatsume, was released.

Transformation

Protocols for transformations of rice are well known. Biolistic and *Agrobacterium*-mediated transformation have greatly facilitated the production of transgenic rice. Transgenic plants were first produced by three groups: Toriya et al (1988), Zhang et al (1988), and Zhang and Wu (1988). Since then, transgenic plants have been produced in many laboratories in both indica and japonica rice carrying genes for herbicide tolerance, resistance to stem borer, virus tolerance, resistance to fungal and bacterial pathogens, and other agronomic traits.

Goto et al (1999) introduced the ferritin gene into rice. The transgenic plants showed increased accumulation of iron in the grain. Further studies are needed to determine the usefulness of engineered rice as a source of dietary iron. Several laboratories have produced transgenic rice, mainly through protoplast-mediated DNA transformation but also via microprojectile bombardment. Cheng et al (1998) produced more than 2,600 transgenic rice plants through *Agrobacterium*-mediated transformation. Chen et al (1998) produced transgenic rice carrying multiple transgenes after co-

bombarding embryogenic cells with a mixture of 14 different puc-based plasmids. Eighty-five percent of the R_0 plants contained more than two and another 17% had more than nine of the target genes. Plants containing multiple transgenes had normal morphology and 63% set viable seeds.

Tu et al (2000) evaluated transgenic elite commercial hybrid rice expressing the *Bacillus thuringiensis* (*Bt*) genes *cryIA(b)* and *cryIA(c)* under field conditions. The transgenic plants showed a high level of protection to both leaffolder and yellow stem borer. More recently, Ye et al (2000) produced transgenic rice ("golden rice") with the provitamin-A (β -carotene) biosynthetic pathway engineered into the rice endosperm. *Agrobacterium*-mediated cotransformation was used to introduce three genes: phytoene synthase (*psy*), phytoene desaturase (*crtl*), and lycopene β -carotene (*lcy*). The genes *psy* and *lcy* originated from daffodil (*Narcissus pseudonarcissus*) and *crtl* from the bacterium *Erwinia uredovora*. High-performance liquid chromatography (HPLC) analysis of transgenic rice seeds showed the presence of β -carotene. Major efforts are under way to introduce genes for improved yield potential, disease and insect resistance, and abiotic stress tolerance into rice.

Integration of transgenes and enhancement of transgene expression are being investigated. Takano et al (1997) found illegitimate recombination accompanying rearrangement in transgenic plants. Kohli et al (1998) reported that transgene integration in rice is a two-phase mechanism in which the original site of transgene integration acts as a hot spot, facilitating subsequent integration of successive transgenic molecules at the same locus. Transformation through particle bombardment generally results in a single transgenic locus as a result of this two-phase integration mechanism. Further analysis of transgenic rice lines carrying a range of transforming plasmid rearrangements revealed a recombination hot spot in the CaMV 35S promoter with predominance of microhomology-mediated recombination (Kohli et al 1999). Vain et al (1999) analyzed the role of matrix attachment regions (MAR) in gene expression and suggested the use of one or several MAR sequences to flank the genes of interest to maximize high-level expression of transgenes.

Molecular linkage maps

The construction of a comprehensive molecular genetic map of rice containing more than 2,200 DNA markers has been a major advance in rice genetics. A molecular genetic map of rice containing 135 markers based on RFLPs was developed at Cornell University (Ithaca, New York, USA) in collaboration with IRRI (McCouch et al 1988). The map was generated from an indica \times tropical japonica F_2 population. Primary trisomics were used to assign linkage groups to each of the 12 chromosomes. A second RFLP map containing 322 markers based on an indica \times japonica cross was prepared by Saito et al (1991). Causse et al (1994) developed the map, which had 726 markers. The mapping population was derived from the cross of cultivated rice (*O. sativa*) and wild species *O. longistaminata*. Kurata et al (1994b) developed a map consisting of 1,383 DNA markers under the Rice Genome Research Program in Ja-

pan. The markers, distributed along 1,575 cM on 12 linkage groups, had 883 cDNAs, 265 genomic DNAs, 147 randomly amplified polymorphic DNAs (RAPDs), and 88 other DNA markers. Harushima et al (1998) constructed a comprehensive map consisting of 2,275 markers using a Nipponbare (japonica) × Kasalath (indica) F₂ population. Singh et al (1996a) mapped centromeres on the molecular genetic map of rice and determined the correct orientation of linkage groups. More than 170 RFLP markers were assigned to specific chromosome arms through gene dosage analysis using secondary and telotrisomics and positions of centromeres were mapped on all 12 linkage groups.

Gene tagging and QTL mapping

The availability of comprehensive molecular maps in rice has opened new avenues to tag genes governing agronomic traits with molecular markers. This has led to major advances in marker-assisted selection and pyramiding of useful genes. McCouch et al (1991) and Yu et al (1991) were the first to tag genes for bacterial blight and blast resistance with molecular markers. Since then, many genes for disease and insect resistance and for other agronomic traits have been tagged with molecular markers (Table 2) (Mohan et al 1997, Khush and Brar 1998). Some examples include genes for blast resistance—*Pi1*, *Pi2(t)*, *Pita*, *Pi5t*, *Pi7(t)*, *Pi9(t)*, *Pi10(t)*, *Pi11(t)*, and *Pib*; bacterial blight resistance—*Xa1*, *Xa2*, *Xa3*, *Xa4*, *xa5*, *Xa10*, *xa13*, and *Xa21*; brown planthopper resistance—*Bph1* and *Bph10*; gall midge resistance—*Gm1*, *Gm2*, *gm3*, *Gm4*, *Gm5*, and *Gm6(t)*; striped virus resistance (*Stvb1*); yellow mottle virus resistance; submergence tolerance (*sub1*); thermosensitive male sterility (*tms2*, *tms3*); photoperiod sensitivity (*Se1*, *Se3*); wide compatibility (*WC*); and fragrance.

Although several important characters are controlled by loci having a major effect on phenotype, several agronomically important traits such as yield, quality, and tolerance for abiotic stresses (drought, salinity, submergence, etc.) are quantitative in nature. The genes governing such traits, called polygenes or minor genes, also show Mendelian inheritance but are greatly influenced by the environment. The advent of molecular markers has made it possible to map such quantitative trait loci (QTLs). QTLs have been mapped for blast resistance, submergence tolerance, drought-related traits, and for several other agronomic traits such as days to heading, days to maturity, panicle length, spikelets per panicle, and grains per plant. Recently, Courtois et al (2000) identified QTLs for drought-related traits: 11 for leaf rolling, 10 for leaf drying, 11 for relative water content, and 10 for relative growth rate under stress.

Another major development involves the use of molecular markers to identify QTLs from unadapted germplasm or wild species that can enhance the grain yield of rice. Two yield-enhancing loci (*yld1*, *yld2*) located on chromosome 1 and 2 of *O. rufipogon* have been identified (Xiao et al 1996). In a similar experiment, Xiao et al (1998) identified 68 QTLs, of which 35 had beneficial alleles derived from a phenotypically inferior *O. rufipogon* parent.

Table 2. Some examples of mapping genes of agronomic importance with molecular markers in rice. Modified from Khush and Brar (1998).

Gene	Trait	Chromosome	Linked marker
<i>Pi1</i>	Blast resistance	11	Npb181
<i>Pi2(t)</i>	Blast resistance	6	RG64
<i>Pi4</i>	Blast resistance	12	RG869
<i>Pi5(t)</i>	Blast resistance	12	RZ397
<i>Pi5(t)</i>	Blast resistance	4	RG498, RG788
<i>Pi6(t)</i>	Blast resistance	12	RG869
<i>Pi7(t)</i>	Blast resistance	11	RG103
<i>Pi9(t)</i>	Blast resistance	6	RG16
<i>Pi10(t)</i>	Blast resistance	5	RRF6, RRH18
<i>Pi11(t)</i>	Blast resistance	8	BP127
<i>Pib</i>	Blast resistance	2	RZ123
<i>Pi20</i>	Blast resistance	12	XNbp88
<i>Pik^m</i>	Blast resistance	11	R1506
<i>Xa1</i>	Bacterial blight resistance	4	Npb235, Npb197
<i>Xa2</i>	Bacterial blight resistance	4	Npb235, Npb197
<i>Xa3</i>	Bacterial blight resistance	11	Npb181, Npb78
<i>Xa4</i>	Bacterial blight resistance	11	Npb181, Npb78
<i>xa5</i>	Bacterial blight resistance	5	RG556
<i>Xa10</i>	Bacterial blight resistance	11	OPO7 ²⁰⁰⁰
<i>xa13</i>	Bacterial blight resistance	8	RZ390, RG136
<i>Xa21</i>	Bacterial blight resistance	11	RG103
<i>Xa22(t)</i>	Bacterial blight resistance	-	RZ536
<i>RTSV</i>	Rice tungro spherical virus resistance	4	RZ262
<i>RYMV</i>	Rice yellow mottle virus resistance	12	RG341
<i>Stvb1</i>	Stripe virus resistance	12	XNpb220
<i>Bph1</i>	Brown planthopper resistance	12	XNpb248
<i>Bph10</i>	Brown planthopper resistance	12	RG457
<i>ef</i>	Early flowering	10	CD098
<i>fgr</i>	Fragrance	8	RG28, RM223
<i>Wph1</i>	Whitebacked planthopper resistance	7	-
<i>WBPH</i>	Whitebacked planthopper resistance	11	RG103
<i>Gm1</i>	Gall midge resistance	-	OPK7
<i>Gm2</i>	Gall midge resistance	4	BG329
<i>gm3</i>	Gall midge resistance	4	OPQ12
<i>Gm4</i>	Gall midge resistance	8	OPM12, RG476
<i>Gm5</i>	Gall midge resistance	12	OPB14
<i>Gm6(t)</i>	Gall midge resistance	4	RG214
<i>Rf1</i>	Fertility restoration	10	OSRRF
<i>Rf2</i>	Fertility restoration	1	CD0686/RZ58
<i>Rf5</i>	Fertility restoration	1	RG374
<i>Rf3</i>	Fertility restoration	1	RG532
<i>S5</i>	Wide compatibility	6	RG213
<i>Se1</i>	Photoperiod sensitivity	6	RG64
<i>Se3</i>	Photoperiod sensitivity	5	A19
<i>Sdg(t)</i>	Semidwarf	5	RZ182
<i>Sd1</i>	Semidwarf	1	RG109
<i>tms1</i>	Thermosensitive male sterility	8	-
<i>tms3(t)</i>	Thermosensitive male sterility	6	OPAC3 ₆₄₀
<i>tms4(t)</i>	Thermosensitive male sterility	2	RM27
<i>pms1</i>	Photoperiod-sensitive male sterility	7	RG477

continued on next page

Table 2 continued.

Gene	Trait	Chromosome	Linked marker
<i>pms2</i>	Photoperiod-sensitive male sterility	3	RG191
<i>pms3</i>	Photoperiod-sensitive male sterility	12	C751/RZ261
<i>Sub1(t)</i>	Submergence tolerance	9	RZ698
<i>Salt</i>	Salt tolerance	7	RG64
<i>OSA3</i>	Salt tolerance	12	RG457

Marker-assisted selection and gene pyramiding

The availability of comprehensive molecular linkage maps, tight linkage of target genes with molecular markers, and rapid development of polymerase chain reaction (PCR)-based DNA markers have facilitated the employment of marker-assisted selection (MAS) in rice breeding. In MAS, individuals carrying target genes are selected in a segregating population based on linked markers rather than on their phenotype. Thus, the population can be screened at any stage of growth and in various environments. MAS increases the efficiency of a breeding program by selecting for markers linked to target traits or QTLs. As mentioned, several genes for resistance to bacterial blight, blast, and gall midge have been tagged with molecular markers. Protocols for PCR-based MAS have been developed (Zheng et al 1995).

Yoshimura et al (1995) selected lines carrying *Xa4* + *xa5* and *Xa4* + *Xa10* using RFLP and RAPD markers linked to bacterial blight resistance genes. Lines carrying *Xa4* + *xa5* were more resistant to isolates of race 4 than were either of the parental lines. Huang et al (1997) used MAS to pyramid four genes for bacterial blight resistance—*Xa4*, *xa5*, *xa13*, and *Xa21*—into the background of IR24. Sanchez et al (2000) used sequence-tagged site (STS) markers to pyramid these three genes in an elite breeding line of new plant type rice. The pyramided lines having three or four genes in combination showed an increased and wider spectrum of resistance to bacterial blight than those having a single resistance gene. Such pyramided lines with different gene combinations are useful for developing varieties with durable resistance. Singh et al (2001) also used MAS to pyramid genes for bacterial blight resistance into a high-yielding indica rice cultivar, PR106, that is susceptible to bacterial blight. MAS has been useful in pyramiding recessive genes for resistance to bacterial blight such as *xa5* and *xa13* with a dominant gene *Xa21*, which confers resistance to many races and thus masks the resistance conferred by recessive genes.

MAS was also employed to pyramid genes for resistance to blast (Hittalmani et al 2000) and gall midge (Katiyar et al 2001). MAS also provides new opportunities to transfer and combine QTLs into agronomically desirable genotypes.

Physical maps

BAC and YAC libraries have facilitated the construction of physical maps of the rice genome. Wang et al (1995) and Umehara et al (1995) were the first to develop BAC

and YAC libraries, respectively. The BAC library consisted of 11,000 clones with an average DNA insert size of 125 kb. Twelve clones hybridized with three DNA markers closely linked to the *Xa21* locus. Yang et al (1997) developed a BAC library in the high-yielding and widely grown indica cultivar IR64. The library contained 18,432 clones with an average size of 107 kb. Several overlapping BAC clones were identified via colony hybridization with RFLP markers on chromosome 4. Zhang et al (1996) constructed two rice BAC libraries containing 22,000 clones with an average insert size of 130 and 150 kb, respectively. DNA markers and BAC-FISH (fluorescence *in situ* hybridization) technologies are becoming important for facilitating the generation and verification of the physical maps of rice.

YAC clones carrying several hundred kb to 1 Mb of rice genomic DNA have become important in physical mapping. The YAC library has an average insert size of 350 kb, ranging from 40 to more than 1,000 kb. Umehara et al (1996) constructed YAC libraries in japonica rice variety Nipponbare consisting of inserts covering about 6 times the genomic length. These two YAC libraries represented a *Not1* fragment library and the *EcoR1* partially digested fragment library. The 1,883 rice DNA markers were used to anchor YAC clones and ordered YAC libraries covering regions of all 12 rice chromosomes were constructed. YAC screening resulted in the identification of 5,701 YAC clones, of which 2,443 YACs have been arranged on distinct positions of the rice chromosomes.

Kurata et al (1997) described the arrangement of minimal overlapping YAC contigs and 188 YAC islands with multiple YACs on single marker positions were generated. The total coverage of all anchored YACs is estimated to be 215.8 Mb, corresponding to half the total length of the rice genome. YAC ordering showed that more than 30 DNA markers were distributed in the same order on both YAC clone arrays arranged on chromosomes 11 and 12 and that the two regions cover almost identical physical lengths on each chromosome. This is considered to be one of the longest duplications of the chromosome segment in the rice genome. Small segment duplications were also detected during the course of YAC selection with multiple-copy DNA sequences.

Recently, Saji et al (2001) constructed a physical map of rice with YAC clones covering 63% of the 12 rice chromosomes.

Map-based cloning of genes

The high-density genetic map coupled with the development of BAC and YAC libraries have been important discoveries leading to the isolation of rice genes (*Xa1*, *Xa21*, *Pib*). Song et al (1995) isolated the *Xa21* gene by positional cloning. The sequence of the predicted protein, which carries both a leucine-rich repeat (LRR) motif and a serine-threonine kinase (STK)-like domain, suggests a role in cell surface recognition of a pathogen ligand and subsequent activation of an intracellular defense response. The STK domain of *Xa21* is most similar to the tomato *Pto* resistance-gene product. Yoshimura et al (1998) cloned *Xa1* conferring resistance to bacterial blight. The deduced amino acid sequence of the *Xa1* gene product contains nucleotide-binding sites (NBS) and a new type of LRR. *Xa1* is a member of the NBS-LRR class of

plant resistance genes but is quite different from *Xa21*. It is interesting to note that *Xal* gene expression, unlike other isolated resistance genes that show constitutive expression, is induced by pathogen infection and wound. Wang et al (1999) cloned the *Pib* blast resistance gene. The deduced amino acid sequence of the *Pib* gene product contains NBS and LRR. A duplication of the kinase 1a, 2, and 3a motifs of the NBS region was found in the N-terminal half of the *Pib* protein. Eight cysteine residues are clustered in the middle of the LRR, which has not been reported for other R genes. *Pib* gene expression was induced upon altered environmental conditions such as altered temperature and darkness. Sanchez et al (1999) identified BAC contigs flanking the *xa13* locus for bacterial blight resistance. Hayano-Saito (2000) identified overlapping BAC clones flanking *Stvb1*, the gene for stripe virus resistance.

Syntenic relationships

The development of molecular genetic maps has been of great value in understanding the homoeologous relationships between the genomes of various crop plants. Ahn et al (1993) found extensive homoeologies in several regions of the genomes of wheat, rice, and maize. Kurata et al (1994a) analyzed synteny between rice and wheat and found that many wheat chromosomes contained homoeologous genes and genomic DNA fragments in an order similar to that found in rice. Comparative genome mapping in rice, maize, wheat, barley, sorghum, foxtail millet, and sugarcane into a single synthesis demonstrates that gene content and order are highly conserved at both the map and megabase level between different species within the grass family, but the amount and organization of repetitive sequences have diverged considerably (Devos and Gale 1997). Microsynteny analysis using the rice YAC clones of several hundred kilobases has also revealed remarkable similarities in marker orders between rice and barley or wheat.

Comparative genomics reveals that cereal genomes are composed of similar genomic building stocks (linkage blocks). The genomes of major cereals have been aligned by dissecting the individual chromosome into segments and rearranging these blocks into highly similar structures. Based on comparative mapping, gene location in one species can be used to predict the presence and location of orthologous loci in other species. Comparative mapping is accelerating map-based cloning of orthologous genes. The synteny relationships among cereals have resulted in the discovery of common genes such as the dwarf phenotype in maize (*D8*) and wheat (*Rht1*) based on genomic information derived from rice.

Alien introgression

Wild species of *Oryza* are an important reservoir of useful genes for resistance to major diseases and insects, tolerance for abiotic stresses, and a new source of cytoplasmic male sterility (CMS). A series of interspecific hybrids between rice and various wild species, monosomic alien addition lines ($2n=25$, MAAL), and introgression lines ($2n=24$) have been produced. The first two examples of the transfer of a useful

gene from wild species is the introgression of a gene for grassy stunt virus resistance from *O. nivara* into cultivated rice varieties (Khush et al 1977) and the transfer of cytoplasmic male sterility from wild rice, *O. sativa* f. *spontanea*, to develop CMS lines for commercial hybrid rice production (Lin and Yuan 1980). Since then, several useful genes for resistance to brown planthopper, whitebacked planthopper, bacterial blight, blast, and tungro have been introgressed from various wild species representing AA, BBCC, CC, CCDD, EE, and FF genomes into cultivated rice (Brar and Khush 1997). Some of the introgressed alien genes have been tagged with molecular markers. Molecular analysis reveals the introgression of small alien chromosome segments into the rice genome (Jena et al 1992, Brar et al 1996). FISH techniques have been employed to characterize parental genomes in interspecific hybrids and to detect homoeologous pairing and introgression of alien chromosome segments (Abbasi et al 1999). Wild species of *Oryza* are being explored for introgression of yield-enhancing loci/QTLs into rice (Xiao et al 1996, 1998, Khush and Brar, unpublished).

Structural genomics

Rice has become a model system for genomics research. Factors contributing to this situation include the comparatively smaller size of the rice genome, the synteny of its genome with those of other cereals, the availability of densely populated molecular maps containing more than 2,300 DNA markers, well-characterized YAC and BAC libraries, large-scale analysis of expressed sequence tags (ESTs), the vast amount of genetic resources (mutant markers, genetic stocks, wild species, mapping populations, introgression lines), and the comparative ease of transformation.

Major advances have been made in sequencing the rice genome under the International Rice Genome Sequencing Project (IRGSP) in Tsukuba, Japan, which began in 1998. This collaborative effort among 15 laboratories in 10 countries aims to produce publicly available sequence data for the complete rice genome. Each country has been assigned responsibility to sequence a specific rice chromosome (number 1—Japan, Korea; 2—UK; 3—USA; 4—China; 5—Taiwan; 6, 7, 8—Japan; 9—Thailand, Canada; 10—USA; 11—USA, India; 12—France, Brazil) and share the sequence data. As of April 2001, the public effort has generated 42 Mb of sequence data (10% of the genome). Two private sequencing efforts by Monsanto and Syngenta have resulted in working draft sequences for the complete rice genome. In August 2000, Monsanto transferred its rice genome draft sequence data to IRGSP. In IRGSP, a PAC library used as a vector to establish a genomics library for sequencing contained 70,000 clones of *Sau3* AI fragments of DNA with an average insert size of 112 kb (Baba et al 2000). A total of 105,000 sequence-tagged connectors (STC) from Nipponbare BAC libraries (*Hind*III and *Eco*RI fragments) have been constructed by Clemson University and 55,000 STC from the IRGSP. At Tsukuba, 48 PAC on chromosome 1, 12 PAC on chromosome 6, two PAC on chromosome 2, one PAC on chromosome 3, and one PAC on chromosome 8 had been completely sequenced and annotated (Sasaki et al, this volume). A total of 102 PAC/BAC had been sequenced, covering 15.0 Mb of the rice genome. These sequences have been registered in the DNA data bank of Japan

and the information is freely available on the World Wide Web. An additional 100 PAC/BAC covering another 15 Mb have also been sequenced although several gap regions remain to be filled. These sequencing efforts by the IRGSP and other public and private organizations will provide sequences for the estimated 30,000 rice genes and for the intergenic DNA that plays an important but poorly understood role in gene expression, DNA replication, chromosome organization, recombination, specialization, and evolution.

Functional genomics

The availability of rice sequence data has ushered in the era of functional genomics. The *AC-DS* maize transposable elements, retrotransposons, miniature inverted repeat transposable elements (MITEs), and T-DNA insertions have provided a wealth of genetic resources for functional genomics. Some notable examples are T-DNA-tagged insertional mutants with 30,000 lines carrying 42,000 T-DNA inserts (An et al, this volume), Tos17 retrotransposon insertional mutants with about 30,000 lines carrying more than 250,000 Tos17s (Hirochika et al, this volume), and more than 40,000 deletion mutants produced by fast-neutron, gamma radiation, and chemical mutagenesis (Leung et al, this volume). These plant materials have provided the necessary link between structural or sequence data and gene function through forward and reverse genetics. Furthermore, a large number of ESTs from various cDNA libraries have been produced for microarray expression experiments. Maize transposon constructs have been used in the transformation of japonica and indica cultivars for knockout and gene detection insertion. A green fluorescent protein (GFP) excision assay is used to determine transposon excision in a variety of tissues. The functional genomic methodology is changing from forward genetics to reverse genetics. One of the major challenges is to determine the function of previously unknown rice genes revealed by sequencing. The second is to understand the functions of apparently redundant rice genes that may have different roles in different tissues or in response to different environments.

New high-throughput methods are being developed for expression analysis. Biochips are being used to follow changes in gene expression in response to abiotic stresses. Using gene chips or microarrays, the representative genes of rice can be analyzed on a glass slide and used in RNA hybridization to reveal gene expression patterns and identify pathways by association. A combination of mRNA and proteomics will precisely reveal the function of rice genes involved in the phenotypic expression of different agronomic traits.

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Notes

Authors' address: Plant Breeding, Genetics, and Biochemistry Division, International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines.

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