# 1 Rice

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## 1.1 Introduction

Rice is the world's single most important crop and a primary food source for half of the world's population. Rice, wheat, and maize provide 49% of the calories consumed by the human population. Of those 23% are provided by rice, 17% by wheat and 9% by maize. Thus almost one fourth of the calories consumed by the entire world population come from rice. More than 90% of the world's rice is grown and consumed in Asia, where 60% of the earth's people live. Rice is planted to about 154 million hectares annually, or on 11% of the world's cultivated land. World rice production was 600 million tons in 2000. India has the largest area under rice (45 million hectares), and China is the largest producer of rice (190 million tons). Other major rice-producing countries are Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Japan, and the Philippines (Table 1). The importance of rice in the diet varies among countries. It accounts for over 70% of the daily calories intake in countries such as Bangladesh, Cambodia, Laos, Myanmar, and Vietnam but drops to about 40% in countries such as China and India, whose northern areas consume primarily wheat.

### 1.1.1 Taxonomy and Origin of Cultivated Rice

Of the two cultivated species, Asian cultivated rice, *Oryza sativa* , is grown worldwide. *Oryza glaberrima*, the African cultivated rice, is grown on a limited scale in West Africa. Like other cereals such as wheat, maize, barley, sorghum, oats, and rye, which feed the world, rice belongs to the grass family Gramineae. The genus *Oryza*, to which cultivated rice belongs, probably originated at least 130 million years ago and spread as a wild grass in Gondwanaland, the super continent that eventually broke up and drifted apart to become Asia, Africa, Australia, and Antarctica (Chang 1976). This explains the distribution of *Oryza* species on all of these continents except Antarctica (Table 2).

There are 22 wild species of genus *Oryza*. Nine of the wild species are tetraploid. The remaining wild species and the two cultivated species are diploid. Ten different genomes (Table 2) have been assigned to the different species based on chromosome pairing in interspecific hybrids or based on total DNA hybridization and molecular divergence.

The common rice, *Oryza sativa,* and the African rice, *Oryza glaberrima,* are thought to be examples of parallel evolution in crop plants. The wild progenitor of *O. sativa* is the Asian common wild rice, *O. rufipogon*, which shows a range of variation from perennial to annual types. Annual types, also given the specific name of *O. nivara*, were domesticated to become *O. sativa* (Khush 1997). In a parallel evolutionary path, *O. glaberrima* was domesticated from annual *O. breviligulata*, which in turn evolved from perennial *O. longistaminata* (Fig. 1).

Domestication of wild rices probably started about 9,000 years ago. Development of annuals at different elevations in East India, northern Southeast Asia, and western China was enhanced by alternating periods of drought and variations in temperature during the Neothermal Age about 10,000 to 15,000 years ago (Whyte 1972). Domestication in Asia could have occurred independently and concurrently at several sites within or bordering a broad belt that extends from the plains below the eastern foothills of the Himalayas in India through upper Myanmar, northern Thailand, Laos, and Vietnam to southwestern or southern China (Roschevitz 1931; Chang 1976). The earliest and most convincing archeological evidence for domestication of rice in Southeast Asia

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**Table 1.** Total area planted, coverage of high-yielding varieties, and increase in rice production in selected countries in Asia

| Country | Total area planted (million ha) | | Area planted with HYVs | Production (million tons) | | Increase in production |
|---|---|---|---|---|---|---|
| | 1966 | 2000 | (%) | 1966 | 2000 | (%) |
| Bangladesh | 9.1 | 10.7 | 65 | 14.3 | 35.8 | 150 |
| China | 31.3 | 30.5 | 100 | 98.5 | 190.1 | 93 |
| India | 35.2 | 44.6 | 73 | 45.6 | 134.1 | 194 |
| Indonesia | 7.7 | 11.5 | 77 | 13.6 | 51.0 | 275 |
| Myanmar | 4.5 | 6.3 | 72 | 6.6 | 21.3 | 222 |
| Pakistan | 1.4 | 2.3 | 42 | 2.0 | 7.0 | 250 |
| Philippines | 3.1 | 4.0 | 89 | 4.1 | 12.4 | 202 |
| Sri Lanka | 0.5 | 0.8 | 91 | 1.0 | 2.8 | 180 |
| Thailand | 7.3 | 10.0 | 68 | 13.5 | 23.4 | 73 |
| Vietnam | 4.7 | 7.6 | 80 | 8.5 | 32.5 | 282 |

was discovered by Welhelm G. Solheim II in 1966 (Solheim 1972). Pottery sherds bearing the imprints of grain and husks of *O. sativa* were discovered at Non Nok Tha in the Korat area of Thailand. The remains were dated to about 4000 BC. The oldest carbonized grains found in India date to about 6750 BC (Sharma and Nanda 1980). The oldest remains of cultivated rice in China date to five centuries before Christ. Carbonized rice grains from Tongxieng County of Zejiang province were identified as being 7,040 years old.

The African cultivar *O. glaberrima* originated in the Niger River delta. The primary center of diversity for *O. glaberrima* is the swampy basin of the upper Niger River and two secondary centers to the southwest near the Guinean coast. The primary center was probably formed around 1500 BC, while the secondary centers were formed 500 years later (Porteres 1956).

### 1.1.2 Dispersal of Cultivated Rice

From the Himalayan foothills rice spread to western and northern India, to Afghanistan and Iran and south to Sri Lanka. The date of 2500 BC has been established for Mohenjodaro in Pakistan, while in Sri Lanka rice was a major crop as early as 1000 BC. The rice crop may well have been introduced to Greece and neighboring countries of Mediterranean by returning members of Alexander the Great's expedition to India in 324 BC. However, in all probability rice did not become an established crop in Europe until much later, perhaps in the 15th century. Rice was introduced from India to Madagascar, to East Africa, and then to countries of West Africa. Indica rices also spread eastward to Southeast Asia and north to China.

The japonica rice was most likely domesticated somewhere in northern parts of Southeast Asia or southern China. It moved north to become a temperate japonica. From China temperate japonicas were introduced into Korea and from Korea to Japan around the beginning of the first century. In the hilly areas of Southeast Asia, japonica rices were grown under upland culture as a component of shifting cultivation before the upland tribes moved into lowlands and introduced the japonicas into lowland culture. From mainland Southeast Asia, both indica and japonica rices were introduced into Malaysia, the Philippines, and Indonesia and from the Philippines to Taiwan. Migrating Malays from Indonesia introduced tropical japonicas to Madagascar in the 5th or 6th century. Portuguese priests introduced the tropical japonicas from Indonesia to Guinea Bissau, and from there they spread to other West African countries. Thus most of the upland rice varieties grown in West Africa are tropical japonicas. The Portuguese also introduced tropical japonicas and lowland indicas to Brazil, and Spanish-speaking people brought them to other Latin American countries. Thus in Brazil today most of the upland varieties are tropical japonicas and the lowland varieties are indicas (Khush et al. 2003). The first record of rice in the U.S. dates from 1685, and it was probably introduced from Madagascar with the slave trade.

**Table 2.** Chromosome number , genomic composition , and geographical distribution of *Oryza* species

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

### 1.1.3 Varietal Diversity of Rice

From its subtropical origin rice is now cultivated between 55° N in China and 36° S in Chile. Cultivation and farmer selection for centuries under varied growing conditions have resulted in a myriad of rice varieties. An estimated 120,000 distinct rice varieties exist in the world. Approximately 80,000 are preserved in the Gene Bank of the International Rice Research Institute (IRRI) in the Philippines. China has about 40,000 and India about 25,000 in their gene banks. Other countries have smaller selections.

Rice varieties differ in numerous morphological and physiological traits and have been selected for adaptation to different growing conditions. Some mature in less than 80 d from sowing. Others, like Rayada rices of Bangladesh, have a growth cycle of about 280 d. These are photoperiod-sensitive deepwater rices and are planted with the onset of rains in March and harvested in December. Rice varieties also differ in endosperm traits, which determine their acceptability to various consumer groups. While the vast majority of rice varieties are nonglutinous, glutinous varieties form the everyday diet of the people of Laos and northeast Thailand. Most of the major rice-growing countries have a few aromatic varieties that are prized on the market. Varieties differ in the level of cold tolerance and tolerance to other abiotic stresses such as drought, submergence, and salinity. There are

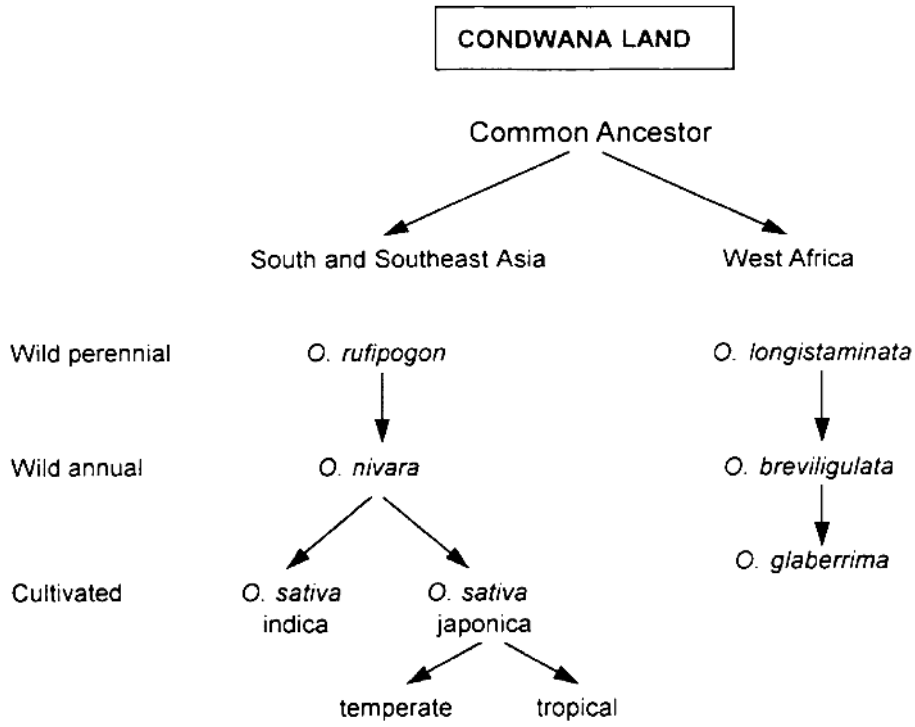



**Fig. 1.** Evolutionary pathways for two cultivated species of rice

differences in resistance to diseases and insects. In some countries, varieties are classified according to the season in which they are grown. For example, in Bangladesh, where rice is grown throughout the year, varieties have been selected for adaptation to following seasons (Khush 1997).

1. Boro: Winter rice, transplanted, cold tolerant, grown December to May
2. Aus: Summer rice, broadcast, sown, drought tolerant, short life cycle, grown April to July
3. Transplanted Aman: Autumn sown, transplanted, photoperiod sensitive, grown July to December
4. Broadcast Aman: Deepwater, photoperiod sensitive, grown March to December
5. Ryadas: Deepwater, photoperiod sensitive, very long duration, grown March to December
6. Ashina: Deepwater aus, broadcast sown, grown April to August
7. Hill Rice: Grown on upland fields, usually on sloping hillsides, direct seeded, grown June to September

Similar varietal differentiations exist in southern India and Sri Lanka, where rice is grown throughout the year.

### 1.1.4 Rice Varietal Improvement

Since its domestication about 10,000 years ago, rice has undergone tremendous modifications so much as a result of human selection for improved traits that domesticated rice varieties can no longer survive in the wild state. The simple acts of reaping and sowing are selective. Our ancestors may not have known it, but they started the first rice-breeding programs when they began to grow rice plants for their use. Most farmers have a keen eye and a sensitive feeling for plants. Millions of farmers have applied this keen insight and sensitivity for thousands of years to select diverse varieties. Selection was first practiced on the variable and heterogeneous wild and semiwild populations, which must have narrowed the genetic variability. However, several mechanisms in primitive agriculture, such as the introduction of varieties from one region to another and occasional natural crosses, enhanced variability for further selection. Natural crosses between domesticated crop and the weed complexes were another source of variability. The third source of variability was varietal mixtures that primitive agriculturists grew as a protection against disease epidemics. Occasional intercrosses between component varieties generated variability. This

conscious and unconscious selection by humans led to the development of over 120,000 rice varieties grown around the world.

Thus farmers themselves were responsible for most rice improvement from the time of its domestication to about 1900. The best known examples are the "rono" varieties such as "Shinriki" that Japanese farmers selected in the 1890s. The rono varieties are shorter and therefore responded to nutrient inputs with higher yields. Rice-breeding stations were established in China, India, and Japan in the early 20th century. Rice breeders' initial activities were the purification of existing varieties (landraces) through pure line selection. This resulted in pure line varieties. Up to the 1960s rice farmers in tropical and subtropical Asia grew thousands of landraces or pure line varieties, and few had been touched by modern agricultural science. These varieties were tall and weak stemmed and late maturing. When nitrogenous fertilizer was applied at rates exceeding 40 kg/ha, traditional varieties tillered profusely, grew excessively tall, lodged early, and yielded less than they would have with lower fertilizer inputs.

The International Rice Research Institute (IRRI) was established in 1960 in the Philippines to address the problems of stagnant yields. A major breakthrough in raising the yield potential of tropical rice came with the development of IR8 at IRRI in 1966, which resulted in a doubling of the yield potential of rice. IR8 has a short stature and a combination of several other agronomic traits such as sturdy stems for lodging resistance, dark green and erect leaves, and high tillering capacity. Because of lodging resistance it is highly responsive to fertilizer. Since the development of IR8 a series of improved rice varieties have been developed at IRRI and by the National Agricultural Research Systems (NARS). These varieties have been improved in many other traits such as grain quality, disease and insect resistance, growth duration, and tolerance to abiotic stresses. More than 300 varieties have been selected from the breeding materials developed at IRRI (Khush and Virk 2002). These and others developed by NARS are now planted on 80% of the world's rice land. Because of widescale adoption of these varieties and associated technology, world rice production increased 135% in a 35-year period from 257 million tons in 1966 to 600 million tons in 2000, and, during the same period, average rice yield increased from 2.1 t/ha to 3.9 t/ha. Most of the major rice-growing countries achieved self-sufficiency in rice.

During this intensive breeding effort rice varieties have been developed that have genes from various ecotypes of rice. Even the genes from wild species have been introduced into modern varieties. Thus the ecotypic differentiation present in the landraces of rices no longer exists in the improved varieties. Genes from numerous landraces have been incorporated into new varieties. For example, widely grown IR64 has 20 landraces in its ancestry (Khush 1987).

### 1.1.5 Rice-Breeding Challenges in the 21st Century

World population continues to increase by 75 million people a year, an annual growth rate of 1.3%, with 90% of this increase occurring in the developing countries of Asia, Africa, and Latin America. Providing for population growth now requires an expansion in world grain production of 26 million tons per year. Moreover, owing to rising living standards, food habits are changing in many countries, particularly in Asia, and people are eating more high-value foods such as meat, eggs, and milk. This is driving the demand for grain at a rapid rate. A kilogram of beef produced in the feedlot requires 7 kg of grain, a kilogram of pork needs 4 kg, and a kilogram of poultry needs just over 2 kg (Brown 1997).

More than a billion people in developing countries live below the poverty line and have poor access to food. As poverty-alleviation programs in developing countries make an impact, the purchasing power of poor people will increase, as will the demand for food grains. Based on population projections and improved consumption patterns in developing countries, it is estimated that rice production must increase by 40% during the next 20 to 25 years or at the rate of about 1.1% a year. This increase will have to be achieved from less land, with less water, less labor, and fewer chemicals.

To feed 5 billion rice consumers in 2025, we have to develop rice varieties with higher yield potential and greater yield stability. Crop cultivars with higher yield potential are the key to increased productivity. Conventional hybridization and selection procedures will continue to be employed, but breakthroughs in cellular and molecular biology will be increasingly used in rice improvement. Transformation techniques allow us to introduce novel genes from unrelated sources to accomplish breeding objectives not possible through conventional breeding approaches. For example, none of the rice varieties or related wild species has beta

carotene, a precursor of vitamin A, and rice varieties with vitamin A could not be developed. Ye et al. (2000) introduced three genes, two from the daffodil (*Narcissus pseudonarcissus*) and one from the bacterium *Erwinia uredovora* into rice variety Taipei 309. This led to the establishment of a biosynthetic pathway for the production of beta carotene in rice endosperm. This so-called "golden rice" will have a great impact in alleviating vitamin A deficiency among poor rice consumers.

## 1.2 Construction of Molecular Linkage Maps in Rice

Genetic mapping means the identification of the location of polymorphism between parental lines that generate progenies used for statistical analysis of recombination frequency. The polymorphisms used are observed both in appearance and nucleotide sequence in genomic DNA. It is well known that Gregor Mendel succeeded in establishing the law of inheritance because he used nearly genetically pure common pea lines for target traits such as plant height or roundness of seed in his experiments. For genomewide mapping using polymorphisms in the nucleotide sequence, the parental lines must be genetically pure or homogeneous as well in terms of the target loci. In the case of rice, homogeneity in the genetic background of the parental lines can be achieved by repeated self-pollination for 5 to 6 generations. In non-self-pollinating plant species, genetic analysis can be performed by a pseudotest cross-analysis method using $F_1$ siblings. The basic idea in generating genetic maps of both self-pollinating and non-self-pollinating plants is to detect recombination between markers of phenotype or DNA. This chapter focuses on the genetic analysis of rice, which is a purely self-pollinating plant.

Historically, mapping of rice was tried first by linkage analysis of appearance, or phenotype (Nagao and Takahashi 1963). Several phenotypes that could be easily identified and evaluated, such as waxy, dwarfism, chlorosis, or disease resistance, were chosen for genetic mapping, which led to the development of the 12 linkage groups of rice. After this remarkable work, improvement of the linkage map was achieved using isozymes such as esterase instead of phenotypes (Nakagahra 1977). The use of isozyme was the first step in innovating the linkage map by molecular tools. The correspondence of linkage groups and chromosomes was achieved by using trisomic rice plants with representative phenotype (Iwata and Omura 1984; Khush et al. 1984). The current numbering of chromosomes and linkage groups was unified in 1990 at the 2nd International Rice Genetics Conference (Khush 1990).

In 1986, the utility of polymorphism in genomic nucleotide sequences was first shown to be effective in tagging the human inheritable disease Huntington's disease (Botstein et al. 1980). This linkage analysis of phenotype with DNA markers led to the success in identification of the gene controlling the corresponding phenotype. Subsequently, several efforts focused on detecting polymorphisms of nucleotide sequence to generate many DNA markers distributed all over the genome of a target species. The most reliable polymorphism is restriction fragment length polymorphism (RFLP) because it can be detected as a codominant trait in Southern hybridization. Other conventional polymorphisms such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP), which are less time consuming but costly and less reliable as compared to RFLP, are more widely used in linkage analysis. In the case of rice, the first molecular linkage map with 135 loci defined by RFLP markers was published in 1988 using 50 progenies derived between a cross of japonica and indica cultivars of *O. sativa* (McCouch et al. 1988). This pioneered the possibility of molecular genetic analysis of the rice genome further promising the gene identification corresponding to phenotype.

After this first endeavor, several groups in Japan continued the effort to increase the number of RFLP markers for a more detailed and accurate genetic anatomy of the rice genome. In Japan, one of the countries where rice is a main staple, a pilot project of development of genetic maps with genomic RFLP markers (Saito et al. 1991) was immediately followed in 1991 by a large-scale and systematic construction of a map with high-density DNA markers mainly of RFLP. The analyzed population was 186 $F_2$ plants obtained by a cross between japonica cultivar Nipponbare and indica cultivar Kasalath , and the first map constructed carried a total of 883 markers (Kurata et al. 1994). The markers were mainly derived from rice cDNAs randomly selected from several libraries and partially sequenced from both ends. These markers, which correspond to expressed genes from the rice genome, are more advantageous than random genomic DNAs because their mapped positions will

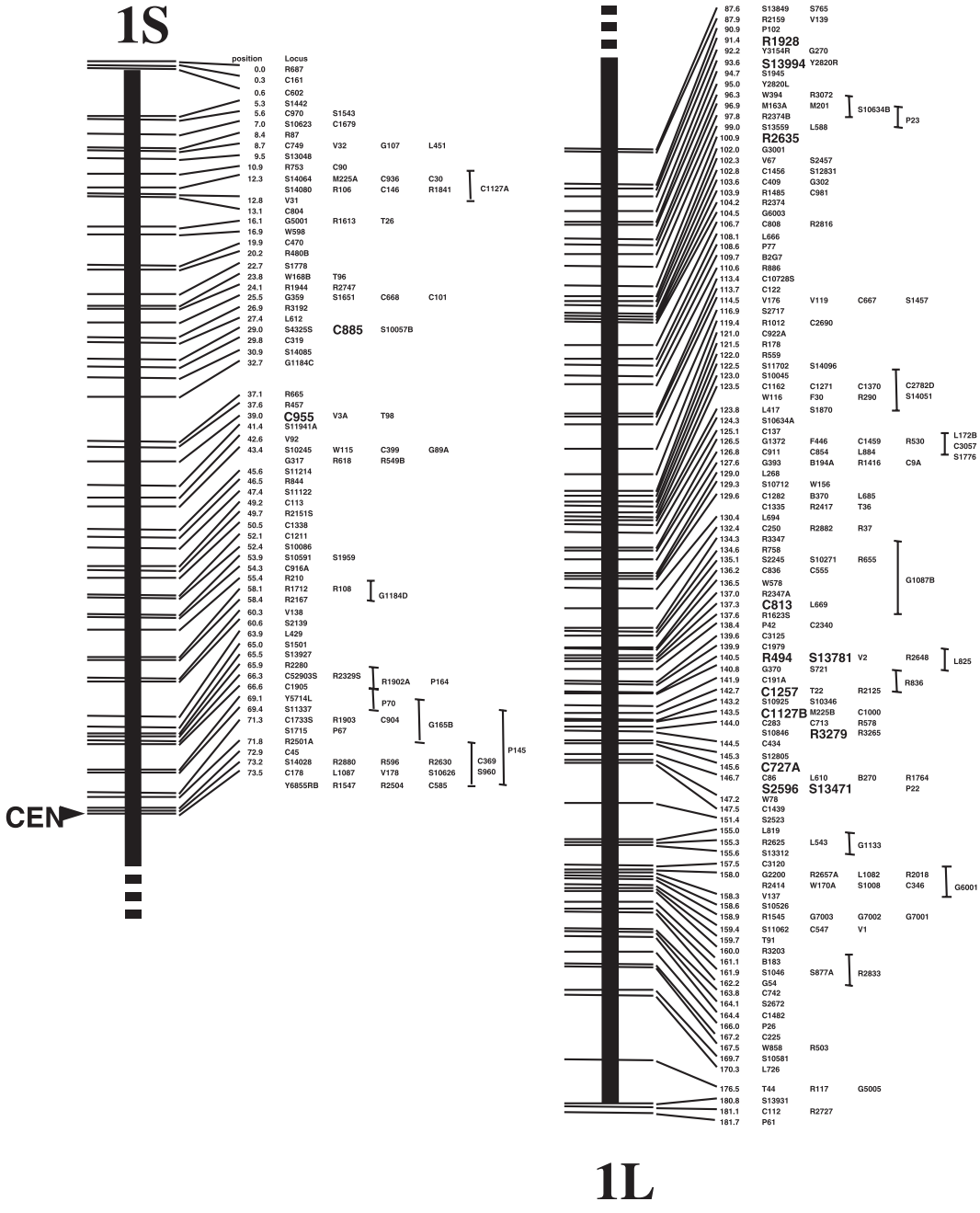
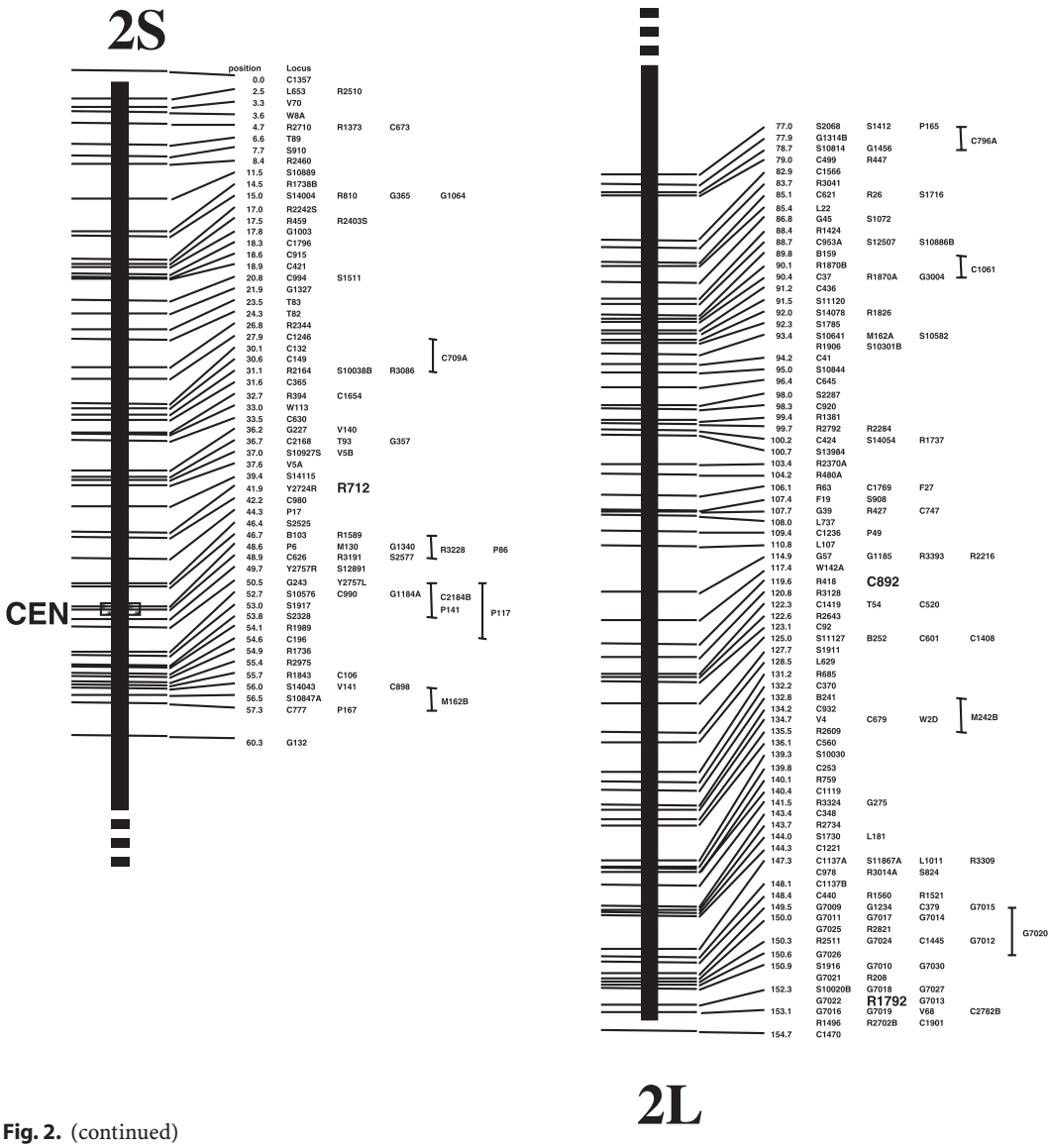



**Fig. 2.** Rice molecular genetic map with 2,275 markers (Harushima et al. 1998) developed using 186 $F_2$ population from the cross Nipponbare × Kasalath

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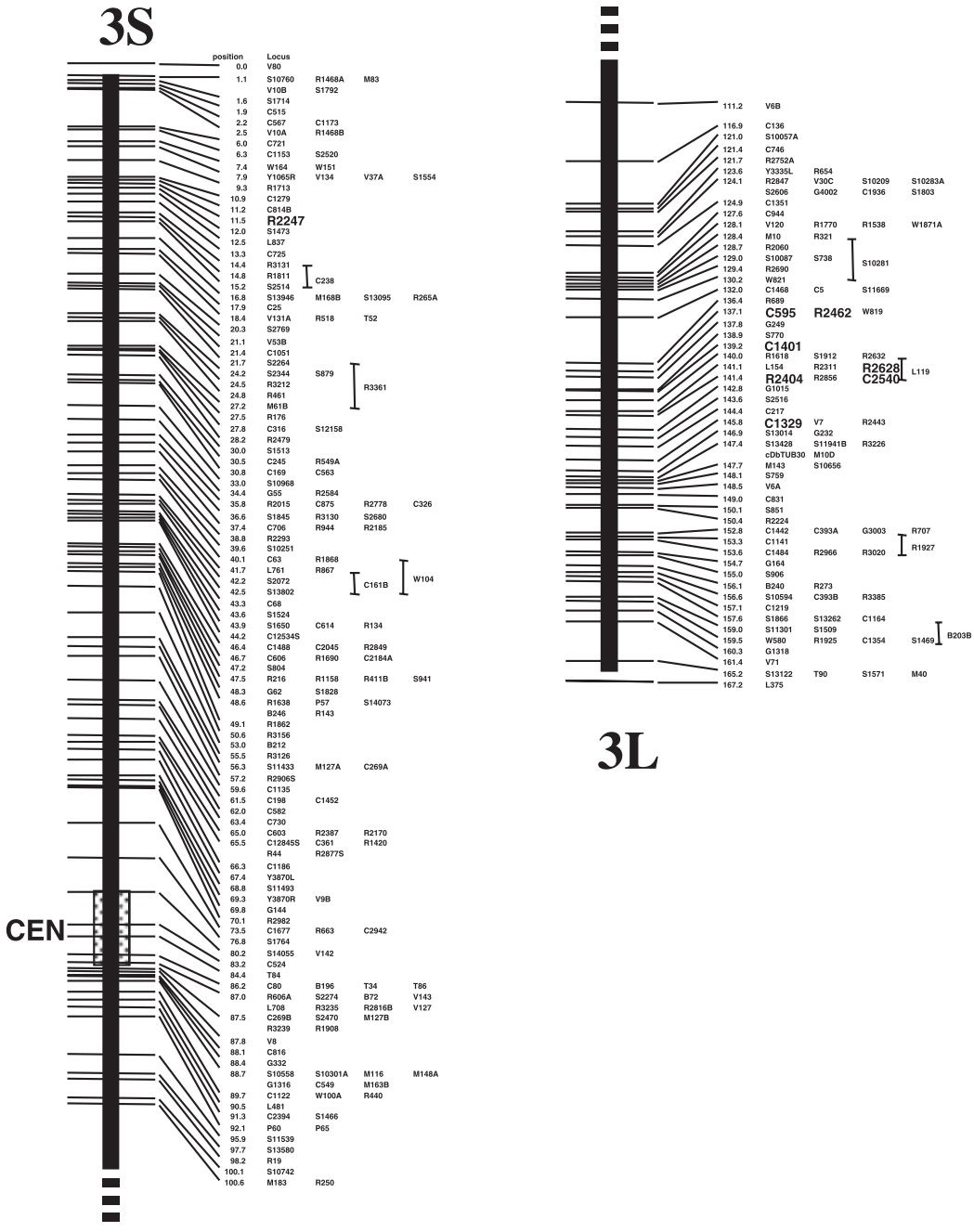


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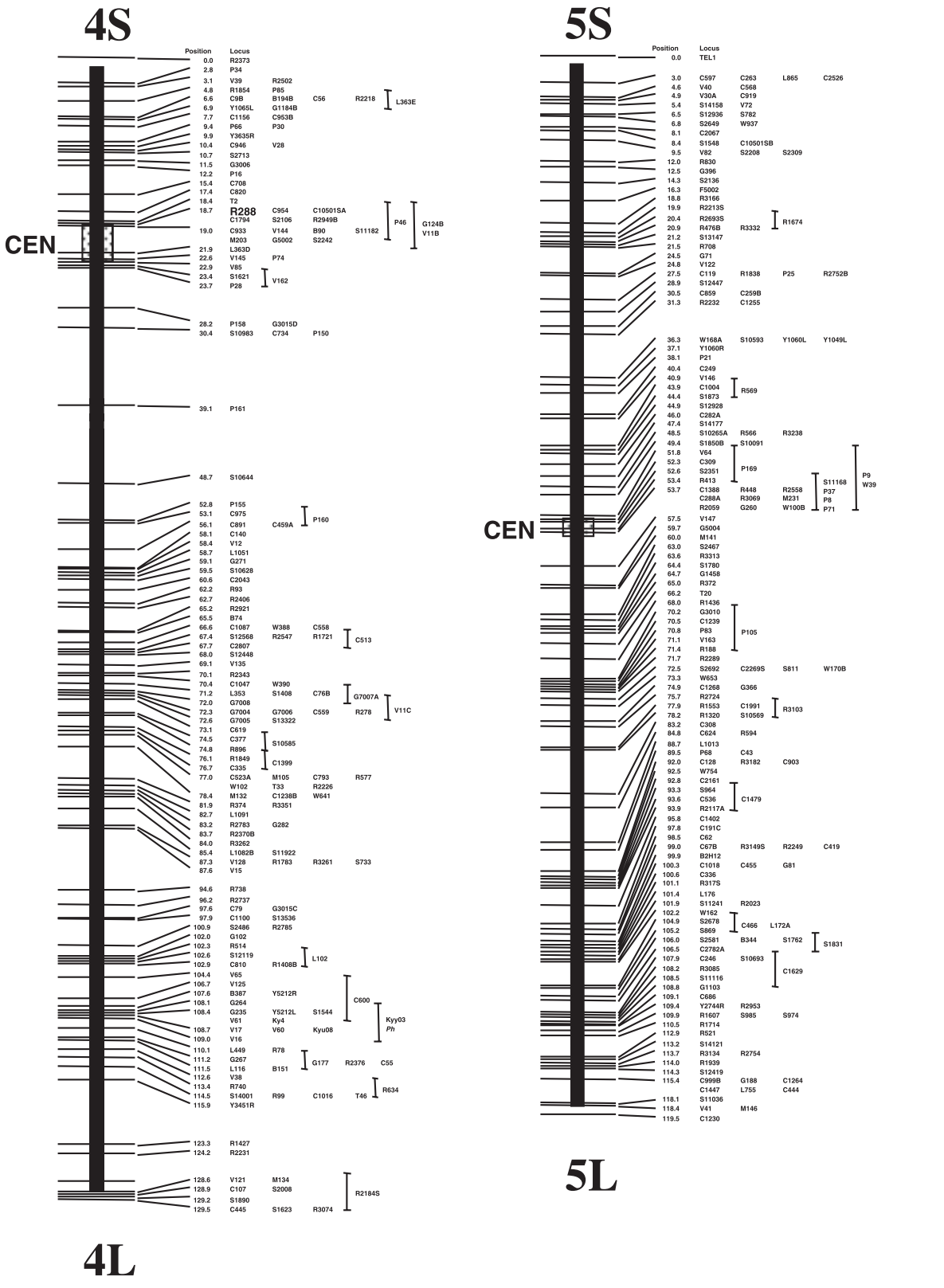


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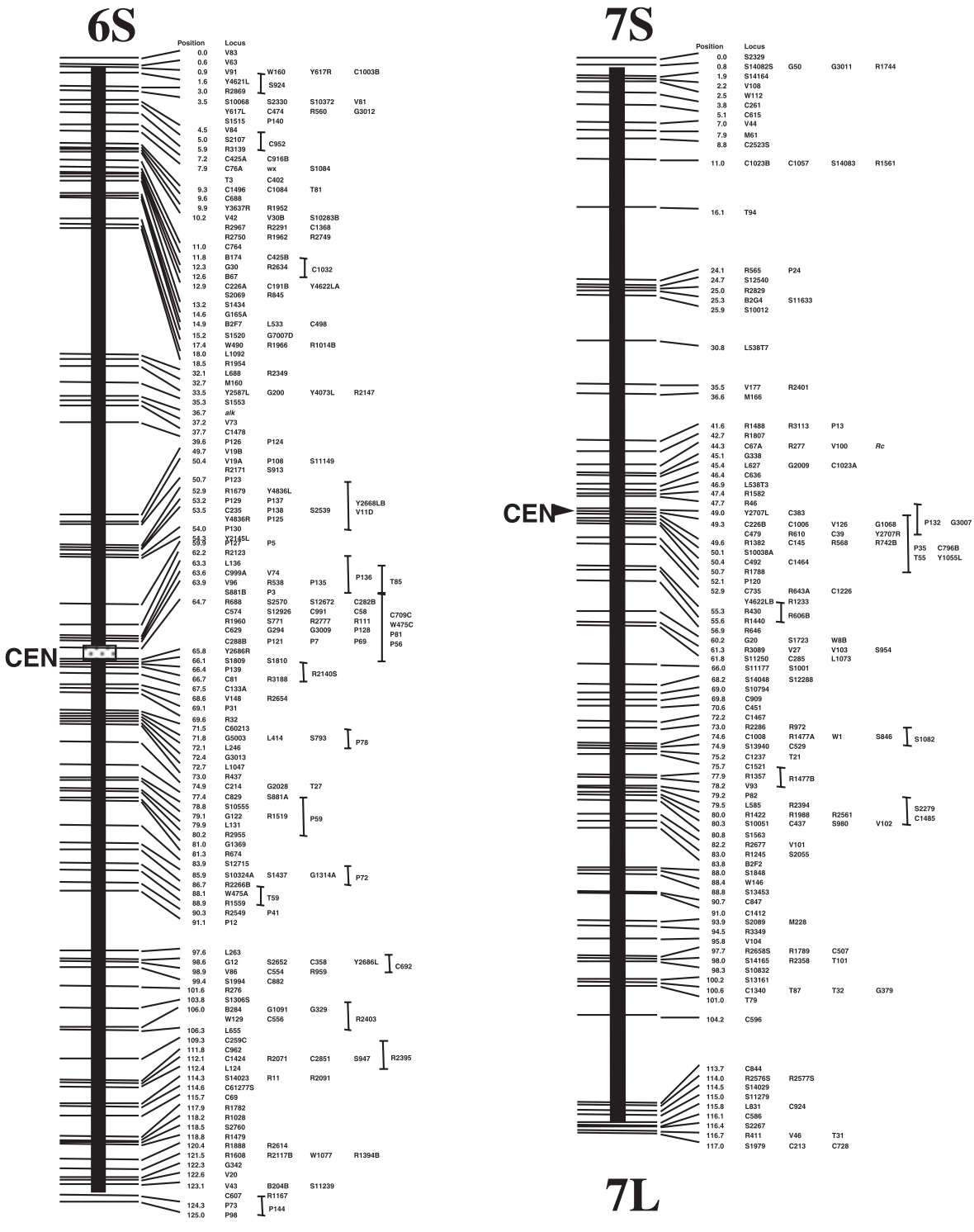


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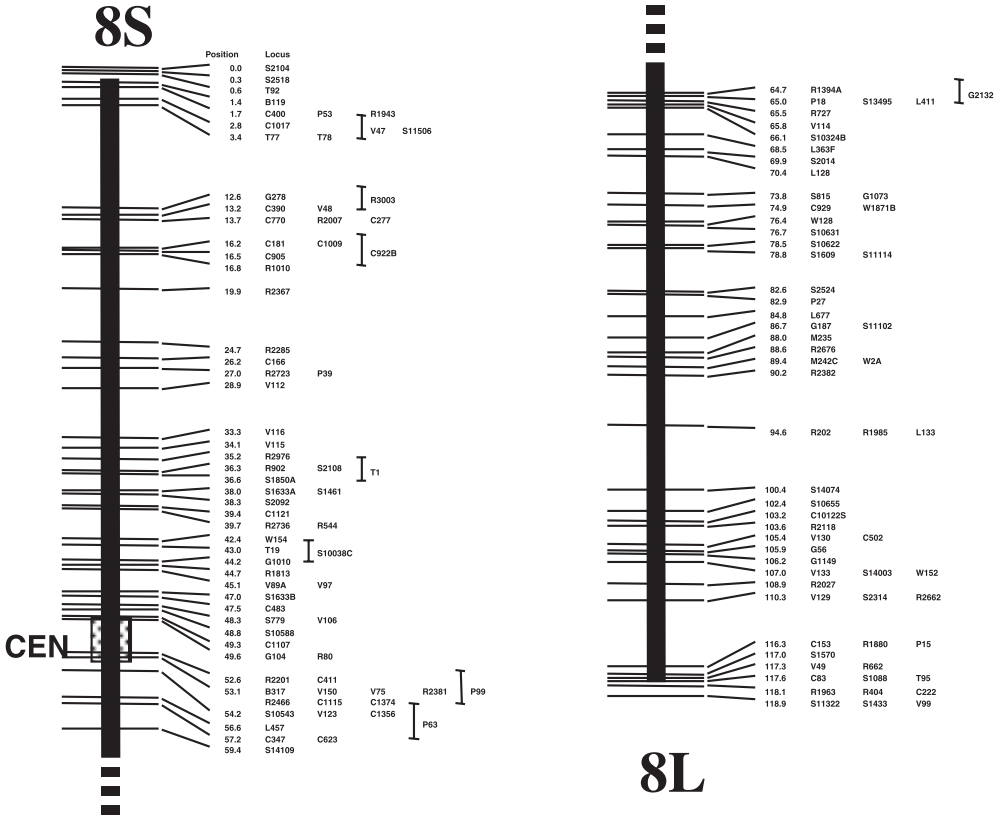


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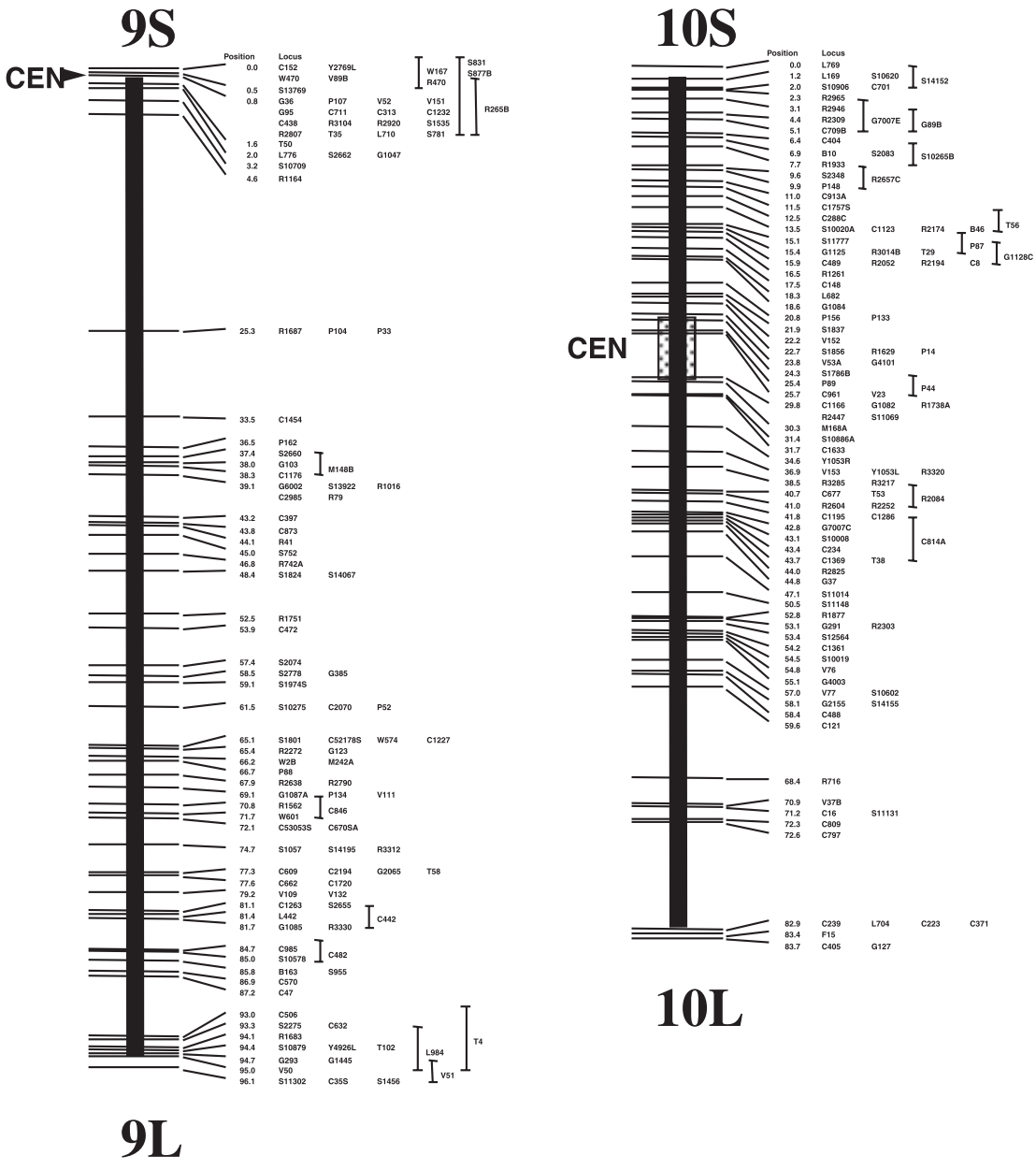


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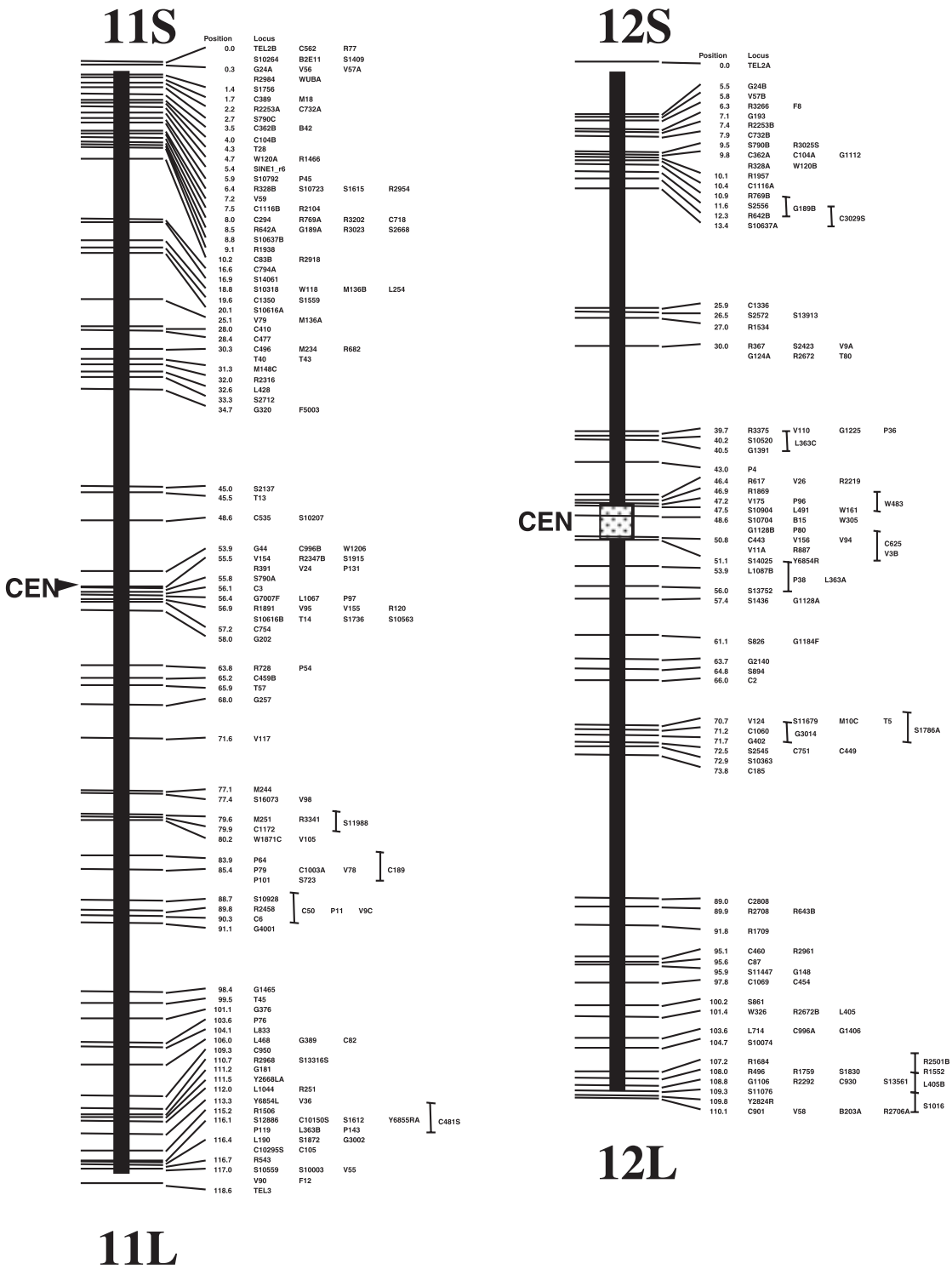


**Fig. 2.** (continued)

indicate the relative position of the gene with respect to other genes in the genome. The second genetic map constructed by the Japanese group (Harushima et al. 1998) is shown in Figs. 2 and 3. The information derived from these maps have become indispensable in gene cloning by providing clues for the tagging of target phenotypes. As in the case of cloning a gene, *Xa21*, for resistance against bacterial blight disease (Song et al. 1995) many mapped cDNAs provided a pivotal point for hitting the target gene. The latest high-density genetic map constructed by the Japanese team consists of 3,267 DNA markers including RFLP, simple sequence repeat (SSR), single-strand conformational polymorphism (SSCP), and cleaved amplified polymorphic sequence (CAPS) markers (http://rgp.dna.affrc.go.jp/publicdata/geneticmap2000/index.html). About 70% of these markers were derived from rice and the remaining from other cereals such as barley, wheat, and maize.

Other genetic maps constructed for rice consist mainly of RFLP markers (Causse et al. 1994; Xiong et al. 1997; Cho et al. 1998). Among them, the map constructed by a team at Cornell University is unique in aiming at the development of universal DNA markers applicable to important cereal crops, such as maize, barley, and oat, other than rice (Causse et al. 1994). This idea was brought about by the fact that there exists colinearity of gene order among these cereals, which belong to the grass family Poaceae. These species diverged from a common ancestor about 60 to 70 million years ago, and current descendants still share common ancestral characteristics in their genomes (Kellog 2001). This collinearity in genetic traits, called synteny among grasses, has been fundamental in rationalizing rice as a model or reference plant among the grass species. In addition, it has the smallest genome size and is one of the most well and deeply studied cereal crops. The rice genetic map by Cornell University carries such DNA markers as to show a distinct hybridization pattern with other major cereals.

The latest genetic map uses markers easily reproduced by polymerase chain reaction (PCR). This characteristic is prerequisite, not only for basic research, but also for the practical application of markers to breeding, such as easy selection of siblings with preferable traits once identified by DNA markers or by the gene itself. RFLP markers are the most accurate because of their codominancy, but it is very tedious and costly to perform Southern hybridization to detect


**Fig. 3.** Rice varieties (*left*: Nipponbare, *right*: Kasalath)

RFLP. On the other hand, PCR is relatively cheaper and easy to use once specific primers for amplification are developed. For satisfying this demand, the best marker, the simple sequence repeat (SSR) marker, has been generated since the beginning of molecular genetic analysis. However, the discovery of Class I SSR, which is less than 20 nucleotides long and shows polymorphism among rice cultivars, requires several laborious steps. Very recently, high-quality genome sequences of japonica cultivar Nipponbare (Feng et al. 2002; Sasaki et al. 2002b; Rice Chromosome 10 sequencing Consortium 2003), a draft sequence of indica cultivar 93-11 (Yu et al. 2002a), and BAC end sequences of indica cultivar Kasalath (Katagiri et al. 2004) have become publicly available. This information offers the opportunity to survey SSRs all over the rice genome and to discover effective SSRs closely linked to target phenotypes. The most extensive and detailed genetic map using SSRs as markers was published in 2002 (McCouch et al. 2002). This map should be useful for map-based cloning and marker-based breeding in the near future.

The molecular genetic map is a required tool for changing the strategy of genetic analysis and breeding of important crops, especially self-pollinating ones.

In the case of corn, which is also a very important cereal crop with a wide range of uses in both industry and agriculture, genetic mapping has been pursued by several private companies. However, these maps have been mainly for in-house use, and the public sector has been rendered to undertake redundant efforts to construct publicly available genetic maps. The University of Missouri-Columbia coordinates the efforts of the public sector and publishes a map with 1,736 loci, including 1,156 loci probed by cDNAs (Davis et al. 1999). Genetic maps have also been constructed for other cereal crops such as wheat (Qi et al. 2004a), barley (Kleinhofs 2004), sorghum (Bowers et al. 2003), and pearl millet (Qi et al. 2004b) by utilizing previously established DNA markers from rice and other crops. For example, wheat and barley are closely related species and DNA markers of both could be shared for mapping. A similar situation is found in the case of mapping of sorghum by using maize DNA markers as a common tool. The detailed genetic map information with images of polymorphisms is now available through Internet databases such as GrainGenes (http://wheat.pw.usda.gov) or Gramene (http://www.gramene.org).

Sharing DNA markers derived from cDNAs of each target cereal species could reveal the existence of significant remnants of ancestral genome structure (Ahn and Tanksley 1993; Moore et al. 1993). The family Poaceae is thought to have diverged about 60 to 70 million years ago from an ancestor common to many current grass species (Kellog 2001). During this long period, each species evolved to adapt to each habitat under natural and, in the case of cultivated species, artificial selection pressures. Molecular genetic analysis using expressed genes as tools for mapping could prove the existence of their common ancestry, although they have undergone different evolutionary pathways. The existence of colinearity among the grass species could be very useful in clarifying the existence of one gene in other species with a syntenous genome structure. For example, the orthologous genes of a waxy gene on rice chromosome 6 are found on chromosome 9 of maize and chromosome 7 of wheat, which are proved to be a syntenic part of these genomes (Devos and Gale 1997). This was first shown in 1993 based on genetic mapping or recombination events. Subsequently, many researchers have sought to apply synteny to tag the phenotype or to isolate the gene in wheat, barley, or maize corresponding to a similar phenotype in rice. The success or failure of this strategy is highly dependent on the existence of true synteny at the target genomic region and the extent of saturation of DNA markers in the genetic map used for analysis. Recent progress in genome sequencing of the whole rice genome and partial maize genome enables detailed evaluation of synteny between them (Lai et al. 2004). Also, genome sequences of a limited genomic area of sorghum (Draye et al. 2001), barley (Dubcovsky et al. 2001; Caldwell et al. 2004), and wheat (Feuillet and Keller 1999) were used for this evaluation. As a result, microlevel synteny based on sequence comparison is in most cases not valid because of rearrangement, insertion by transposable element, or translocation to other chromosomes (Bennetzsen and Ma 2003). However, synteny observed by mapping of orthologous genes is still very important for interpreting the evolution of Poaceae and to understand microlevel synteny as a clue for research.

## 1.3 Molecular Mapping of Simple and Complex Traits in Rice

In comparison to the classical morphological markers and isozymes, DNA markers are now becoming an essential tool for genetic investigations because of ability to generate and track an unlimited number of loci that can be linked to any trait of interest. Aside from RFLP, a variety of DNA markers such as RAPD (Williams et al. 1990), SSR (Litt and Lutty 1989), sequence tagged sites (STS) (Olson et al. 1989), sequence characterized amplified region (SCAR) (Martin et al. 1991), CAPS (Koniecyzn and Asubel 1993), and AFLP (Vos et al. 1995) have been developed. Unlike RFLP, most of these recently developed markers are PCR-based with simplified protocols and require minute quantities of DNA. However, the dominant nature of some PCR markers like RAPD and AFLP makes distinguishing homozygotes from heterozygotes difficult. Currently, SSR markers are the most preferred class of markers for marker-assisted selection (MAS) because of its codominant nature, simpler protocols, abundance, and higher level of polymorphism. Due to the availability of considerable amounts of sequence data, single nucleotide polymorphism (SNP) (Brookes 1999) is gaining momentum as an excellent tool to navigate the genome due to its simplicity, abundance, and amenability for automation. A number of softwares such as Mapmaker/QTL (Lincoln et al. 1992), Qgene (Nelson 1997), QTL map-

per (Wang et al. 1999a), QTL Cartographer (Basten et al. 2001), PLABQTL (Utz and Melchinger 1996), and MQTL (Tinker and Mather 1995) have been developed to detect quantitative trait loci (QTL). DNA markers and their usefulness in crop improvement have been widely reviewed (Paterson et al. 1991; Burrow and Blake 1998; Brar 2002; Subudhi and Nguyen 2004). In this section, we will provide an update on molecular marker utilization to investigate both simple and complex traits in rice.

### 1.3.1 Disease Resistance

The advent of molecular markers greatly facilitated genetic analysis of disease resistance genes in rice. In the case of rice blast (*Magnaporthe grisea*) and bacterial leaf blight (BLB) (*Xanthomonas oryzae* pv. *oryzae*), a large number of major genes had been earlier identified by classical genetic studies and thus targeted for mapping investigations using a variety of marker systems and approaches. Besides blast and bacterial leaf blight, sheath blight caused by *Rhizoctonia solani* Kühn also limits rice productivity significantly. Few reports are available on the mapping of genes responsible for resistance to sheath blight, rice yellow mottle virus, stem rot, bacterial leaf streak, rice stripe disease, and rice tungro virus.

In most of these studies, either near isogenic lines (NIL) or bulked segregant approach (BSA) (Michelmore et al. 1991) was extensively preferred for identifying markers linked to the resistant genes. Additionally, segregating populations were developed from crosses involving resistant and susceptible cultivars to develop closely linked markers for MAS. Most of the BLB resistance genes are major genes, and though a similar trend was followed for some time to analyze blast resistance, more emphasis is currently given to identify QTL for partial resistance that, in combination with major genes, can improve the durability of resistance. The candidate-gene approach is also demonstrated as an efficient way of mapping resistance genes or resistance QTL in rice (Wang Z et al. 2001).

#### Bacterial Leaf Blight

To date, more than 20 resistance genes against various strains of *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*) have been identified. Many of those genes have been assigned to rice chromosomes using molecular markers (Table 3). Mapping of these genes facilitated pyramiding of a number of genes through MAS providing a higher degree of resistance in rice-breeding programs. Physical mapping, cloning, and characterization of resistant genes were also possible in many cases. Despite the clustering of several bacterial blight resistance genes on chromosome 11 along with some blast resistance genes, clear identity of those genes has been demonstrated by their unique location on the rice chromosomes. Other BLB resistance genes were mapped on rice chromosomes 4, 5, 6, and 8. To date, three BLB resistance genes, *Xa-1*, *Xa-21*, and *Xa-26*, have been isolated by map-based cloning (Song et al. 1995; Yoshimura et al. 1998; Sun et al. 2004). High-resolution genetic maps of the *Xa-7*- and *Xa-27(t)*-carrying genomic regions have been constructed to expedite cloning of these genes (Porter et al. 2003; Gu et al. 2004). Considerable progress has been made in the physical mapping of BLB resistance genes: *Xa-4* (Wang W et al. 2001; Sun et al. 2003), *xa-5* (Yang et al. 1998; Blair et al. 2003),*xa-13* (Sanchez et al. 1999), and *Xa-22 (t)* (Wang et al. 2003a). BAC clones carrying these resistant genes have been identified or candidate genes have been identified from sequence information obtained from the target BAC clone.

Though most of the genes discussed above involve qualitative resistance, quantitative component of resistance was also investigated (Li et al. 1999, 2001c). Using NIL sets for four BLB genes, *Xa-4*, *xa-5*, *xa-13*, and *Xa-21*, Li et al. (2001a) demonstrated that a qualitative component of the resistance genes is reflected by their large effects against corresponding avirulent *Xoo* races and the quantitative component is their residual effect against corresponding virulent races and their epistatic effects. Another study by Li et al. (1999a) involving a recombinant inbred line (RIL) population from a cross Teqing × Lemont and three strains of *Xoo*, CR4, CR6, CXO 8, revealed several QTL for resistance, and interestingly a major gene, *Xa-4*, was mapped onto chromosome 11. Lemont was susceptible to all three strains while Teqing was resistant to CR4 and CX8 but susceptible to CR6. Teqing allele at the *Xa-4* locus behaved like a dominant resistance gene against CR4 and CXO8.

#### Blast

More than 40 major blast resistance genes have been identified and deployed in rice-breeding programs. Despite considerable progress in the mapping and identification of a number of blast resistance genes

**Table 3.** Molecular marker facilitated tagging of disease resistance genes in rice

| Gene | Source of resistance | Linked markers | Chromosome | Reference |
|---|---|---|---|---|
| **Bacterial blight** | | | | |
| *Xa-1* | Kogyoku | XNpb 235 | 4 | Yoshimura et al. 1996 |
| *Xa-3* | Chugoku45 | XNpb 181 | 11 | Yoshimura et al. 1995 |
| *Xa-4* | IR20 | XNpb 181 | 11 | Yoshimura et al. 1995 |
| *xa-5* | IR1545-339 | RG 556 | 5 | Yoshimura et al. 1995 |
| *Xa-7* | IRBB7 | M5 | 6 | Porter et al. 2003 |
| *Xa-10* | IRBB10 | CDO365 | 11 | Yoshimura et al. 1995 |
| *xa-13* | IR66699-5-5-4-2 | RG 136 | 8 | Zhang et al. 1996a |
| *Xa-21* | *O. longistaminata* | RG 103 | 11 | Ronald et al. 1992 |
| *Xa-22(t)* | Zhachanglong | R1506 | 11 | Lin et al. 1996b |
| *Xa-26(t)* | Minghui 63 | R1506, M224 | 11 | Yang et al. 2003 |
| *Xa-27(t)* | *O. minuta* | M631/M1230 | 6 | Gu et al. 2004 |
| **Blast** | | | | |
| *Pi-1* | LAC23 | RZ536 | 11 | Yu et al. 1996 |
| *Pi-2* | 5173 | RG64 | 6 | Yu et al. 1991 |
| *Pi-4* | Tetep | RG869 | 12 | Yu et al. 1991 |
| *Pi-5* | Moroberekan | RG498 | 4 | Wang et al. 1994 |
| *Pi-7* | Moroberekan | RG16, G103A | 11 | Wang et al. 1994 |
| *Pi-10* | Tongil | RRF6, RRH18 | 5 | Naqvi et al. 1995 |
| *Pi-11 (Pi-zh)* | Zhai-Ye-Qing 8 | BP127 | 8 | Zhu et al. 1993 |
| *Pi-12* | Hong-jiao-zhan | RG869 | 12 | Zheng et al. 1996 |
| *Pi-18* | Suweon 365 | RZ536 | 11 | Ahn et al. 2000 |
| *Pi-20* | IR24 | XNpb88 | 12 | Imbe et al. 1997 |
| *Pi-21* | Owarihatamochi | G271/G317 | 4 | Fukuoka and Okuno 2001 |
| *Pi-24(t)* | Azucena | K5 | 1 | Sallaud et al. 2003 |
| *Pi-25(t)* | IR64 | RG520 | 2 | Sallaud et al. 2003 |
| *Pi-26(t)* | Azucena | RG313 | 5 | Sallaud et al. 2003 |
| *Pi-27(t)* | IR64 | Est-2 | 6 | Sallaud et al. 2003 |
| *Pi-28(t)* | Azucena | RZ617/RGA-IR86 | 8 | Sallaud et al. 2003 |
| *Pi-29(t)* | IR64 | RZ500 | 10 | Sallaud et al. 2003 |
| *Pi-30(t)* | IR64 | OpZ11-f RGA-IR14 | 11 | Sallaud et al. 2003 |
| *Pi-31(t)* | IR64 | O10-800 | 12 | Sallaud et al. 2003 |
| *Pi-32(t)* | IR64 | AF6 | 12 | Sallaud et al. 2003 |
| *Pi-33* | IR64, Bala | R1813 | 8 | Berruyer et al. 2003 |
| *Pi-44* | RIL276 from Co 39 × Moroberekan | CDO520 | 11 | Chen et al. 1999 |
| *Pi-b* | Tjahaja | RZ213 | 2 | Miyamoto et al. 1996 |
| *Pi-ta, Pi-ta*$^2$ | Tadukan | XNpb088 | 12 | Rybka et al. 1997 |
| *Pi-k*$^m$ | Tsuyuake | G181 | 11 | Kaji and Ogawa 1996 |
| *Pi-ar* | SC09 | OPK17 | – | deAraújo et al. 2004 |
| *Pi-g(t)* | Guangchangzhan | RM166, RM208 | 2 | Zhou et al. 2004 |
| **Leaf and neck blast** | | | | |
| *Pi-24(t)* | Zhong 156 | $RGA3_{620}$ | 12 | Zhuang et al. 2002 |
| *Pi-25(t)* | Gumei 2 | $RGA7_{470}$ | 6 | Zhuang et al. 2002 |
| **Panicle blast** | | | | |
| *Pb-1* | – | CDO 226 | 11 | Fuji et al. 1995 |
| **Rice Stripe disease** | | | | |
| *Stv-b(i)* | Modan | ST10, XNpb257 | 11 | Hayano-Saito et al. 1998 |

**Table 3.** (continued)

| Gene | Source of resistance | Linked markers | Chromosome | Reference |
|---|---|---|---|---|
| **Rice Tungro Spherical virus** | | | | |
| *RTSV* | ARC11554 | RZ262 | 4 | Sebastian et al. 1996 |
| **Yellow mottle virus** | | | | |
| *RYMV* | Azucena | RG341/RG869 | 12 | Ghesquiere et al. 1997 |

(Table 3), only two such resistance genes, *Pi-b* and *Pi-ta*, have been isolated and characterized (Wang et al. 1999b; Bryan et al. 2000). Due to the mapping of several blast resistance genes to the same rice genomic region, there is confusion regarding the identity of those genes. For example, the allelic relationship between *Pi-4* and *Pi-ta* is not clear (Inukai et al. 1994). Rybka et al. (1997) could not separate the genes *Pi-ta* and *Pi-ta2* even in a large mapping population. Physical mapping is now revealing information on the allelic nature of these genes. A physical mapping study by Jeon et al. (2003) suggested that *Pi-3(t)* and *Pi-5(t)* are the same resistance gene. Similarly, a detailed physical map of the *Pi-2(t)* (Jiang and Wang 2002; Liu et al. 2002a) revealed that *Pi-9(t)* and *Pi-2(t)* are either allelic or tightly linked.

The first comprehensive QTL mapping study by Wang GL et al. (1994) elucidated the number and nature of blast resistance genes. Ten QTLs conferring partial resistance based on the number of lesions, lesion size, or the diseased leaf area (DLA) were mapped in an RIL population developed from a cross Moroberekan (with durable resistance) × Co 39 (susceptible). Two dominant blast resistance genes, *Pi-5(t)* and *Pi-7(t)*, were associated with these QTLs for partial resistance. The researchers' data suggest that stable resistance in Moroberekan is due to the combination of genes conferring both partial and complete resistance, and some QTL for partial resistance may be alleles of the major resistant loci. Since then, QTLs for field resistance to blast have been identified using several different mapping populations (Fukuoka and Okuno 2001; Sirithunya et al. 2002; Tabien et al. 2002; Sallaud et al. 2003; Talukder et al. 2004; Wu et al. 2004). Sallaud et al. (2003) mapped nine unlinked loci [*Pi-24(t)* to *Pi-32(t)*] in a double haploid (DH) population of the cross IR64 × Azucena. A major gene conferring partial resistance against leaf blast has been demonstrated (Zenbayashi et al. 2002).

Several conclusions can be drawn from these mapping studies involving blast and bacterial blight resistance. (1) Although breeding blast-resistant cultivars through deployment of these major resistance genes is simple, the major limitation is the lack of durability, which can be improved by pyramiding of multiple resistance genes. Both QTL and major genes are required for durable resistance. (2) Since many of these QTL were localized in the vicinity of many major resistance genes, it reinforces the hypothesis that QTL and major genes are probably different alleles of the same loci (Robertson 1985). (3) Partial resistance genes might be defeated major genes with residual effectiveness and race specificity.

## Sheath Blight

The major obstacle in breeding rice cultivars resistant to sheath blight disease is lack of resistance sources. Quantitatively inherited resistance can be helpful in protecting rice crop from this disease in field conditions. The first QTL study was conducted by Li et al. (1995a) in an $F_4$ bulked population from a cross between the susceptible variety "Lemont" and the resistant variety "Teqing". Six QTLs contributing to resistance were located on six chromosomes and collectively explained approximately 47% of the phenotypic variation. Except for one QTL (*QSbr4a*), which accounted for 6% of the genotypic variation, the other five putative resistance loci (*QSbr2a, QSbr3a, QSbr8a, QSbr9a,* and *QSbr12a*) colocalized with QTLs for morphological traits.

Zou et al. (2000) used an $F_2$ clonal population of another cross Jasmine 85 × Lemont and, based on field disease evaluations for 2 years, six QTLs, *qSB-2, qSB-3, qSB-7, qSB-9-1, qSB-9-2*, and *qSB-11*, were located on chromosomes 2, 3, 7, 9, and 11, respectively. The QTLs *qSB-2, qSB-3, qSB-7*, and *qSB-9-2* from Jasmine 85 explained 21.2%, 26.5%, 22.2%, and 10.1% of the total

phenotypic variation, respectively; while *qSB-9-1* and *qSB-11* from Lemont were responsible for 9.8% and 31.2% of the total phenotypic variation. Contrary to the observation of Li et al. (1995a), this study did not demonstrate any linkage of detected resistance loci to the loci for heading date or plant height. A dominant sheath blight resistant gene *Rsb1,* carried by a transgenic cultivar "4011," was mapped recently on rice chromosome 5 near RM39 (Che et al. 2003).

### Rice Yellow Mottle Virus , Stem Rot , Bacterial Leaf Streak , and Rice Stripe Disease

Ghesquiere et al. (1997) identified a QTL for rice yellow mottle virus (RYMV) resistance on chromosome 12 in two DH rice populations developed from crosses IR64 × Azucena and IRAT177 x Apura, and it corresponded to regions known to harbor major blast resistance genes. In the former DH population, Albar et al. (1998) detected 15 QTLs for RYMV resistance on seven chromosomes, and most of the resistant QTL alleles were from the resistant parent "Azucena". Resistance was correlated to plant morphology. There was one QTL of resistance on chromosome 12 independent of plant morphology that interacted with a QTL on chromosome 7 to control the virus content (Pressoir et al. 1998).

A selective genotyping approach was used to map two loci for resistance to stem rot (*Sclerotium oryzae*) in populations developed from the crosses between an *O. rufipogon* derived resistant line and susceptible line (Ni et al. 2001). These two loci on chromosome 2 (AFLP marker TAA/GTA167 and near RZ166 and RG139) and chromosome 3 (near RM232) jointly explained 50% of the phenotypic variation.

Using both $F_2$ and RIL population tested over 2 years, Tang et al. (2000) mapped 11 QTLs conferring resistance to bacterial leaf streak on six chromosomes. Six of the QTLs were detected in both seasons. Five QTLs with the largest effects were significant in both seasons. The detected QTLs explained 85% of the genetic variation in 1997. Bulked segregant analysis of the extremes of the $F_2$ population identified three QTLs of large effect.

Graphical genotyping and linkage analyses with molecular markers were used by Hayano-Saito et al. (1998), who determined the chromosomal location of the rice stripe disease resistance gene *Stvb(i)* from indica rice cv "Modan" on chromosome 11 between XNpb220 and XNpb257/XNpb254. A tightly linked marker, ST10, was developed on the basis of the results of RAPD analysis for MAS. Hayano-Saito et al. (2000) also physically mapped *Stvb(i)* in an approximately 286-kb region covering two overlapping BAC clones.

### 1.3.2 Insect Resistance

Major insect pests of rice include gall midge, stem borer, brown plant hopper, and green leafhopper. While many of these insects damage rice crop by feeding others, particularly leafhoppers, act as vectors of many viruses, spreading viral diseases in rice crop. Host plant resistance is an ideal and environmentally friendly approach to lessening the damage to rice crop, and a large number of germplasms with resistance to various insect species have been identified accordingly, and inheritance of insect resistance has been elucidated (Khush and Brar 1991).

Progress has been made in breeding for resistance to gall midge and different plant and leafhoppers (Table 4). For stem borer, a damaging pest in most rice-growing areas of the world, a resistance source has been rare in available germplasms. Selvi et al. (2002), however, identified RAPD markers $K6_{695}$ and $AH5_{660}$ linked to yellow stem borer resistance at distances of 12.8 cM and 14.9 cM, respectively, using BSA.

Tan et al. (2004) mapped two white-backed plant hopper (WBPH) resistant genes, which are the same as *Qbp1* and *Qbp2* genes for brown plant hopper resistance in an RIL population from B5 × Minghui 63. Of the two WBPH resistance genes, one designated as *Wbph7(t)* was located within a 1.1-cM region between R1925 and G1318 on chromosome 3, and the other designated as *Wbph8(t)* was within a 0.3 cM region flanked by R288 and S11182 on chromosome 4. Yamasaki et al. (1999) used ovicidal response as a criterion for resistance against WBPH and mapped the traits, percentage of watery lesions (PWL), and WBPH egg mortality (EM) in an RIL population developed from a cross of japonica cultivar Asominori and indica cultivar IR24. Out of a total of 10 QTLs for ovicidal response, QTL on chromosome 6 (R1954-L688) was most significantly associated with the ovicidal response and accounted for 69.9% of phenotypic variance for PWL and 46% of phenotypic variance for EM.

The first green leafhopper mapping study by Sebastian et al. (1996) revealed a dominant gene conferring resistance to GLH and RTSV located within 5.5 cM of RFLP marker RZ 262 on rice chromosome 4.

**Table 4.** Molecular-marker-facilitated tagging of insect resistance genes in rice

| Resistance Gene | Source | Linked markers | Chromosome | Reference |
|---|---|---|---|---|
| **Gall midge** | | | | |
| *Gm2* | Phalguna, ARC6650 | RG329, RG476 | 4 | Mohan et al. 1994 |
| *Gm4(t)* | Abhaya | R1813 | 8 | Mohan et al. 1997b |
| *Gm6(t)* | Duokong #1 | RG214 | 4 | Katiyar et al. 2001a |
| **Brown plant hopper** | | | | |
| *Bph1* | Mudgo | XNpb248, em5814N, em2802N | 12L | Hirabayashi and Ogawa 1995; Sharma et al. 2003 |
| *Bph?* | IR64 | Sdh-1, RG463 | 12 | Huang et al. 1997b |
| *Bph10* | *O. australiensis* | RG457 | 12 | Ishii et al. 1994 |
| *Bph (t)* | Sanguizhan | RZ404 | 9 | Mei et al. 1996 |
| *bph2* | IR1154-243 | KAM4 | 12L | Murata et al. 1998; Murai et al. 2001 |
| *Bph9* | Pokkali | OPR04 | 12L | Murata et al. 2000 |
| **White backed plant hopper** | | | | |
| *Wbph-1* | – | RG146B | 7 | McCouch et al. 1991 |
| *Wbph?* | Asominori | R1954, L668 | 6 | Yamasaki et al. 1999 |
| *Wbph?* | IR64 | RG103, RG167 | 11 | Kadirvel et al. 1999 |
| *Wbph7(t)* | B5 | R1925, G1318 | 3 | Tan et al. 2004 |
| *Wbph8(t)* | B5 | R288, S11182 | 4 | Tan et al. 2004 |
| **Green leaf hopper and Green rice leaf hopper** | | | | |
| *GLH* | ARC11554 | RZ262 | 4 | Sebastian et al. 1996 |
| $Glh_{Ib1}$ | Ptb8 | $OPA19_{320}$ | – | Padmavathi et al. 2001 |
| *Grlp3/Grh4* | DV85 Norin PL-6 | XNpb144 | 3 | Yasui and Yoshimura 1999 Fukuta et al. 1998 |
| *Grlp11/Grh2* | DV85 Norin PL-6 | G1465 | 11 | Yasui and Yoshimura 1999 Fukuta et al. 1998 |
| *Grh1* | IR24 | C309 | 5 | Yasui and Yoshimura 1999 |
| | Norin PL2 | R566 | 5 | Tamura et al. 1999 |

Padmavathi et al. (2001) mapped a gene $glh_{Ib1}$ of Ptb8 conferring resistance to the Indian biotype of green leafhopper using RAPD markers. The resistance locus was closely linked with three QTLs controlling total tiller number, effective tiller number, and 100-grain weight. Wang et al. (2004) recently conducted a QTL mapping study of antibiosis to green leafhopper in an RIL population of rice developed from the cross Taichung65 (susceptible) × ARC10313 (resistant) and identified four QTLs on chromosomes 3, 5, 11, and 12. Two major QTLs on chromosomes 3 and 11 explained 25.3% and 56.8% of phenotypic variance, respectively, and were localized close to two green rice leafhopper (*Nephotettix cincticeps*) resistance genes, *Grh4* and *Grh2*, mapped earlier onto the same position using different sources (Fukuta et al. 1998; Yasui and Yoshimura 1999).

Thirteen biotypes of gall midge (*Orseolia oryzae* Wood-Mason) and seven gall midge resistance genes are reported in the literature (Sardesai et al. 2001). Despite the emergence of new biotypes, the development of resistant varieties has been possible due to the involvement of a single dominant gene in most cases and the identification of resistance sources among available germplasms. Several *Gm* genes have been tagged with molecular markers (Table 4), and MAS is practiced in some cases (Sardesai et al. 2001). *Gm2* is the first example of a mapped gall midge resistant

gene from "Phalguna" on chromosome 4 that confers resistance to biotypes 1, 2, and 5 (Mohan et al. 1994). Another gall midge resistant gene *Gm4(t)* from the source "Abhaya" was mapped on chromosome 8 (Mohan et al. 1997b). Katiyar et al. (2001a) mapped *Gm6(t)* from Chinese rice cultivar Duokang # 1, which confers resistance against four biotypes of Asian rice gall midge in China on chromosome 4, and demonstrated that it is nonallelic to *Gm2* located in its vicinity (Mohan et al. 1994). *Gm6(t)* was later pyramided with *Gm2* by traditional breeding (Katiyar et al. 2001b). YAC and BAC clones encompassing the genes have been identified as being able to clone *Gm2* and *Gm6(t)* (Rajyashri et al. 1998; Katiyar et al. 2001a).

At least 12 major brown plant hopper (BPH) (*Nilaparvata lugens* Stål) resistance genes have been identified in indica rice cultivars and two wild species of rice, *O. australiensis* and *O. officinalis*. *Bph10* from *O. australiensis* was mapped onto chromosome 12 (Ishii et al. 1994). Jena et al. (2003) and Renganayaki et al. (2002) mapped two BPH genes resistant to an Indian biotype and biotype 4 onto chromosomes 11 and 3, respectively, using lines with resistance genes introgressed from *O. officinalis*. In both cases, BSA was used in conjunction with RAPD markers. One of these genes was designated *Bph13 (t)* (Renganayaki et al. 2002). *Bph1* locus was mapped onto chromosome 12 at a distance of 10.7 cM from XNpb248 (Hirabayashi and Ogawa 1995) and mapped near *bph2* (Murata et al. 1998). A high-resolution map of this region (Murai et al. 2001) demonstrated that *Bph1* and *bph2* were nonallelic, and an AFLP marker KAM4 completely cosegregated with *bph2*. Four additional genes, *bph4, bph9, bph11(t),* and *bph12(t),* have been mapped onto chromosomes 6, 12, 3, and 4, respectively (Hirabayashi et al. 1998, 1999; Murata et al. 2000; Kawaguchi et al. 2001).

The quantitative nature of resistance to BPH has been demonstrated in a number of studies (Alam and Cohen 1998; Yamasaki et al. 2000; Huang et al. 2001; Xu XF et al. 2002). Alam and Cohen (1998) first reported the mapping of seven QTLs associated with resistance to two Philippine BPH populations in a DH population developed from the cross IR64 × Azucena. These QTLs are located on chromosomes 1, 2, 3, 4, 6, and 8 and individually accounted for 5.1 to 16.6% of the phenotypic variance. Most of these QTLs were derived from IR64 and conferred a relatively durable resistance under field conditions. Yamasaki et al. (2000) used an RIL population derived from a cross between a japonica variety Asominori with ovicidal response and an indica variety IR24 without ovicidal response and detected two QTLs each on 1L and 6S for both grades of watery lesions (GWL) and egg mortality (EM). The 6S QTL explained 72.1% and 85.1% of the phenotypic variance for GWL and EM, respectively. The QTL on 1L explained 19.8% and 17.8% of the phenotypic variations for GWL and EM, respectively. Both alleles from Asominori increased GWL and EM. The Asominori allele at the 6S QTL was essential for the ovicidal response to BPH, and the Asominori allele at the 1L QTL could increase the EM of BPH in the presence of the Asominori allele at the 6S QTL. Two RFLP loci, R1954 linked to 6S QTL and C112 linked to 1L QTL, can be used for MAS. Using $F_3$ families from a B5 × Minghui 63 cross, Huang et al. (2001) identified two QTLs on chromosome 3 (*Qbp1*) and chromosome 4 (*Qbp2*) for BPH resistance that explained 26.4% and 14.3% of the phenotypic variation and are different from at least nine of the ten previously identified BPH resistance genes. Xu et al. (2002b) mapped seven main-effect QTLs and many epistatic QTL pairs onto 12 rice chromosomes in an RIL population from the Lemont × Teqing cross. The main-effect and epistatic QTLs together accounted for more than 70% of the total phenotypic variation in damage scores. Teqing contributed the resistance allele at four main-effect QTLs, and the Lemont allele resulted in resistance at the other three. The Teqing allele controlling leaf and stem pubescence was associated with resistance, while the Lemont allele for glabrous stem and leaves was associated with susceptibility, indicating that this gene might have contributed to resistance through antixenosis. These studies revealed that there are many major genes and QTLs on the rice genome that are conferring BPH resistance and should be pyramided to provide durable resistance to this pest.

### 1.3.3 Traits Relevant for Hybrid Rice Breeding

Development of semidwarf rice varieties beginning in the early 1960s was a significant accomplishment in improving rice productivity in all rice-growing areas. With growing demand for more food for the increasing world population, new strategies need to be developed to further elevate the stagnant rice productivity plateau. Exploitation of heterosis is one such strategy that has been demonstrated well in China to shift the yield ceiling beyond the level of current semidwarf rice cultivars (Yuan et al. 1994). Although yield advantage of hybrids is about 20% over the inbred va-

rieties, the high cost of hybrid seeds is reducing the profit margins of rice farmers. Many challenges and opportunities still exist to reap the benefits of this technology in rice. A number of genetic tools, such as cytoplasmic genetic male sterility, environment-sensitive genic male sterility, and wide compatibility, are being employed and refined to facilitate hybrid rice breeding. Significant progress has been made on molecular marker utilization to accelerate hybrid rice breeding (Table 5).

### CMS-Fertility Restoration

Cytoplasmic genetic male sterility (CMS) is the most effective male sterility system to produce hybrid seeds in rice (Virmani 1996). Despite the discovery of numerous CMS systems, the wild-abortive (WA) CMS is still commonly used in commercial rice hybrids because it gives stable CMS lines for which fertility restorers are available in abundance (Virmani 1999). The usefulness of other CMS systems (CMS-TN, CMS-MS577, CMS-*O. perrenis*, CMS-O. *glumaepetula*) has been limited because of the nonavailability of restorer lines in developing rice hybrids. Reports regarding the number, chromosome location, and effects of fertility restorer genes are conflicting. Bharaj et al. (1995), from a trisomic analysis, reported the involvement of two *Rf* loci on chromosomes 7 and 10. Using a population from the cross of two isogenic lines, Zhang et al. (1997) mapped one locus *Rf3* on chromosome 1. Another study by Yao et al. (1997) using an $F_2$ population of a cross between Zhengshan 97A and Minghui 63 identified two loci on chromosomes 1 and 10, and the locus on chromosome 1 was the same as that reported by Zhang et al. (1997). Since then, Ichikawa et al. (1997) mapped a restorer locus for BT-CMS system in a region near by, but further investigation is needed to clarify whether it is same as or different from that of Yao et al. (1997). Tan et al. (1998) used QTL strategy to map two restorer loci on chromosome 10, of which one QTL linked to marker C1361 explained 71.5% of the phenotypic variance, and the second QTL located between RFLP makers R2309 and RG257 explained 27.3% of the phenotypic variance. As many as four loci on chromosomes 2, 3, 4, and 5 were associated with fertility-restoring ability to WA cytoplasm (Zhu et al. 1996). Liu et al. (2004b) studied a novel type of gametophytic CMS system, called Honglian CMS (CMS-HL) used in hybrid rice production in China, and mapped two fertility restorer loci, *Rf5* and *Rf6(t),* on chromosome 10. A rice nuclear gene *Rf-1* was recently fine mapped and has been subsequently cloned (Komori et al. 2003, 2004).

### Environmental Genetic Male Sterility

Commercial production of hybrid seeds in CMS systems is cumbersome due to the involvement of three different lines, A (male sterile), B (maintainer), and R (fertility restorer) lines. Moreover, application of a CMS system is limited in germplasm in which maintainer and restorers are scarce. Due to the discovery of nuclear sterility factors that are regulated by environmental factors, viz., temperature and/or photoperiod, simplification of hybrid seed production is now possible. Several temperature-sensitive genetic male sterile (TGMS) lines have been developed through irradiation (Virmani 1996). These mutants are male sterile under high temperature but revert to partial to full fertility under low-temperature conditions. In all TGMS lines, male sterility is controlled by a single recessive gene (Virmani 1999). So far, seven TGMS genes have been mapped on rice chromosomes 8, 7, 6, 2, 9 (Wang et al. 1995; Subudhi et al. 1997; Yamaguchi et al. 1997; Koh et al. 1999; Dong et al. 2000; Reddy et al. 2000; Wang et al. 2003b) (Table 5). A reverse TGMS gene in line J207S was mapped using the AFLP technique combined with BSA (Jia et al. 2001). This *rtms1* gene was mapped between RM239 and RG257 with a genetic distance of 3.6 cM and 4.0 cM, respectively. The reverse-TGMS rice exhibits sterility at lower temperature and will have applications in a much larger area. AFLP and RAPD markers were utilized in this study to tag the genes, and then the linked markers were mapped onto specific rice chromosomes using a reference mapping population.

Male fertility in photoperiod-sensitive genetic male sterile (PGMS) rice is regulated by photoperiod length. The first PGMS rice was a spontaneous mutant in japonica rice cultivar Nongken58. PGMS rice can be multiplied under short-day conditions but is to be planted under long-day conditions to produce hybrid seeds. Zhang et al. (1994a) used bulked DNA from the extreme fertile and extreme sterile individuals of a large $F_2$ mapping population developed from a cross 32001S (PGMS line) $\times$ Minghui 63 and mapped two loci, *pms1* and *pms2,* on chromosome 7 and 3, respectively. The effect of *pms1* was two to three times larger than that of *pms2,* and the dominance was almost complete at both loci. The PGMS line "32001S" was developed by transferring the PGMS trait from the original Nongken58S. Later, Mei et al. (1999a,b) used

**Table 5.** Molecular-marker-facilitated tagging of genes for hybrid breeding in rice

| Genes | Source | Linked markers | Chromosome | References |
|---|---|---|---|---|
| **TGMS** | | | | |
| *tms1* | 5460S | RZ562 | 8 | Wang et al. 1995 |
| *tms2* | Norin PL12 | R643A, R2440 | 7 | Yamaguchi et al. 1997 |
| *tms3* | IR32364TGMS | RZ144 | 6 | Subudhi et al. 1997 |
| *tms4* | TGMS-VN1 | RM27 | 2 | Dong et al. 2000 |
| *tms5* | AnnongS-1 | C365-1 | 2 | Wang YG et al. 2003 |
| *tgms* | SA2 | RM257 | 9 | Reddy et al. 2000 |
| **Reverse TGMS** | | | | |
| *rtms1* | J207S | RM239 | 10 | Jia et al. 2001 |
| **PGMS** | | | | |
| *pms-1* | 32001S | RG477 | 7 | Zhang et al. 1994a |
| *pms-2* | 32001S | RG191 | 3 | Zhang et al. 1994a |
| *pms-3* | Nongken58S | C751, RZ261 | 12 | Mei et al. 1999a,b |
| **Male sterility** | | | | |
| *ms-h(t)* | Hwacheong ms-h | RG451, RZ404 | 9 | Koh et al. 1999 |
| **Fertility restorer (BT)** | | | | |
| *Rf-1* | MTC10R | OSRRF | 10 | Akagi et al. 1996 |
| *Rf2* | IR64 | CDO686 | 2 | Yang et al. 1997 |
| **Fertility restorer (WA)** | | | | |
| *Rf-3* | IR24 | RG532 | 1 | Zhang et al. 1997 |
| *Rf?* | IR24 | C1361 | 10 | Tan et al. 1998 |
| *Rf5* | T24 | RG374, RG394 | 1 | Shen et al. 1998 |
| *Rfu* | Minghui 63 | C4003 | 10 | Yao et al. 1997 |
| **Fertility restorer (HL)** | | | | |
| *Rf5* | MY23 | RM3150 | 10 | Liu et al. 2004b |
| *Rf6(t)* | 93-11 | RM5373 | 10 | Liu et al. 2004b |
| **Wide compatibility** | | | | |
| $S-5$ | 02428 | R2349 | 6 | Liu et al. 1997 |
| $S-5$ | Nekken 2 | RG213 | 6 | Yanagihara et al. 1995 |
| **Hybrid breakdown** | | | | |
| *Hwd1* | Siborunauli, Col.No.15 | C701, R2309 | 10 | Fukuoka et al. 1998 |
| *Hwd1* | Siborunauli, Col.No.15 | C796B, R1382, C492, C145 | 7 | Fukuoka et al. 1998 |

the PGMS line Nongken58S in two crosses and identified two PGMS loci. One locus was *pms1,* identified previously by Zhang et al. (1994b), and the second locus designated as *pms3* was on chromosome 12, which was later fine mapped by Li et al. (2001a). Both had a strong effect on fertility and behaved like a pair of duplicated genes in controlling sterility. A comparison of the *pms3* region between Nongken58S and 32001S indicated that there was no transfer of this region from Nongken58S. This implies that transfer of a complete set of PGMS genes is not necessary for the development of PGMS lines. On the contrary, Wang et al. (1997) showed that a mutation on the *pms1* locus did not result in the sterility of the PGMS line Nongken58S. Another study by He et al. (1999b) revealed that both stability of sterility and reversibility of fertility are the joint effects of the additive effects of the QTL and additive-by-additive components of two-locus interactions.

## Wide-Compatibility Genes

To enhance the level of heterosis for yield in rice, intersubspecific crosses (e.g., indica/japonica) were

proposed. But hybrid sterility observed in those crosses was a major deterrent in the utilization of heterosis at the subspecific level. The discovery of wide-compatibility varieties (WCV) (Ikehasi and Araki 1984) offered the possibility of realizing high-yield heterosis through the production of fertile hybrids in many indica/japonica crosses. The wide-compatibility locus was named $S_5$, and a three-allele system ($S_5^i$, $S_5^j$, and $S_5^n$ to represent indica, japonica, and neutral alleles at the $S_5$ locus, respectively) was proposed to explain the partial hybrid sterility in those crosses (Ikehasi and Araki 1986). Hybrid sterility is observed in the $S_5^i$–$S_5^j$ combination but not in the $S_5^n$–$S_5^i$ or $S_5^n$–$S_5^j$ combination. $S_5$ locus was closely linked to marker genes C and *wx* (Ikehasi and Araki 1987) and to isozymes *Amp3* and *Est2* (Malik and Khush 1996) on chromosome 6. The map location was also confirmed using RFLP markers (Liu et al. 1992; Yanagihara et al. 1995). A genomewide mapping by Liu et al. (1997) revealed a tightly linked marker R2349 for the $S_5$ locus and two more additional minor loci on chromosome 2 and 12, whose combined effect could lead to partial sterility even in the presence of the wide-compatibility gene.

The complex genetic basis of wide compatibility in different WCVs is evident from several studies (Li HB et al. 1997a; Wang et al. 1998). In a QTL study involving the rice cultivar "Dular" with a high level of wide compatibility, Wang et al. (1998) identified five loci on chromosomes 1, 3, 5, 6, and 8 with significant effect on fertility segregation. These loci jointly explained 55.5% of the phenotypic variation, and the location of locus on chromosome 6 was the same as the earlier mapped $S_5$; the locus with largest effect was on chromosome 5. Two complex interactions between two loci and three loci were proposed to explain the level of hybrid fertility. Interactions between loci have also been detected to play a role in the expression of hybrid sterility in indica/japonica crosses (Wu et al. 1996; Li HB et al. 1997a). Zhuang et al. (2002) mapped a locus *S–c* closely linked to an RFLP marker RG227 on chromosome 3 and suggested that the "one-locus sporogametophytic" model could explain $F_1$ hybrid pollen sterility in cultivated rice.

### Understanding Heterosis

A fundamental assumption for hybrid breeding is the advantage of heterozygotes. Although two major hypotheses, the dominance hypothesis and the overdominance hypothesis, were proposed earlier to explain the genetic basis of heterosis, no consensus has been reached to date. With the help of molecular marker technology and high-density molecular linkage maps, it is now possible to critically evaluate those hypotheses in rice (Xiao et al. 1995; Yu et al. 1997; Li et al. 2001b; Luo et al. 2001; Hua et al. 2002, 2003). Recently, Hua et al. (2003) investigated the genetic basis of heterosis by using an "immortalized $F_2$" population developed by randomly permutated intermating of 240 recombinant inbred lines derived from the cross between the parents of the elite hybrid Shanyou 63. Using a 231 marker linkage map in conjunction with the data gathered from a field trial of the hybrids and parental recombinant lines over 2 years, 33 heterotic loci were detected for four traits–yield, tillers per plant, grains per panicle, and thousand grain weight. Because of little overlapping of the QTLs for the traits with the heterotic loci, the involvement of a different group of factors for heterosis and trait performance is expected. It was concluded that all kinds of genetic effects, including partial, full, and overdominance at single-locus level and all three forms of digenic interactions (additive by additive, additive by dominance, and dominance by dominance) contributed to heterosis and that these genetic components were not mutually exclusive as explanation of the manifestation of heterosis. Heterosis in Shanyou 63 could be explained by heterotic effects at the single-locus level combined with the marginal advantages of double heterozygotes caused by dominance-by-dominance interaction at the two-locus level.

Using the same immortalized $F_2$ population Hua et al. (2002) concluded that heterozygotes were not necessarily advantageous for trait performance even among genotypes derived from the above highly heterotic hybrid. Earlier in an intersubspecific cross of rice, Xiao et al. (1995) suggested that dominance was the genetic basis of heterosis in rice and both dominance and overdominance hypotheses may be based on a single-locus theory. On the contrary, epistasis and overdominance are primarily responsible for explaining inbreeding depression and heterosis (Li et al. 2001b; Luo et al. 2001). Yu et al. (1997) investigated an $F_{2:3}$ population derived from a highly heterotic rice cross combination and detected a high level of digenic interactions involving loci that are distributed all over the rice genome in expression of heterosis. In most studies (Xiao et al. 1995; Li et al. 2001b; Luo et al. 2001) backcrossed recombinant inbred lines were used, but such populations do not provide estimates

for some genetic components at both single- and multilocus levels to study the genetic basis of heterosis.

### Predicting Heterotic Crosses

The selection of parental lines that would result in improved performance of rice hybrids is a challenging task for hybrid rice breeders. Hybrid rice breeding would be accelerated phenomenally if a reliable, simple, and efficient method could replace large-scale crossing and field evaluation to predict heterotic cross combinations. A number of criteria commonly used to breed heterotic rice hybrids are *per se* performance, combining ability, and genetic diversity. During the last two decades, with the advent of molecular markers, genetic diversity is now estimated more efficiently compared with morphological variation. Several investigations in rice have been conducted to define the correlation between a hybrid performance and the molecular divergence in the parental lines. Two different measures of $F_1$ heterozygosity based on molecular data are general heterozygosity and specific heterozygosity. General heterozygosity is based on all molecular markers used in the study, whereas specific heterozygosity involves only those markers that affect a trait in a significant way. The results from the studies undertaken so far are conflicting. For example, Zhang et al. (1994b, 1995b) evaluated a diallel set of 28 indica × indica hybrids and detected high correlation between specific heterozygosity and heterosis for yield and its component traits. But Xiao et al. (1996b) found genetic distance measures useful for predicting yield and heterosis of intrasubspecific hybrids but not of intersubspecific hybrids. Similar conclusions were drawn in two other studies involving a wide assembly of germplasms (Zhang et al. 1996b; Zhao et al. 1999). These studies make clear that the correlation between genetic distance and heterosis is not of universal occurrence and the degree of correlation is variable because of germplasm diversity and the complex genetic basis of heterosis.

Since a moderate level of genotypic divergence between parents of intersubspecfic hybrids plays an important role in heterosis (Li et al. 1998), Liu and Wu (1998) suggested optimal accumulation of favorable alleles and removal of unfavorable alleles in parental lines using MAS rather than broadening the genetic diversity or heterozygosity in indica/japonica hybrid breeding programs. The discovery of favorable alleles and unfavorable alleles may be useful for hybrid rice breeding (Liu et al. 2002b).

## 1.3.4 Grain Quality

The preference for cooking, processing, and eating quality rice differs greatly around the world. To meet the consumer demand for rice of a specific quality, breeders' objective to improve grain quality changes accordingly. Rice grain quality can be defined in many ways. The major components of rice grain quality include appearance, milling, cooking, eating, and nutritional qualities; they are determined by physical and chemical characteristics. Most grain quality mapping studies have involved the *O. sativa* germplasm (He et al. 1999a; Tan et al. 1999, 2000, 2001a; Li Z et al. 2003b; Zhou et al. 2003b). Three recent reports concerned crosses involving *O. rufipogon* (Septiningsih et al. 2003a) and African rice *O. glaberrima* (Aluko et al. 2004; Li et al. 2004). Although most *O. rufipogon* alleles are inferior, information about the inferior grain quality QTLs can be useful in reducing the linkage drag while introgressing yield-improving QTLs from wild species. Similarly, the new QTL from *O. glaberrima* and a high level of transgressive variation in *O. sativa* × African rice *O. glaberrima* should provide further opportunity to improve grain quality.

Major grain quality genes mapped in rice include aroma, cooked kernel elongation, and waxy gene. Using an NIL developed by introgression of the scent gene (*fgr*) from Della in Lemont background, Ahn et al. (1992) identified a marker, RG28, linked to this gene on chromosome 8 at a distance of 4.5 cM. Later this gene was found to be linked to cooked kernel elongation QTL located in the proximity of RZ323 in a line, B8462T3-710, derived from Basmati 370 (Ahn et al. 1993). A similar gene for scented kernel (*sk-2*) was mapped on chromosome 8 near markers RG28 and XNpb369 (Yano et al. 1991). The major component of rice aroma is a compound 2-acetyl-1-pyrolline (AcPy) (Buttery et al. 1983), and the gene regulating this compound was also mapped near RG28 on chromosome 8 in a DH population derived from IR64 x Azucena (Lorieux et al. 1996).

The grain quality QTL studies are listed in Table 6. Rice milling quality is judged by three main factors: brown rice percentage, milled rice percentage, and head-milled rice. Grain length, grain width, width-length ratio, grain shape, and degree of chalkiness determine the quality of appearance of rice. Red pericarp in rice was studied in both classical mutants (Kinoshita 1998) and QTL studies (Bres-Patry et al. 2001; Septiningsih et al. 2003a), and major genes/QTL

were localized on chromosomes 1 and 7 and possibly a modifier on chromosome 12. The protein content and the fat content in rice grains were also mapped to facilitate improvement of the nutritional quality (Tan et al. 2001b; Hu et al. 2004).

Amylose content (AC) is one of the most important determinants of rice cooking and eating quality and is known to be controlled by a major locus *waxy* (*wx*) on chromosome 6 (Wang et al. 1992; He et al. 1999a; Tan et al. 1999). Sano et al. (1986) identified two different alleles of the *wx* locus corresponding to the indica and japonica subspecies using RFLP markers. In a study involving 89 nonglutionous rice cultivars, Ayres et al. (1997) identified eight different alleles of waxy genes that accounted for more than 85% of the variation in amylose content. But involvement of some minor genes in modifying this major locus has also been reported (McKenzie and Rutger 1983). AC of rice grain affects the gelatinization temperature (GT) and gel consistency (GC). A QTL study in both $F_2$ and RIL populations derived from a cross Zhenshan 97 x Minghui 63 indicated that AC, GC, and GT are controlled by the *wx* locus or surrounding region on chromosome 6 (Tan et al. 1999). Improvement of four quality traits, such as AC, GC, GT, and chalky endosperm in Zhenshan 97, an elite parent of hybrid rice, by introgressing the waxy region from Minghui 63 through molecular marker MAS, further testifies to the importance of this chromosome 6 region (Zhou et al. 2003b). A recent study by Larkin et al. (2003) indicated that the waxy gene encoding granule bound starch synthase affects viscosity characteristics significantly, whereas a tight-linked starch synthase locus has a lesser effect. By contrast, Han et al. (2004) reported the contribution of starch branching enzymes to viscosity characteristics. A single QTL study involving jasmine rice KDML 105 indicated involvement of several QTLs for controlling AC, GC, and GT (Lanceras et al. 2000).

### 1.3.5 Abiotic Stress Tolerance

#### Drought

Drought imposes serious limitations on rice productivity in rainfed ecosystems. In rainfed rice-growing areas, yield is greatly determined by the amount and distribution of rainfall. There is a tremendous amount of genetic variation for drought tolerance among world rice germplasms because some genotypes perform remarkably better than others under drought conditions (Price et al. 2002a). Some of these lines use drought escape mechanisms, while others have an inherent ability to fight drought. Both shoot- and root-related traits contributing toward drought tolerance have been recently reviewed (Pathan et al. 2004). Shoot-related traits that are important in the context of drought tolerance are osmotic adjustment (OA), leaf water potential (LWP), cell-membrane stability (CMS), osmolytes, leaf rolling, leaf drying, and relative water content. A number of root morphological attributes to improve drought tolerance are root thickness, root weight, root length, root number (penetrated and total), and root penetration index. Most studies use $F_2$, DHL, or RIL as mapping populations to study root and shoot traits related to drought tolerance (Table 7).

Osmotic adjustment (OA) allows plants to maintain higher turgor to sustain normal physiological functions. The indica cultivars are known to have high OA capacity compared to japonica cultivars. There has been no attempt to exploit the existing genetic variation in breeding programs because the methods of OA measurement are both time consuming and labor intensive and also there is no distinct relationship between OA and rice productivity under drought. In the first report, Lilley et al. (1996) mapped a major QTL for OA onto chromosome 8 between markers RG978 and RG1. Of five QTLs for dehydration tolerance on chromosomes 1, 3, 7, and 8, two QTLs on chromosomes 3 and 7 overlapped with QTL for leaf rolling (Champoux et al. 1995) and total root number (Ray et al. 1996). Subsequently, Zhang et al. (2001) and Robin et al. (2003) used DH and advanced backcross populations to map several QTLs for OA. A comparison of these results revealed that there is some consistency in QTL locations (Pathan et al. 2004). For example, the QTL for OA on chromosome 8 (Lilley et al. 1996) was mapped in the same chromosomal region by both Zhang et al. (2001) and Robin et al. (2003). Similarly, another QTL on chromosome 1 (RG140-ME2_12) was consistent in both reports. On chromosome 3, one QTL for OA was detected between markers RZ313 and RG224 in two rice populations (Zhang et al. 2001; Robin et al. 2003). Tripathy et al. (2000) detected nine QTLs for cell membrane stability (CMS) using the DH population developed from the cross CT9993 × IR62266.

On comparing the location of QTL (Champoux et al. 1995; Courtois et al. 2000; Price et al. 2002b) involving drought-avoidance traits like leaf rolling,

**Table 6.** Grain quality QTL studies in rice

| Reference | Populations | Number of lines and population type | No. and types of markers used | Methods/software used | Traits | Number of QTL | Chromosomal location of QTL | Variance % explained |
|---|---|---|---|---|---|---|---|---|
| He et al. 1999a | ZYQ 8 × JX 17 | 132, DHL | 243 RFLP and SSR | Mapmaker/QTL | Amylose content | 2 | 5,6 | 11.8–91.1 |
| | | | | | Gel consistency | 2 | 2,7 | 14.2–20.2 |
| | | | | | Alkali Spreading score | 2 | Both on 6 | 24.6–82.4 |
| | | | | | Percentage of grain with white core | 2 | 8,12 | 10.0–21.9 |
| | | | | | Square of white core | 1 | 3 | 8.8 |
| Lanceras et al. 2000 | CT9993 × KDML105 | 141, RIL | 191 RFLP, SSR, and AFLP | MQTL | Amylose content | 4 | 3,4,6,7 | 9.2–58.7 |
| | | | | | Gel consistency | 3 | 6,6,7 | 10.5–53.1 |
| | | | | | Gelatinization temperature | 3 | 2,6,6 | 8.6–60.3 |
| Bao et al. 2000 | ZYQ 8 × JX 17 | 132, DHL | 243 RFLP and SSR | Mapmaker/QTL | Paste viscosity (Rapid Visco Analyzer profile) | 20 | 1,2,5,6,7,12 | 10.0–63.7 |
| Tan et al. 2000 | Zhenshan 97 x Minghui 63 | 241, $F_2$ | 150 RFLP, and SSR | Mapmaker/QTL and QTL Cartographer | Grain length | 3 | 2,3,7 | 6.5–63.8 |
| | | | | | Grain width | 3 | 1,5,6 | 10.4–55.2 |
| | | | | | Length-width ratio | 2 | 3,5 | 36.4–37.8 |
| | Zhenshan 97 × Minghui 63 | 238, RIL | 171 RFLP, and SSR | Mapmaker/QTL and QTL Cartographer | Grain length | 2 | 3,11 | 7.2–57.6 |
| | | | | | Grain width | 2 | 5,6 | 4.6–44.0 |
| | | | | | Length-width ratio | 2 | 3,5 | 25.4–33.3 |
| Tan et al. 2001a | Zhenshan 97 × Minghui 63 | 238, RIL | 162 RFLP and SSR | Mapmaker/QTL and QTL Cartographer | Brown rice (%) | 1 | 5 | 10.0 |
| | | | | | Milled rice (%) | 2 | 3,5 | 4.8–7.0 |
| | | | | | Head rice (%) | 1 | 3 | 10.1 |
| | | | | | Protein (%) | 2 | 6,7 | 6.0–13.0 |
| | | | | | Flour color L* | 9 | 5,6,8,4,7,1,3,6,8 | 4.3–25.4 |
| Yoshida et al. 2002 | Reiho × Yamadanishiki | 91, DHL | 145 RAPD, AFLP, and SSR | QGENE | Grain length | 4 | 1,4,6,11 | 11.9–23.4 |
| | | | | | Grain width | 4 | 3,4,5,6 | 8.7–27.1 |
| | | | | | Grain thickness | 3 | 2,4,5 | 9.7–19.6 |
| | | | | | White core (%) | 2 | 12, unknown | 14.2–17.3 |
| | | | | | White belly (%) | 3 | All three on 5 | 8.7–20.7 |
| | | | | | Cracked grains (%) | 4 | 1,3,11,12 | 9.7–14.3 |
| | | | | | Amylose content | 1 | 8 | 9.9 |
| | | | | | Protein content (brown rice) | 4 | 2,3,11,12 | 11.0–15.1 |
| | | | | | Protein content (polished rice) | 5 | 1,3,4,11,12 | 9.9–18.2 |

**Table 6.** (continued)

| Reference | Populations | Number of lines and population type | No. and types of markers used | Methods/software used | Traits | Number of QTL | Chromosomal location of QTL | Variance % explained |
|---|---|---|---|---|---|---|---|---|
| Septiningsih et al. 2003b | IR64 × *O. rufipogon* (IRGC 105491) | 285 $BC_2F_2$ families | 165 SSR and RFLP | QGENE and QTL Cartographer | Filled/total rough rice | 1 | 8 | 5.1 |
| | | | | | Grain density | 2 | 4,8 | 10.1–11.2 |
| | | | | | Dehusked rice grain | 1 | 10 | 6.5 |
| | | | | | Green grain | 2 | 7,12 | 5.1–5.3 |
| | | | | | Damaged-yellow rice grain | 3 | 7,9,10 | 4.9–28.3 |
| | | | | | Red grain | 2 | 7,12 | 5.5–43.0 |
| | | | | | Milled rice grain | 1 | 10 | 5.8 |
| | | | | | Head rice grain | 3 | 1,2,5 | 5.2–5.5 |
| | | | | | Broken rice grain | 4 | 1,1,2,5 | 5.4–6.4 |
| | | | | | Crushed rice grain | 2 | 2,3 | 4.9–5.2 |
| | | | | | Amylose content | 1 | 6 | 21.9 |
| | | | | | Gel consistency | 1 | 6 | 6.6 |
| Aluko et al. 2004 | Caiapo × *O. glaberrima* (IRGC 103544) | 312, DHL from a $BC_3F_1$ population | 100 SSR | Mapmaker/QTL and QTL Cartographer | Brown rice (%) | 3 | 1,7,8 | 2.8–4.9 |
| | | | | | Head rice (%) | 5 | 1,3,6,8,11 | 7.6–54.1 |
| | | | | | Rice bran (%) | 4 | 2,4,7,10 | 4.0–39.7 |
| | | | | | Milled rice (%) | 2 | 5,7 | 5.3–6.1 |
| | | | | | Amylose (%) | 3 | 3,6,8 | 10.9–73.7 |
| | | | | | Alkali spread score | 2 | both on 6 | 44.0–50.1 |
| | | | | | Protein (%) | 4 | 1,2,6,11 | 4.5–15.0 |
| | | | | | Grain length | 2 | 3,6 | 4.7–12.5 |
| | | | | | Grain l/w ratio | 2 | 1,6 | 4.0–14.0 |
| Hu et al. 2004 | Gui 630 × 02428 | 81, DHL | 232 RFLP | Mapmaker/QTL | Rice protein content | 5 | 1,4,5,6,7 | 6.9–35.0 |
| | | | | | Rice fat content | 3 | 1,2,5 | 7.7–25.5 |
| Li et al. 2004 | V20A × *O. glaberrima* (IRGC 103544) | 308 $BC_3F_1$ | 110 RFLP and 20 SSR | QGENE and QTL Cartographer | Amylose content | 3 | 2,6,12 | 7–8 |
| | | | | | Kernel elongation | 1 | 3 | 6 |
| | | | | | Grain length | 3 | 1,3,10 | 5–27 |
| | | | | | Grain width | 3 | 1,11,12 | 12–33 |
| | | | | | Length:width ratio | 3 | 3,4,12 | 13–14 |
| | | | | | Brown rice yield | 2 | 7,12 | 5–22 |
| | | | | | Crude protein content | 1 | 8 | 10 |
| | | | | | Gel consistency | 2 | 2,7 | 69–70 |

**Table 7.** Quantitative trait loci studies for drought stress tolerance and associated traits in rice

| Reference | Parents and cross | Mapping population | No. and types of markers used | Methods/software used | Traits scored | Number of QTL | Chromosomal location of QTL | Variance % explained |
|---|---|---|---|---|---|---|---|---|
| Champoux et al. 1995 | Co 39 × Moroberekan | 203 RILs | 127 RFLP | Mapmaker/QTL | Root thickness | 18 | 1,2,3,4,6,7,8,9,10,11,12 | 13–33 |
| | | | | | Root shoot ratio | 16 | 1,2,3,4,6,7,8,9,10,11,12 | 9–22 |
| | | | | | Root dry weight/tiller | 14 | 1,2,3,4,6,7,8,9,12 | 11–18 |
| | | | | | Deep root weight | 8 | 2,3,4,7,8,9,,12 | 6–17 |
| Ray et al. 1996 | Co 39 × Moroberekan | 203 RILs | 125 RFLP | Mapmaker/QTL | Total root number | 19 | 1,2,3,4,5,6,7,8,9,11,12 | 8–19 |
| | | | | | Root penetration index | 6 | 2,4,5,6,11 | 7–13 |
| | | | | | Tiller number | 10 | 1,2,4,6,8,11,12 | 7–14 |
| | | | | | Penetrated root number | 4 | 1,3,6,12 | 6–8 |
| Lilley et al. 1996 | Co 39 × Moroberekan | 52 RILs | 127 RFLP | ANOVA and Mapmaker/QTL | Osmotic adjustment | 1 | 8 | 32 |
| | | | | | Dehydration tolerance | 5 | 1,3,7,8 | 27–36 |
| Quarrie et al. 1997 | IR20 × 63-83 | 123 $F_2$ | 228 RFLP, AFLP | ANOVA and QTL Cartograoher | Leaf weight | 7 | 2,4,8 | Total 54% |
| | | | | | ABA accumulation | 10 | 2,3,4,6,7,9 | Total 55% |
| Price and Tomos 1997 | Bala × Azucena | 178 $F_2$ | 71 RFLP | Mapmaker/QTL | Root length | 17 | 1,2,3.5,6,10,11 | 5–38 |
| | | | | | Root cell length | 1 | – | 10 |
| | | | | | Root thickness | 3 | 2,3,5 | 7–21 |
| | | | | | Root volume | 3 | 1,8,12 | 6–10 |
| Yadav et al. 1997 | IR64 × Azucena | 125 DHLs | 175 RFLP, RAPD, isozyme | ANOVA | Total root weight | 6 | 1,5,6,7,9 | 5–11 |
| | | | | | Deep root weight | 5 | 1,6,7,9 | 4–15 |
| | | | | | Deep root weight to shoot ratio | 9 | 1,2,5,6,7,8,9 | 4–22 |
| | | | | | Deep root weight per tiller | 6 | 1,2,6,7,8,9 | 4–20 |
| | | | | | Maximum root length | 8 | 1,2,6,7,8,9 | 4–21 |
| | | | | | Root thickness | 5 | 1,2,5,8 | 4–10 |
| Courtois et al. 2000 | IR64 × Azucena | 135 DHLs | 175 RFLP, AFLP, Isozyme | QTL Cartographer | Leaf rolling | 11 | 1,3,4,5,7,9 | 5–23 |
| | | | | | Leaf drying | 10 | 1,4,5,6,7,10,11,12 | 5–19 |
| | | | | | Relative water content (RWC) | 11 | 1,3,5,6,9,12 | 6–19 |
| | | | | | Relative growth rate under stress | 10 | 1,2,3,5,6,7,9 | 8–17 |
| Hemamalini et al. 2000 | IR64 × Azucena | 56 DHLs | 175 RFLP, AFLP, Isozyme | Mapmaker/QTL | Morphological and physiological traits | 36 | 1,2,3,4,5,6,7,8,9,10,12 | 11–29 |
| Tripathy et al. 2000 | CT9993 × IR62266 | 154 DHLs | 315 RFLP, AFLP, SSR | PLABQTL | Cell-membrane stability | 9 | 1,3,7,8,9,11,12 | 13–42 |

**Table 7.** (continued)

| Reference | Parents and cross | Mapping population | No. and types of markers used | Methods/software used | Traits scored | Number of QTL | Chromosomal location of QTL | Variance % explained |
|---|---|---|---|---|---|---|---|---|
| Price et al. 2000 | Bala × Azucena | 205 RILs | 135 RFLP, AFLP | QTL Cartographer | Total root number | 3 | 1,10 | 5–10 |
| | | | | | Penetrated root number | 7 | 2,3,5,10,11 | 5–17 |
| | | | | | Penetration ratio | 7 | 2,3,5,10,11 | 7–18 |
| | | | | | Tiller number | 1 | 1 | 12 |
| Zheng et al. 2000 | IR64 × Azucena | 109 DHLs | 175 RFLP, RAPD, isozyme | Mapmaker/QTL | Root penetration index | 4 | 2,3,7,8 | 9–14 |
| | | | | | Penetrated root number | 2 | 2,7 | 8–9 |
| | | | | | Total root number | 2 | 1,7 | 9–14 |
| | | | | | Penetrated root thickness | 4 | 1,3,4,9 | 10–16 |
| Ali et al. 2000 | IR58821x IR52561 | 166 RILs | 399 RFLP, AFLP | Mapmaker/QTL | Total root number | 2 | 3,7 | 9–12 |
| | | | | | Penetrated root number | 7 | 1,2,3 | 7–27 |
| | | | | | Root penetration index | 6 | 2,3,10 | 8–26 |
| | | | | | Penetrated root thickness | 8 | 1,2,4,6,7,10 | 6–14 |
| | | | | | Penetrated root length | 5 | 1,2,3,7,11 | 6–13 |
| Zhang et al. 2001 | CT9993 × IR62266 | 154 DHLs | 315 RFLP, AFLP, SSR | Mapmaker/QTL | Osmotic adjustment | 5 | 1,2,3,8,9 | 8–13 |
| | | | | | Root penetration index | 4 | 3,4,12 | 8–11 |
| | | | | | Basal root thickness | 6 | 2,3,4,8,9,12 | 9–38 |
| | | | | | Penetrated root thickness | 11 | 1,2,4,6,7,9,12 | 9–31 |
| | | | | | Penetrated root length | 1 | 11 | 17 |
| | | | | | Total root dry weight | 5 | 1,2,4,6,10 | 9–20 |
| | | | | | Penetrated root dry wt. | 3 | 4,9,12 | 11–17 |
| | | | | | Root pulling force | 6 | 2,3,4,5,11 | 9–20 |
| Kamoshita et al. 2002a | CT9993 × IR62266 | 154 DHLs | 315 RFLP, AFLP, SSR | QTLMapper | Shoot biomass | 7 | 2,6,11,12 | 9–57 |
| | | | | | Deep root mass | 7 | 1,2,3,7,11 | 9–36 |
| | | | | | Deep root ratio | 6 | 1,2,5,11 | 8–52 |
| | | | | | Deep root per tiller | 6 | 1,2,4,5,11 | 5–40 |
| | | | | | Rooting depth | 9 | 1,2,5,7,9,11 | 5–17 |
| | | | | | Root thickness 0–10cm | 6 | 1,2,4,8,11 | 7–36 |
| | | | | | Root thickness 20–25cm | 3 | 2,4 | 15–22 |

**Table 7.** (continued)

| Reference | Parents and cross | Mapping population | No. and types of markers used | Methods/software used | Traits scored | Number of QTL | Chromosomal location of QTL | Variance % explained |
|---|---|---|---|---|---|---|---|---|
| Kamoshita et al. 2002b | IR58821 × IR52561 | 184 RILs | 399 RFLP, AFLP | QTLMapper | Shoot biomass | 2 | 4,8 | 13–14 |
| | | | | | Deep root mass | 5 | 2,3,4,9,11 | 7–21 |
| | | | | | Deep root ratio | 5 | 2,3,4,9,11 | 6–27 |
| | | | | | Deep root per tiller | 6 | 2,4,6,7 | 9–22 |
| | | | | | Rooting depth | 5 | 1,4 | 6–30 |
| | | | | | Root thickness 0–10 cm | 6 | 1,3,8,9 | 6–15 |
| | | | | | Root thickness 20–25 cm | 2 | 4 | 12–23 |
| Price et al. 2002b | Bala × Azucena | 205 RILs | 135 RFLP, AFLP | QTL Cartographer | Leaf rolling | 5 | 1,3,5,6,7 | 5–20 |
| | | | | | Leaf drying | 11 | 1,2,3,4,5,7,8,11,12 | 6–18 |
| | | | | | RWC | 8 | 1,3,4,5,6,8,9,10 | 9–26 |
| Courtois et al. 2003 | IAC165 × Co39 | 125 RIL | 182 RFLP, SSR | QTL Cartographer | Maximum root length | 3 | 1,9,12 | 7–14 |
| | | | | | Root thickness | 4 | 1,4,7 | 6–24 |
| | | | | | Root weight (0–30 cm) | 5 | 1,6,7,11,12 | 8–22 |
| | | | | | Root weight (30–60 cm) | 2 | 4,8 | 9–11 |
| | | | | | Root weight (60–90 cm) | 3 | 4,10,11 | 7–10 |
| | | | | | Total root weight | 3 | 1,3,11 | 10–12 |
| | | | | | Deep root weight | 3 | 4,7,8 | 11–12 |
| Zheng et al. 2003 | IR1552 × Azucena | 96 RILs | 239 RFLP, AFLP, SSR | QGENE | Seminal root length | 4 | 1,2,7,9 | 11–14 |
| | | | | | Adventious root number | 8 | 1,2,3,4,9 | 11–20 |
| | | | | | Lateral root length | 5 | 1,3,5,6 | 12–14 |
| | | | | | Lateral root number | 3 | 3,4,6 | 12–13 |

leaf drying, and relative water content, it is evident that there is consistency of several QTLs across genotypes, screening environments, and years (Pathan et al. 2004). Of the 18 QTLs for drought avoidance at seedling, early vegetative, and late vegetative stages in the field (Champoux et al. 1995), five were consistently identified during three different growth stages and four across at least two growth stages. Courtois et al. (2000) detected 11 QTLs for leaf rolling, 10 for leaf drying, and 11 for RWC. Many of these QTLs were detected across different trials. For example, of 11 QTLs for leaf rolling, three QTLs, one each on chromosomes 1, 5, and 9, were common in three trials. Using an RIL population developed from the cross Bala × Azucena, Price et al. (2002b) detected 17 QTLs for leaf rolling, leaf drying, and relative water content in two different years and in two different locations. When QTLs of all drought-avoidance traits were examined, QTLs for leaf rolling and RWC on chromosome 1 (RG331-RZ14) were consistent in both Co 39 x Moroberekan and IR64 × Azucena populations. The QTLs for OA and root traits were also mapped in this region across different genetic backgrounds. The region of chromosome 3 between RZ519 and CDO795 carried QTLs for leaf rolling, leaf drying, and RWC in all three populations. A similar overlapping of QTLs for many of these shoot- and root-related traits were also evident (Pathan et al. 2004).

Root morphology is fundamentally important for improving drought tolerance in rice. A large number of QTL studies involving root-related traits such as thickness, weight, length, number (penetrated and total), and root penetration index are available (Table 7). Most japonica cultivars have well-developed root systems compared to indica cultivars. Root morphology and drought avoidance in rice under both field and greenhouse conditions were first investigated in an RIL population derived from the cross Co 39 x Moroberekan (Champoux et al. 1995). They showed that 12 of 14 QTLs were associated with field drought tolerance and overlapped with QTLs for root morphology (root thickness, root/shoot ratio, and root dry weight). Later, Ray et al. (1996) used the same RILs to locate QTLs associated with root penetration ability in rice. Additionally, five more populations have been used for QTL mapping of root traits (Table 7). Despite different experimental conditions, several QTLs for root traits were consistent across different mapping populations (Pathan et al. 2004). The most recent study of Courtois et al. (2003) involved an RIL population developed from the cross IAC165 x Co 39 in which root traits (maximum length, thickness, and dry weight in various layers) were measured in greenhouse. For each trait, one to four QTLs were detected and each QTL on chromosomes 1, 4, 9, 11, and 12 explained 5.5 to 24.8% of the phenotypic variation. Most QTLs in this population overlapped with one or more root traits in earlier studied populations (Courtois et al. 2003). It is thus necessary to test the utility of this QTL information under natural field situations.

The above populations were used in a number of studies to establish a relationship between an individual drought tolerance trait and yield under drought stress. Babu et al. (2003) used a DH population of 154 rice lines from the cross CT9993-5-10-1-M × IR62266-42-6-2 in three field experiments at two locations and identified 47 QTLs, individually explaining 5 to 59% of the phenotype variation for various plant water stress indicators, phenology, and production traits. A region on chromosome 4 (RG939-RG476-RG214) with root-related traits was observed to have a pleiotropic effect on yield traits under stress. Venuprasad et al. (2002) used a similar strategy employing an IR64 × Azucena DH population and found a positive correlation between maximum root length and grain yield under stress but a negative correlation under unstressed conditions. They further reported that QTLs responsible for grain yield and component traits were not pleiotropic with loci for desirable root morphology under low-moisture stress at the vegetative stage, and so it may be possible to combine higher grain yield and desirable root morphological traits to improve rice productivity in rainfed ecosystems.

Zheng et al. (2003) used an RIL population derived from a cross between the lowland rice variety IR1552 and the upland rice variety Azucena and compared the QTL results with earlier reports. In all these studies Azucena provided positive alleles for root elongation. The researchers screened several candidate genes from expressed sequence tags (EST) and cDNA-AFLP and mapped two genes for cell expansion, four cDNA-AFLP clones from root tissues of Azucena in the QTL region for seminal root length (SRL), and lateral root length (LRL) under upland conditions, respectively. Nguyen TT et al. (2004) used differential display to identify candidate genes and mapped several of these adjacent to the QTLs for root thickness and OA capacity in a CT9993 × IR62266 population.

## Submergence

Rice varieties tolerant to flooding or submergence are needed to improve productivity in rainfed lowland and flood-prone areas of South and Southeast Asia. An Indian cultivar FR13A is the most widely used source of flooding tolerance. Although considered earlier as a polygenic trait, a number of molecular marker analyses (Table 8 A) indicated that a major locus, *Sub1*, located on chromosome 9 and controlling 69% of the phenotypic variation, is responsible for this trait (Xu and Mackill 1996). This major locus was confirmed along with the discovery of four additional QTLs using an RIL population developed from the cross IR74 x FR13A (Nandi et al. 1997). A high-resolution map of this major locus was constructed (Xu et al. 2000). Sripongpangkul et al. (2000) used another submergence-tolerant traditional Indian cultivar Jalamagna and mapped a QTL for submergence tolerance with large effect onto the same position on chromosome 9. Among six more genomic regions for leaf and stem elongation, the most important QTL *QIne1* located near *sd1* on chromosome 1 had a large effect on internode elongation and contributed significantly to the submergence tolerance under flooding. From this study it is evident that genes for submergence tolerance are different from genes for elongation ability. Using three different mapping populations the same group Toojinda et al. (2003) identified the same major QTL on chromosome 9 consistently in different years and different genetic backgrounds. Several other QTLs specifically expressed under certain environments or genetic backgrounds were also mapped on chromosomes 1, 2, 5, 7, 10, and 11.

## Salinity

Salinity is a major constraint on rice productivity, affecting 20% of irrigated land worldwide. There has been rapid growth in understanding of the component traits for salt tolerance and mechanism in other plant species, such as production of compatible solutes, salt compartmentation, sodium uptake, and preference for potassium to sodium. However, this knowledge has not been translated into improvements in salt tolerance in cereal crops including rice (Flowers and Yeo 1995). The major bottleneck is the complex genetic and physiological mechanism along with high environmental influence associated with salinity tolerance. Because of the involvement of large number of genes for this complex trait and its associated components, a QTL approach may be ideal to dissect the component traits to enhance salinity tolerance.

A number of QTL studies in rice to dissect various component traits of salinity are listed in Table 8 B. There is hardly any agreement in those reports regarding the map position of the identified loci for component traits of salt tolerance. Koyama et al. (2001) described QTLs responsible for sodium uptake, potassium uptake, and regulation of the $Na^+$:$K^+$ ratio that are independent of vegetative growth. Because of localization of QTLs for $Na^+$: and $K^+$: uptake on different chromosomes, the uptake pathways are independent like the $Na^+$::$K^+$ ratio. The independence of QTL location for $Na^+$ and $K^+$ transport was also reported by Lin et al. (2004), who mapped eight QTLs of which two major QTLs, *qSNC-7* and *qSKC-1*, explained 48.5% and 40.1% of the total phenotypic variation, respectively. Zhang et al. (1995a) detected one gene for salt tolerance on chromosome 7 near RG4. Flowers et al. (2000) opined that transferability of markers linked to physiological traits like ion transport and selectivity across populations is not possible, and thus novel protocols to identify the differentially expressed genes would be necessary.

Few reports are available on genetic control of aluminum (Al) tolerance (Wu et al. 2000; Nguyen et al. 2001, 2002, 2003) and ferrous iron toxicity tolerance (Wu et al. 1997; Wan et al. 2003) in rice (Table 8 C). One locus for tolerance against ferrous iron toxicity on chromosome 1 appeared to be identical in both reports, with a large effect. The molecular mechanism of aluminum tolerance is little understood and the aforementioned QTL reports indicated a complex genetic basis for this trait. Wu et al. (2000) identified several QTLs conferring Al tolerance in an RIL population developed from the cross IR1552 (sensitive) × Azucena (tolerant). Their results showed that an additive effect is important for Al tolerance in younger seedlings but an epistatic effect is important in older seedlings. Nguyen et al. (2001) identified five QTLs for Al tolerance scattered over five chromosomes with a major QTL located on chromosome 1, whereas in another study involving a DH population from the cross CT9993 × IR62266, ten QTLs were localized on nine chromosomes. The Al tolerance QTL on chromosome 1 was found conserved across three genetic backgrounds. Ma et al. (2002) used a population of backcross inbred lines (BIL) derived from the cross between a japonica variety, Koshihikari, and an indica variety, Kasalath, in which Koshihikari showed

**Table 8.** Quantitative trait loci studies for tolerance to submergence, salinity, aluminum, cold and ferrous iron toxicity in rice

| Reference | Parents and cross | Mapping population | No. and types of markers used | Methods/software used | Traits scored | Number of QTL | Chromosomal location of QTL | Variance % explained |
|---|---|---|---|---|---|---|---|---|
| **A. Submergence tolerance** | | | | | | | | |
| Xu and Mackill 1996 | IR40931-26 × PI543851 | 169$F_2$ | RFLP and RAPD | Mapmaker/QTL | Submergence tolerance | One major | 9 | 69 |
| Nandi et al. 1997 | IR74 × FR13A | 74 RILs | 202 AFLP | Mapmaker/QTL | Submergence tolerance | Five | 6,7,9 (major),11,12 | 19–27 |
| Sripongpangkul et al. 2000 | IR74 × Jalamagna | 165 RILs | 144 RFLP, AFLP and Isozyme | QTL Mapper | Submergence tolerance | 13 | 1,3,4,5,7,8,9,10,11,12 | 11–36 |
| | | | | | Leaf elongation | 3 | 4,6,7 | 9–14 |
| | | | | | Internodes elongation | 3 | 1,2,4 | 9–37 |
| Toojinda et al. 2003 | IR49830 × CR6241 | 65 DHLs | 105 RFLP, SSR, AFLP, RAPD | MQTL | % plant survival | 3 | 1,5,9 | 16–48 |
| | | | | | Total shoot elongation | 3 | 2,5,9 | 24–74 |
| | | | | | Tolerance score | 2 | 5,9 | 28–72 |
| | | | | | Leaf senescence | 2 | 5,9 | 30–72 |
| | FR13A × CT6241 | 172 RILs | 183 RFLP, SSR, AFLP, and others | MQTL | % plant survival | 4 | 7,9,10 | 5–77 |
| | | | | | Total shoot elongation | 4 | 9,10 | 10–52 |
| | | | | | Relative shoot elongation | 2 | 9 | 10–12 |
| | | | | | Tolerance score | 9 | 7,9,10 | 8–63 |
| | | | | | Leaf senescence | 4 | 5,7,9,10 | 10–53 |
| | Jao-Hom-Nin × KDML105 | 188 $F_2$ | 99 SSR, STS and others | MQTL | % plant survival | 3 | 10,11 | 3–15 |
| | | | | | Total shoot elongation | 2 | 7,9 | 2–3 |
| | | | | | Relative shoot elongation | 5 | 1,8,10,11,12 | 2–5 |
| | | | | | Leaf senescence | 6 | 1,2,4,9 | 3–19 |
| **B. Salt tolerance** | | | | | | | | |
| Zhang et al. 1995a | M-20 × 77-170 | 85 $F_2$ | 130 RFLP | ANOVA | Salt tolerance | One | 7 | Major gene |
| Koyama et al. 2001 | IR4630 × IR15324 | 118 RILs | RFLP, AFLP, SSR | GENSTAT and QTL Cafe | Ion Conc, ratios, ion uptake, and dry mass | 11 | 1,4,6,9 | 6–20% |
| Lin et al. 2004 | Nona Bokra × Koshihikari | 133$F_2$ | 154 RFLP | Mapmaker/QTL | $Na^+$ and $K^+$ uptake of the roots and shoots | 11 | 1,4,6,7,9 | 12–40 |

**Table 8.** (continued)

| Reference | Parents and cross | Mapping population | No. and types of markers used | Methods/software used | Traits scored | Number of QTL | Chromosomal location of QTL | Variance % explained |
|---|---|---|---|---|---|---|---|---|
| **C. Aluminum tolerance** | | | | | | | | |
| Wu et al. 2000 | IR1552 × Azucena | 150 RILs | 207 RFLP, AFLP | QGENE | Al tolerance after 2 weeks of stress | 3 | 1,3,12 | 9–19 |
| | | | | | Al tolerance after 4 weeks of stress | 3 | 1,9,12 | 9–20 |
| Nguyen VT et al. 2001 | Chiembau × Omon269-65 | 188 $F_3$ families | 164 RFLP | Mapmaker/QTL | Aluminum tolerance | 9 | 1,2,3,5,6,10,11,12 | 6–51 |
| Nguyen VT et al. 2002 | CT9993 × IR62266 | 146 DHLs | 280 RFLP, SSR, AFLP | Mapmaker/QTL | Aluminum tolerance | 19 | 1,2,3,4,6,7,8,9, 10,12 | 9–29 |
| Ma et al. 2002 | Koshihikari × Kasalth | 183 BILs | 162 RFLP | PLABQTL | Aluminum tolerance | 3 | 1,2,6 | 27 (total) |
| Nguyen BD et al. 2003 | IR64 × *O. rufipogon* (Acc 106424) | 171 $F_6$ RILs | 151 RFLP, SSR | Mapmaker/QTL | Aluminum tolerance | 7 | 1,2,3,7,8,9,12 | 9–26 |
| **D. Cold tolerance** | | | | | | | | |
| Takeuchi et al. 2001 | Akihikari × Koshihikari | 212 DHLs | 135 RFLP and 34 RAPD | – | cold tolerance at booting stage | 3 | 1,7, 11 | 5–22 |
| Andaya and Mackill 2003a | M-202 × IR50 | 191 RILs | 175 SSR | PLABQTL | Cold tolerance at the booting stage | 8 | 1,2,3,5,6,7,9,12 | 11–17 |
| Andaya and Mackill 2003b | M-202 × IR50 | 191 RILs | 175 SSR | PLABQTL | Cold tolerance at the vegetative stage | 15 | 1,3,4,6,8,10,11,12 | 9–41 |
| Fujino et al. 2004 | Italica Livorno × Hayamasari | 122 BILs | 186 RFLP, SSR | QTL Cartographer | Low temperature germinability | 3 | 3,4 | 6–35 |
| **E. Ferrous iron toxicity tolerance** | | | | | | | | |
| Wu et al. 1997 | IR64 × Azucena | 123 DHLs | 175 RFLP, RAPD, isozymes | Mapmaker/QTL | Ferrous iron toxicity tolerance | 3 | 1,8 | 11–33 |
| Wan et al. 2003 | Nipponbare/Kasalth //Nipponbare | 96 $BC_1F_9$ backcross inbred lines | 245 RFLP | Mapmaker/QTL | Ferrous iron toxicity tolerance | 4 | 1,3 | 21–48 |

higher tolerance at various Al concentrations than Kasalath, probably because of exclusion mechanisms rather than internal detoxification. Three chromosomal regions on chromosomes 1, 2, and 6 controlling Al tolerance explained about 27% of the phenotypic variation and were confirmed using substitution lines. Kasalath contributed positive alleles at the QTL on chromosome 6 but were unfavorable for loci on chromosomes 1 and 2. Nguyen et al. (2003) used IR 64 × *O. rufipogon* RIL and identified nine QTLs, of which QTLs for relative root length (RRL) located on chromosomes 1 and 9 were consistent across different genetic backgrounds. A major QTL for RRL on chromosome 3 was also reported to be conserved across many cereals.

### Cold Tolerance

Low-temperature stress reduces rice growth and yield because of poor germination, poor seedling growth, delayed heading, and spikelet sterility in most temperate regions and high-elevation areas in tropics. A list of QTL mapping studies on cold tolerance in rice is given in Table 8 D. Andaya and Mackill (2003a,b) employed a QTL mapping strategy to investigate cold tolerance during both the vegetative and booting stages of rice. In an RIL population (temperate japonica M-202 × tropical indica, IR50), they identified a single major QTL, *qCTS12a,* on chromosome 12 that accounted for 41% of the variation and several minor QTLs distributed over eight rice chromosomes. In another study they reported eight QTLs for cold tolerance at the booting stage on chromosomes 1, 2, 3, 5, 6, 7, 9, and 12 with a contribution of 11 to 17% to the total phenotypic variation. Two major QTLs, *qCTB2a* and *qCTB3* from the tolerant parent M-202, explained approximately 17% of the phenotypic variance, and IR50 alleles in two QTLs contributed to cold tolerance. But for the same trait only three QTLs on chromosomes 1, 7, and 11 explained 5 to 22% of the phenotypic variation in a DH population from the cross Akihikari (moderately low-temperature susceptible) × Koshihikari (low-temperature tolerant) (Takeuchi et al. 2001). Saito et al. (2003) introgressed fragments of cold-tolerant variety Silewah into Norin-PL8 and a cold-sensitive variety Kirara 397 background and identified three QTLs on chromosomes 3 and 4 for cold tolerance at the booting stage.

Fujino et al. (2004) mapped three QTLs for low-temperature germinability on chromosomes 3 and 4 in a population of 122 backcross inbred lines (BIL) derived from a cross between temperate japonica varieties, Italica Livorno, and Hayamasari. A major QTL, *qLTG-3-1,* on chromosome 3 accounted for 35.0% of the total phenotypic variation and two additional QTLs, *qLTG-3-2* on chromosome 3 and *qLTG-4* on chromosome 4, explained 17.4% and 5.5% of the total phenotypic variation, respectively. The Italica Livorno alleles contributed toward improvement in germinability at low temperatures. Miura et al. (2001) studied this trait in a BIL population from the cross Nipponbare × Kasalath and identified five putative QTLs on chromosome 2, 4, 5, and 11 explaining 40.7% of the total phenotypic variation.

### Nutrient Deficiency and Toxicity

Few studies have been directed toward mapping of QTL for nutrient deficiency and toxicity (Table 8 E). The tolerance against phosphorous (P) deficiency was investigated by a number of researchers using different mapping populations (Ni et al. 1998; Wissuwa et al. 1998; Ming et al. 2001; Hu et al. 2001). Four QTLs for P-deficiency tolerance were identified on chromosomes 2, 6, 10, and 12 in a BIL population from the cross Nipponbare × Kasalath (Wissuwa et al. 1998). The major QTL for P uptake (*Pup1*) on chromosome 12 that explained 28% of the phenotypic variability was confirmed and fine mapped by employing both NIL and substitution mapping strategy (Wissuwa et al. 2002). The locus *Pup1* cosegregated with the marker S13126. Ni et al. (1998) used the extreme RILs (sensitive and tolerant) from a cross IR20 × IR55178-3B-9-3 (sensitive to P deficiency) in conjunction with AFLP markers and mapped a major QTL for P-deficiency tolerance (*PHO*) on chromosome 12 along with several minor QTLs on chromosomes 1, 6, and 9. Additionally, QTL studies targeted to locate genes for tolerance to ferrous iron ($Fe^{2+}$) toxicity, low potassium stress, and manganese toxicity were available (Wu et al. 1998a,b; Wang et al. 2002).

## 1.3.6 Important Agronomic Traits

Rice breeders usually target yield and yield-attributing traits that have a high impact on improving productivity. Some of the agronomic traits that have been thoroughly investigated in a wide array of populations under different environmental situations are plant height, heading date, yield, and its component traits. The genetic basis of many of these

traits is understood to some degree by determining the number and location of genes/QTLs on the map, gene effects, and interaction with other QTLs and also environment. It is evident from the progress made so far that molecular markers and their application has revolutionized the concept of quantitative traits and breeding strategy to facilitate further genetic gain in rice productivity. Since this aspect has been reviewed earlier (Yano and Sasaki 1997; Zhang and Yu 2000), our discussion will be limited to recently reported QTL mapping studies. For many of these so-called quantitative traits, such as heading date, plant height etc., loci with major effect have been identified and a few of them have been cloned.

## Plant Height

The reduction of plant height through the use of a semidwarfing (*sd*) gene was instrumental in boosting rice productivity. A total of 13 genes responsible for semidwarfism have been plotted onto the molecular linkage map of rice (Huang et al. 1996). The semidwarfing genes located on different rice chromosomes 1 are *d-10*, *sd-1*, and *d-18* (chromosome 1), *d-5*, *d-30*, and *d-32* (chromosome 2), *d-56* (chromosome 3), *d-31* and *d-11* (chromosome 4), *sdg* (chromosome 5), *d-9* (chromosome 6), *d27* (chromosome 11), and *d-33* (chromosome 12) (Zhang and Yu 2000). Besides these qualitative genetic loci, a large number of studies have investigated plant height using a QTL approach and mapped several QTLs distributed over all 12 chromosomes (Table 9). Huang et al. (1996) compared the QTL-mapping results across five populations to analyze the correspondence between the qualitative genes and the QTLs for plant height and found a very strong correspondence between the map positions of QTLs and the major dwarfing genes.

The genetic basis of QTL effects and their interaction with environments for plant height and heading date was investigated by evaluating the DH population of IR64 × Azucena in nine environments in Asia (Li et al. 2003c). Thirty-seven main-effect QTLs and 29 epistatic QTLs were identified, and many of them were detected in multiple environments with consistency in direction but of variable magnitude. Response of some QTLs was different in different environments. Therefore, information regarding the magnitude of QTL x Environment interaction would be essential even for highly heritable traits for effective MAS.

## Heading Date and Photoperiod Sensitivity

Manipulation of the heading date in rice is an important objective in all rice-breeding programs. The vegetative growth duration and photoperiod sensitivity both determine the time of flowering. Although a number of major genes controlling photoperiod sensitivity have been known, few of these genes have been assigned to rice chromosomes: *Se-1, Se3, Se-5* on chromosome 6, *E1* on chromosome 7, and *E-3* on chromosome 3 (Kinoshita 1998). Since the tagging of the first major photosensitive gene *Se-1* with a molecular marker (Mackill et al. 1993), a large number of QTLs with both major and minor effects have been mapped onto rice chromosomes (Table 10) (Li et al. 1995b; Xiao et al. 1995, 1996c; Lin et al. 1996a, 1998; Yano et al. 1997). QTL mapping by Yano et al. (1997) further confirmed that *Se-1* locus was the same as *Hd1,* which explained 67% of the phenotypic variation. Of four additional QT loci, *Hd-2* and *Hd-4* were mapped on chromosome 7 and *Hd-3* and *Hd-5* on chromosomes 6 and 8, respectively. Three additional QTLs were identified using a BIL population of the same cross Nipponbare x Kasalath (Lin et al. 1998). A fine mapping study using an advanced backcross progeny revealed *Hd1, Hd2,* and *Hd3* loci as Mendelian factors (Yamamoto et al. 1998). Further characterization of these QTLs and their interaction were done by developing the QTL-NILs through MAS (Yamamoto et al. 2000; Lin et al. 2000, 2002, 2003; Monna et al. 2002b) ultimately leading to cloning and isolation of some of these QTLs (Yano et al. 2000; Kojima et al. 2002; Takahashi et al. 2001).

## Yield and Yield Components

A few reviews summarizing the progress of QTL mapping of complex agronomic traits in rice are available (Yano and Sasaki 1997; Zhang and Yu 2000). A large number of QTL studies have been directed toward mapping the genes for yield and yield-contributing factors (Table 9). Populations used in those studies were either $F_2$, or RIL or DHL or advanced backcross lines (ABL) derived from either intersubspecific crosses or interspecfic crosses involving wild relatives. The varying number of QTLs identified in different experiments and the differential QTL effects and their contribution to the total phenotypic variation of a specific trait might be due to variable population size or variable statistical threshold to declare the QTL, linkage density, and genotypes. From a practical point of view, comparison of QTLs and their

**Table 9.** Quantitative trait loci studies for yield and yield components involving cultivated rice

| Reference | Parents and cross* | Mapping population | No. and types of markers used | Methods/software used | Traits scored | Number of QTL | Chromosomal location of QTL | Variance % explained |
|---|---|---|---|---|---|---|---|---|
| Lin HX et al. 1996a | Tenasai (I) × CB (I) | 480 $F_2$ | 91 RFLP | Mapmaker/QTL | Grain weight/plant | 3 | 1,2,4 | 9–11 |
| | | | | | No. of panicles/plant | 2 | 2,4 | 9–26 |
| | | | | | Grain number/panicle | 2 | 1,2 | 9–17 |
| | | | | | Spikelet no./panicle | 1 | 8 | 16 |
| | | | | | Spikelet fertility | 1 | 1 | 12 |
| | | | | | 1000-grain weight | 3 | 1,4,5 | 9–15 |
| | | | | | Spikelet density | 1 | 8 | 11 |
| | | | | | No. of first branches/ main panicle | 1 | 8 | 21 |
| | Waiyin 2 (I) × CB (I) | 480 $F_2$ | 101 RFLP | Mapmaker/QTL | Grain weight/plant | 3 | 1,4,5 | 10–22 |
| | | | | | No. of panicles/plant | 2 | 5,6 | 10–12 |
| | | | | | Spikelet no./panicle | 2 | 6,11 | 13 |
| | | | | | Spikelet fertility | 1 | 1 | 9 |
| | | | | | 1000-grain weight | 2 | 2,10 | 15–23 |
| | | | | | Spikelet density | 2 | 2,6 | 12–13 |
| Xiao et al. 1996 | 9024 (I) × LH 422 (J) | 194 RILs | 141 RFLP | ANOVA and Mapmaker/QTL | Plant height | 6 | 1,2,5,6,7,8 | 8–12 |
| | | | | | Days to heading | 3 | 3, 8,11, | 7–51 |
| | | | | | Days to maturity | 2 | 8,11 | 7–74 |
| | | | | | Panicle length | 2 | 7,9 | 6–10 |
| | | | | | Panicles/plant | 1 | 4 | 7 |
| | | | | | Spikelets/panicle | 4 | 3,4,8 | 5–19 |
| | | | | | Spikelet setting density | 3 | 3,4,10 | 7–21 |
| | | | | | Grains/panicle | 3 | 3,4,5 | 14–22 |
| | | | | | Percentage seed set | 1 | 5 | 12 |
| | | | | | 1000-grain weight | 3 | 3,4,5 | 10–15 |
| | | | | | Spikelets/plant | 4 | 3,4,9,11 | 5–11 |
| | | | | | Grains/plant | 3 | 3,4,5 | 15–16 |
| | | | | | Grain yield | 2 | 8,12 | 6–9 |

**Table 9.** (continued)

| Reference | Parents and cross* | Mapping population | No. and types of markers used | Methods/software used | Traits scored | Number of QTL | Chromosomal location of QTL | Variance % explained |
|---|---|---|---|---|---|---|---|---|
| Wu et al. 1996 | Palawan (JV) × IR42 (I) | 231 $F_2$ | 104 RFLP | Mapmaker/QTL | Plant height | 4 | 1,2 | 11–17 |
| | | | | | Tiller number | 2 | 4,12 | 4–7 |
| | | | | | Panicle number | 1 | 4 | 5 |
| | | | | | Panicle length/plant | 2 | 2,6 | 5–6 |
| | | | | | Primary branch no./ panicle/plant | 1 | 4 | 7 |
| | | | | | 1000 Grain weight | 1 | 1 | 10 |
| Lu et al. 1997 | ZYQ8 (I) × JX17 (J) | 132 DHLs | 137 RFLP | Mapmaker/QTL | Heading date | 4 | 1,8,10 | 9–35 |
| | | | | | Plant height | 5 | 3,4,7,8,10 | 9–24 |
| | | | | | 1000-grain weight | 6 | 1,2,3,5,6,8 | 8–19 |
| | | | | | Spikelets/panicle | 2 | 4,6 | 13–25 |
| | | | | | Grains/panicle | 2 | 4,6 | 13–28 |
| | | | | | Seed set % | 3 | 4,5,7 | 10–23 |
| Redona and Mackill 1998 | Labelle (TJ) × Black Gora (I) | 195 $F_2$ | 116 RFLP | PLABQTL | Panicle size | 2 | 3,6 | 8–16 |
| | | | | | Spikelet fertility | 4 | 1,3,5 | 7–11 |
| | | | | | Grain length | 7 | 2,3,4,7,10 | 8–21 |
| | | | | | Grain breadth | 4 | 2,3,7,8 | 3–11 |
| | | | | | Grain shape | 3 | 3,4,7 | 11–26 |
| | | | | | Grain weight | 2 | 4,8 | 8–10 |
| He et al. 2001 | ZYQ8 (I) × JX17 (J) | 107 RIL | 154 RFLP,AFLP, RAPD, SSR | Mapmaker/QTL | Days to heading | 2 | 8,12 | 11–35 |
| | | | | | Plant height | 2 | 1,4 | 12–32 |
| | | | | | No. of spikelets per panicle | 2 | 4,6 | 10–15 |
| | | | | | No. of grains per panicle | 1 | 4 | 13 |
| Yamagishi et al. 2002 | Akihikari (Tmp. J) × Koshihikari (Tmp. J) | 212 DHLs | 169 RFLP, RAPD | QGENE | No. of primary branches per panicle | 5 | 3,5,7,8,11 | 6–19 |
| | | | | | No. of secondary branches per panicle | 3 | 3,6,7 | 5–26 |
| | | | | | No. of spikelets on secondary branch | 1 | 6 | 20 |

**Table 9.** (continued)

| Reference | Parents and cross* | Mapping population | No. and types of markers used | Methods/software used | Traits scored | Number of QTL | Chromosomal location of QTL | Variance % explained |
|---|---|---|---|---|---|---|---|---|
| Hittalmani et al. 2003 | IR 64 (I) × Azucena (J) | 135 DHLs | 175 RFLP, isozyme, RAPD, cloned genes | Mapmaker/QTL | Biomass per plant | 6 | 1,3,4,7 | 9–16 |
| | | | | | Fertility % | 1 | 4 | 8–13 |
| | | | | | Heading date | 7 | 1,3,4,7,8,9 | 8–32 |
| | | | | | Harvest index | 11 | 1,3,4,7,8 | 9–26 |
| | | | | | No. of panicles per plant | 6 | 1,3,4,12 | 8–28 |
| | | | | | No. of spikelets per panicle | 6 | 1,4,7 | 8–41 |
| | | | | | Panicle exsertion | 3 | 1,4,8 | 9–23 |
| | | | | | Plant height | 4 | 1,3,7 | 8–63 |
| | | | | | Panicle length | 8 | 1,3,4,6,7,10 | 8–37 |
| | | | | | 1000 grain weight | 10 | 1,3,6,10 | 8–30 |
| | | | | | Grain yield per plant | 3 | 4,9,10 | 8–15 |
| Mei et al. 2003 | Lemont (J) × Teqing (I) | 254 RILs and two test cross populations | 182 RFLP, morphological markers | QTLMapper | Heading date | 6 | 3,4,7,8,11,12 | 5–17 |
| | | | | | Plant height | 5 | 3,4,6,8 | 6–16 |
| | | | | | Flag leaf length | 2 | 2,3 | 12–19 |
| | | | | | Flag leaf width | 5 | 1,4,6,8,12 | 6–25 |
| | | | | | Panicle length | 3 | 2,8,10 | 9–16 |
| | | | | | Spikelet No. per panicle | 4 | 1,3,6,11 | 5–16 |
| | | | | | Spikelet fertility | 7 | 3,5,6,7,8,10,11 | 7–13 |
| Kobayashi et al. 2003 | M23 (I) × AK (J) | 191 RILs | 182 RFLP | QGENE | Culm length | 31 | 1,2,3,4,5,6,9,10,11,12 | 5–19 |
| | | | | | Panicle length | 59 | 1,2,3,4,5,6,7,8,9,10,11,12 | 5–23 |
| | | | | | Panicle number | 38 | 1,2,3,4,5,6,7,8,11,12 | 5–39 |
| | | | | | Tiller number | 14 | 1,2,3,4,6,9,11 | 5–20 |
| Teng et al. 2004 | ZYQ 8 (I) × JX17 (J) | 127 DHL | 108 RFLP, isozyme | Mapmaker/QTL | Net Photosynthetic rate | 2 | 4,6 | 15–17 |
| | | | | | Chlorophyll content | 3 | 1,3,8 | 11–14 |
| | | | | | Stomatal resistance | 1 | 4 | 11 |
| | | | | | Transpiration rate | 2 | 4,7 | 12–14 |

*I: Indica; J: Japonica; TJ: Tropical Japonica; Tmp. J: Temperate Japonica; JV: Javanica

**Table 10.** Quantitative trait loci analysis of yield and yield components in crosses involving wild species

| Reference | Parents and cross* | Mapping population | No. and types of markers used | Methods/software used | Traits scored | Number of QTL | Chromosomal location of QTL | Variance explained per QTL (%) |
|---|---|---|---|---|---|---|---|---|
| Xiao et al. 1998 | V20A/*O.rufipogon* (IRGC105491) //V20B///V20B ////Ce64 | 300 $BC_2$ testcross | 102 RFLP, 20 SSR | ANOVA | Days to heading | 7 | 1,3,5,6,7,8,12 | 3–15 |
| | | | | | Days to maturity | 8 | 1,3,5,6,7,8,12 | 3–11 |
| | | | | | Plant height | 6 | 1,4,8,9,12 | 8–45 |
| | | | | | Panicle length | 7 | 1,2,4,8,9,12 | 4–14 |
| | | | | | Panicles/plant | 2 | 1,2 | 3–4 |
| | | | | | Spikelets/panicle | 4 | 1.6,9 | 4–8 |
| | | | | | Spikelets/plant | 1 | 1 | 4 |
| | | | | | Grains/panicle | 5 | 1,4,8,12 | 4–13 |
| | | | | | Grains/plant | 6 | 1,2,4,5,8 | 2–7 |
| | | | | | Percentage seed set | 7 | 2,3,4,5,7,8 | 3–15 |
| | | | | | 1000-grain weight | 8 | 2,3,4,5,8,9,11,12 | 3–10 |
| | | | | | Grain yield | 7 | 1,2,4,5,8,12 | 3–5 |
| Xiong et al. 1999 | Aijiao Nante (I) × *O. rufipogon* (Acc. P16) | 172 $F_2$ | 348 RFLP, SSR, AFLP | Mapmaker/QTL | Heading date | 4 | 3,6,8,11 | 2–52 |
| | | | | | Plant height | 4 | 1,7,8,9 | 8–60 |
| | | | | | Tillers/plant | 2 | 1,4 | 9–18 |
| | | | | | Panicle length | 2 | 1,7 | 19–26 |
| | | | | | Secondary branches/panicle | 2 | 1,7 | 8–18 |
| | | | | | Spikelets/panicle | 3 | 1,7,8 | 7–8 |
| | | | | | Spikelet density | 2 | 1,3 | 9–10 |
| Moncada et al. 2001 | *O.rufipogon* (IRGC #105491) × Caiapo //Caiapo///Caiapo | 274 $BC_2F_2$ | 125 RFLP, SSR | QGENE | Days to heading | 4 | 2,3,7 | 5–13 |
| | | | | | Plant height | 6 | 1,2,4,5 | 6–22 |
| | | | | | Panicles/plant | 2 | 6,11 | 8 |
| | | | | | Grains/plant | 4 | 1,2,6,11 | 6–9 |
| | | | | | 1000-grain weight | 5 | 1,3,11 | 5–22 |
| | | | | | Yield/plant | 2 | 1,11 | 7–14 |

**Table 10.** (continued)

| Reference | Parents and cross* | Mapping population | No. and types of markers used | Methods/software used | Traits scored | Number of QTL | Chromosomal location of QTL | Variance explained per QTL (%) |
|---|---|---|---|---|---|---|---|---|
| Brondani et al. 2002 | *O. glumaepatula* (Acc. RS16) × BG90-2 | 96 $BC_2F_2$ | 157 SSR, STS | QGENE | Days to flowering | 11 | 2,3,4,5,7 | 12–26 |
| | | | | | Plant height | 7 | 1,3,5 | 12–21 |
| | | | | | Tiller no. | 6 | 4,5,7,8,11 | 12–33 |
| | | | | | Panicle number | 7 | 5,8,11 | 10–31 |
| | | | | | Panicle length | 9 | 4,5,7,8,11 | 12–19 |
| | | | | | Spikelets/panicle | 7 | 1,2,3,4 | 11–48 |
| | | | | | % filled grains/panicle | 12 | 1,2,3,4,5,6,7,11 | 11–24 |
| | | | | | 100-grain weight | 20 | 2,3,4,5,,7,8,11 | 12–33 |
| | | | | | Grain yield/plant | 18 | 1,2,3,4,5,6,7,11 | 12–39 |
| | | | | | Filled grain number/panicle | 16 | 1,2,3,4,5,7,11 | 12–42 |
| | | | | | Grain yield/panicle | 23 | 1,2,3,4,5,6,7,11 | 11–32 |
| Thomson et al. 2003 | *O.rufipogon* (IRGC #105491) × Jefferson (TJ) | 353 $BC_2F_2$ | 153 SSR, RFLP | QGENE and QTL Cartographer | Days to heading | 11 | 1,2,3,4,7,10 | 6–26 |
| | | | | | Grains/panicle | 10 | 1,2,3,4,8,9,11,12 | 5–11 |
| | | | | | Spikelets/panicle | 6 | 1,2,3,9,12 | 6–10 |
| | | | | | Panicle length | 6 | 1,2,4,9,10,12 | 6–21 |
| | | | | | Percentage Seed Set | 7 | 1,3,4,5,6,10 | 6–16 |
| | | | | | Grain weight | 8 | 1,2,3,5,9,10,12 | 6–15 |
| | | | | | Panicles/plant | 2 | 3,7 | 6–8 |
| | | | | | Yield/plant | 5 | 2,3,6,9 | 7–17 |
| Septiningsih et al. 2003 | IR64 × *O.rufipogon* (IRGC #105491) | 285 $BC_2F_2$ | 165 RFLP, SSR | QGENE and QTL Cartographer | Days to heading | 6 | 2,7,11,12 | 3–6 |
| | | | | | Days to maturity | 4 | 4,7,8 | 2–5 |
| | | | | | Plant height | 5 | 1,4,6,10,11 | 3–56 |
| | | | | | Panicle length | 5 | 1,3,9,10 | 2–7 |
| | | | | | Panicles/plant | 5 | 1,2,11 | 4–10 |
| | | | | | Grains/panicle | 2 | 2,3 | 5 |
| | | | | | Spikelets/panicle | 1 | 3 | 6 |
| | | | | | Percentage Seed Set | 3 | 1,2,11 | 1–8 |
| | | | | | Grains/plant | 3 | 1,2,12 | 2–5 |
| | | | | | Grain weight | 5 | 1,2,3,7 | 5–11 |

*I: Indica; J: Japonica; TJ: Tropical Japonica; Tmp. J: Temperate Japonica; JV: Javanica

map positions across different populations, though useful for marker-assisted improvement of quantitative traits, is inhibited by the use of different sets of DNA markers. Epistasis and QTL × E interaction further limits the use of QTL information for crop improvement. Development of a series of NILs with different combinations of QTLs will be essential to demonstrate the effect of QTL and epistatic interaction. This approach was used to identify the QTLs that could improve yield and lodging resistance in rice (Ishimaru 2003; Kashiwagi and Ishimaru 2004). From an analysis of QTL-NILs in conjunction with candidate gene strategy Ishimaru et al. (2004) identified a new gene sucrose phosphate synthase, which controls plant height.

In addition to the plant type attributes, photosynthetic rate determines the dry matter production and yield. Photosynthesis and its related physiological traits were studied using molecular markers to identify the responsible QTLs (Teng et al. 2004). Because of nonoverlapping of the QTLs for yield components with those for photosynthetic ability, it was concluded that photosynthetic ability does not influence yield (Ishimaru et al. 2001) because many factors other than photosynthetic ability influence grain yield.

### 1.3.7 QTL × Environment Interaction

In most QTL mapping studies, the QTL × Environment (E) interaction factor is often overlooked. Realizing the importance of epistasis and environmental influence in analyzing the genetic basis of quantitative traits, the main effects, epistatic effects, and environmental effects in rice have been characterized in several studies (Li et al. 1997b; Yan et al. 1998, 1999; Cao et al. 2001; Liao et al. 2001; Xing et al. 2002; Yu et al. 2002). The QTL × E interaction is responsible for the fluctuation in the phenotypic expression of traits in different environments, making phenotype-based selection difficult. The most common way to deduce Q × E interaction is by evaluating the segregating mapping population in different environments and then comparing with QTL mapping results. Hittalmani et al. (2003) evaluated a DH population for 11 growth- and yield-related traits in nine different environments across four countries in Asia and identified many QTLs that are stable across environments. They also evaluated the clustering of QTLs for traits like plant height, panicle number, panicle length, and spikelet number in the same chromosomal regions. Thirty-four of 126 QTLs detected for 11 traits were common in more than one environment and were spread over 10 chromosomes. Plant height was least influenced by environment and 0 to 4 QTLs were detected per trait per location. A similar study was conducted earlier by Zhuang et al. (1997) using $F_2/F_3$ populations from an indica/indica cross combination Tenasai2/CB. While this type of study gives an indication about the stability of QTL expression, it does not quantify the individual Q × E interaction effects and the reasons for the instability of QTL in different environments. Evaluating the same IR64 × Azucena DH population at nine locations in Asia, Li et al. (2003c) not only identified the main effect and epistatic QTL but also quantified the Q × E effect for heading date and plant height. QTLs are either not expressed or weakly expressed in multiple environments. This inconsistency is further compounded by the epistasis and significant Q × E interaction with its direction being opposite to QTL main effects. This interaction might be either trait specific or gene specific and thus should be considered before MAS is performed to improve quantitative traits.

#### Seedling Vigor

Cultivars with improved seedling vigor emerge rapidly and uniformly from soil, ensuring an optimum stand establishment in temperate rice-growing areas and high-elevation areas in the tropics and subtropics. A number of quantitatively inherited traits such as long mesocotyls and coleoptiles, rapid root growth, and longer shoots determine the seedling vigor. Though considerable variation exists for these traits, improvement of seedling vigor of modern cultivars through breeding has not been satisfactory. Seedling vigor in general is higher in temperate japonica and indica rices than tropical japonicas. Redona and Mackill (1996) used an $F_2$ population from a cross Labelle (low-vigor japonica) × Black Gora (high-vigor indica) and mapped 13 QTLs distributed on chromosomes 1, 2, 3, 5, 6, 7, and 9, each accounting for 7 to 38% of the phenotypic variation for four seedling characteristics, i.e., shoot length, root length, coleoptile length, and mesocotyl length. Both parents contributed positive alleles to high seedling vigor. In an RIL population, Cui et al. (2002) identified four important genomic regions (RG393-C1087-RZ403 interval on chromosome 3, C246-RM26-C1447 and R830-R3166-RG360-C734b

intervals on chromosome 5, and waxy gene region on chromosome 6) that harbor QTL clusters for a number of traits associated with seedling vigor. Additionally, they mapped a number of biochemical or physiological traits such as total amylase activity, alpha-amylase activity, reducing sugar content, root activity, and seed weight, which are associated with seedling vigor.

### 1.3.8 Utilization of Wild Species for Mapping and Introgression of Agronomic Traits

Most wild relatives of crop species are phenotypically inferior and are often regarded unuseful for crop production. With the current innovative genomics approach, it is now possible to mine previously undiscovered genes in wild species that will have the potential to improve yield, quality, and other agronomic traits (Table 10). By using a unique mapping procedure called advanced backcross QTL analysis (Tanksley et al. 1996), it was possible to discover those masked genes (Xiao et al. 1996b). In a study involving a wild species *O. rufipogon* Acc. IRGC 105491, Xiao et al. (1998) found beneficial alleles from 35 of 68 identified QT loci, and particularly two QTLs, namely, *yld1.1* and *yld2.1* of this wild species, when added to cultivated species, improved yield by 17 to 18% without delaying maturity or increasing plant height. A number of parallel studies involving the same accession of *O. rufipogon* in combination with different rice cultivars from Brazil, USA, and Asia uncovered *O. rufipogon*-derived alleles with the potential to improve rice productivity (Moncada et al. 2001; Septiningsih et al. 2003b; Thomson et al. 2003). *O. glumapaetula*, a diploid wild relative of rice, was used in another study (Brondani et al. 2002) that demonstrated the existence of positive alleles in this species to improve tiller number and panicle number.

## 1.4 Molecular Characterization of Rice Germplasm

Molecular markers have become more common for analyzing germplasm resources in many field crop species including rice. The most familiar application has been the assessment of the amount of genetic variability present in germplasm collections. Additionally, molecular marker technology is helping in the identification of redundancies and gaps in germplasm collections, screening of new potential accessions, variety identification, and purity testing. Several reports document the amount of diversity present in wild species of rice (Qian et al. 2001; Sun et al. 2001; Thomas et al. 2001; Park et al. 2003; Zhou et al. 2003a), whereas in some cases the phylogenetic relationship among *Oryza* species has also been determined (Aggarwal et al.1999; Ge et al. 1999).

Since the use of isozymes in classifying the Asian rice varities (Glaszmann 1987), a number of genetic fingerprinting techniques have been developed to characterize and classify rice accessions. The techniques used involved RFLP (Sun et al. 2001), AFLP (Zhu et al. 1998), RAPD (Mackill 1995), intersimple sequence repeat (ISSR) (Blair et al. 1999; Joshi et al. 2000), STS (Yashitola et al. 2002), SSR (Ni et al. 2002), minisatellites (Zhou and Gustafson 1995), simple repetitive and hypervariable DNA sequences (Ramakrishna et al. 1994, 1995), and restriction landmark genomic scanning (RLGS) (Kawase 1994).

The genetic relationship among rice cultivars was examined using a number of different marker systems (Joshi et al. 2000). Spada et al. (2004) examined the genetic relationship among cultivated Italian rice germplasm using AFLP and SSR markers and grouped them into two main clusters: a small one comprising four exotic accessions and a larger one capable of being split into four subgroups. Song et al. (2003) compared the molecular-marker-based and pedigree-based genetic similarity among Korean rice cultivars. They showed that molecular data are more effective in identifying individual cultivars, and both pedigree data and DNA data are helpful in assessing overall patterns of genetic variation among rice germplasm. Using a set of 55 SSR markers scattered over all 12 chromosomes, Singh et al. (2004) could establish the identity of 23 aromatic rice genotypes including the Basmati types. They also fingerprinted 20 individual plants, grown from the nucleus, breeder, foundation, certified, and farmer-saved seed samples of Pusa Basmati 1 and found no variation among them.

The application of molecular markers is also emphasized for germplasm management (Jackson 1997). It is possible to predict the quantitative variation within rice germplasm using molecular markers to expedite the utilization of biodiversity available and maintained at genebanks (Virk et al. 1996). Zhu et al. (1998) analyzed the biodiversity of 57 rice germplasm

accessions using AFLP and grouped them into three groups that corresponded to isozyme groups I, II, and VI. The utility of PCR-based approaches, amplicon length polymorphism (ALP), and PCR-based RFLP, relative to that of southern-based RFLP, was demonstrated by Ghareyazie et al. (1995) to classify the Iranian rice varieties. Xu et al. (1998) successfully separated japonicas from indica varieties by ALP. To examine the pattern of diversity among 38 US rice cultivars belonging to two rice subspecies, Ni et al. (2002) used 111 SSR markers and concluded that japonica varieties are more diverse than indica cultivars on chromosomes 6 and 7 but less diverse on chromosome 2. Two subsets of around 30 SSR markers could show the same level of discriminating ability as that of 111 markers. In a recent study involving 101 SSR markers, Yu et al. (2003) grouped 193 lines from 26 countries into three major groups and nine subgroups. Group I represented the classical indica subspecies, whereas groups II and III belonged to the japonica subspecies. Most variation (93.5%) in the entire sample was caused by intrasubspecies differences, whereas indica-japonica differentiation contributed only 6.5% to the total variation. The largest number of markers on chromosomes 9 and 12 and the smallest number of markers on chromosomes 4 and 8 distinguished indicas from japonicas. This study revealed that the wide diversity among these rice germplasm was caused by selection for ecogeographical adaptation on multilocus associations.

Germplasm chacterization may be helpful for hybrid rice breeding. Subudhi et al. (1998) characterized 72 CMS lines developed at IRRI with AFLP and showed that resolution was much higher than for those based on qualitative and quantitative phenotypic traits. Genetic grouping of CMS lines based on AFLP may be useful for breeders in selecting genetically diverse CMS lines for hybrid seed production without performing a test cross of individual lines. Although molecular markers are helpful for identification, protection and parentage determination of hybrids (Wang et al. 1994a), molecular-marker-based genetic distance is not yet perfected to predict heterosis for complex traits (Kwon et al. 2002a; Xu et al. 2002a).

## 1.5 Progress in Marker-Assisted Breeding

Molecular marker technology has the potential to accelerate the cultivar development process in a number of ways (Tanksley et al. 1989; Mohan et al. 1997a; Subudhi and Nguyen 2004). Manipulation of most agronomic traits in crop plants is difficult because of complex polygenic inheritance. The ability to dissect and clone factors responsible for those complex traits offers a unique opportunity to improve crop productivity. Transfer of desirable genes among cultivars and precise introgression of novel genes from wild and weedy relatives into cultivars can be hastened significantly by using molecular markers. Pyramiding of genes through conventional plant-breeding approaches is difficult, laborious, and, in most cases, impossible. Durability of resistance can be enhanced if two or three genes for resistance against the same pathogen or insect can be pyramided into a single cultivar using molecular markers. Thus, molecular markers have obvious advantages for efficient selection of target traits under a variety of situations such as (1) when the trait is difficult or expensive to evaluate, (2) when several genes are to be pyramided, (3) when quick and precise transfer of genes is needed by reducing linkage drag, specifically introgression of alien genes, and (4) when selection of desirable plants is needed at early seedling stage.

With the development of high-density molecular linkage maps of rice (Harushima et al. 1998; McCouch et al. 2002), molecular tags for any trait of interest can be found anywhere on the genome and can be used to transfer useful genes from one varietal background to another. It is also possible for the breeders to conduct several cycles of selection in a year using molecular markers. Successful integration of molecular marker technology in plant-breeding programs, however, will require the development of high-throughput, rapid, reliable, and inexpensive genotyping tools that are capable of assaying large breeding populations with little DNA. Besides SSR, single nucleotide polymorphism (SNP) is gradually becoming popular as a high-throughput genotyping tool. Multiplex PCR to target multiple loci and multiple genotypes is being investigated (Fan et al. 2000; Hirschhorn et al. 2000; Buetow et al. 2001). There is progress in the development of new technology to eliminate electrophoresis (Tyagi and Kramer 1996). It is expected that DNA chips capable of expression profiling of several target genes

simultaneously in large population will be designed for use in breeding programs in the near future. This section updates the progress made in marker utilization in rice cultivar development.

### 1.5.1 MAS for Disease Resistance

The impact of DNA-marker-assisted selection on breeding disease-resistant rice cultivar has been impressive as reflected by a number of studies. In lieu of RFLPs, PCR-based molecular tools in the form of STS and SSR have been developed to implement genotypic selection. Two important diseases, BLB and blast, were the prime targets for which a large number of genes have been identified and mapped in a wide range of germplasms. Rice lines with improved resistance against BLB and blast are being developed through successful pyramiding of multiple disease resistance genes. A number of STS and SSR markers linked to various BLB and blast genes and their primer sequences are listed in Table 11.

Among several STS markers generated from AFLP fragments linked to the rice bacterial blight resistance gene *Xa7*, M5 was found to be cosegregating with the gene (Porter et al. 2003). Gu et al. (2004) saturated the *Xa27(t)* genomic region with markers derived from the genomic sequence of *O.sativa* cv. Nipponbare and developed markers, viz., M631, M1230, and M449, that cosegregate with the gene. SSR and STS markers linked to BB resistance genes, *xa5, xa13, Xa21,* have been identified and developed (Ronald et al. 1992; Yoshimura et al. 1995; Zhang et al. 1996a; Blair and McCouch 1997).

In backcross breeding programs, DNA-marker-based selection can hasten the incorporation of desirable genes. Chen et al. (2000, 2001) improved bacterial blight resistance of two elite restorer lines "6078" and "Minghui 63" by incorporating *Xa21* from "IRBB21" through MAS. The hybrids developed using these improved restorer lines showed improvement in yield under disease infestation.

Mapping of many blast resistance genes followed by fine mapping and development of PCR-based markers have significantly accelerated the breeding of blast-resistant cultivars in rice. Analyzing the molecular profile and blast resistance data of the RIL population of Co 39 x Moroberekan (Wang et al. 1994b), three RI lines carrying different genes for complete resistance, and two RI lines with genes for partial resistance were identified for quick development of NILs (Inukai et al. 1996). Liu et al. (2003) demonstrated the utility of MAS by improving the resistance of Zhenshan 97 against rice blast. Selection was performed by using an SSR marker linked to *Pi1* gene on chromosome 11.

For blast resistance gene *Pi10*, Naqvi and Chattoo (1996) developed SCAR markers from linked RAPD fragments. Hittalmani et al. (1995) developed an STS marker for a tightly linked RFLP marker RG64 and detected specific amplicon polymorphism (SAP) between the resistant and the susceptible genotypes upon digestion of the PCR products with a restriction enzyme *Hae*III. Efficiency of selecting resistant plants with this STS marker was 95%, but use of flanking markers improved the selection efficiency to 100%. Pan et al. (2003) developed three RAPD markers, BAR 15486, BAR 15782, and BAR 15844, tightly flanking the *Pi15* gene with recombination frequencies of 0.35%, 0.35%, and 1.1%, respectively, for marker-aided gene pyramiding. A pair of primers that specifically amplified a susceptible *pi-ta* allele was developed to verify the absence of *Pi-ta* gene (Jia et al. 2004).

Using sequence data found in public databases and degenerate primer pairs based on the P-loop, nucleotide binding sites, and kinase domain motifs of previously cloned resistance genes, Conaway-Bormans et al. (2003) developed PCR-based markers that cosegregate with the gene *Pi-z* that confers complete resistance to five races of blast and is located on the short arm of chromosome 6. The ability to identify polymorphism in a wide range of rice germplasms offers a valuable alternative to conventional phenotypic screening for rapid introgression of genes into susceptible varieties as well as the incorporation of multiple genes into individual lines for more-durable blast resistance. Hayashi et al. (2004) surveyed SNPs and insertion-deletions (InDels) in the chromosomal region containing the blast resistance genes *Piz* and *Piz*-t and generated SNP markers to discriminate resistant and susceptible alleles.

### 1.5.2 MAS for Insect Resistance

Among the mapped insect resistance genes (Table 4), considerable progress has been made in the development of PCR-based markers for gall midge resistance genes. Since rice breeders evaluate their segregating population in endemic areas where the pest occurrence is severe in particular parts of the year, it is very time consuming and labor intensive to breed vari-

**Table 11.** Primer sequences of PCR-based markers used for marker-assisted selection in rice

| Gene | Linked marker | Forward primer (5′–3′) | Reverse primer (5′–3′) | Enzyme | Reference |
|---|---|---|---|---|---|
| *xa5* | RG556 | TAG CTG CTG CCG TGC TGT GC | AAT ATT TCA GTG TGC ATC TC | *Mae*II | Yoshimura et al. 1995 |
| *xa5* | RG207 | ATT GTT ACG TTT GGT GGG GG | GCC ATG GCG ACT GTC AGT CG | – | Blair and McCouch 1997 |
| *Xa7* | M5 | CGA TCT TAC TGG CTC TGC AAC TCT GT | GCA TGT CTG TGT CGA TTC GTC CGT ACG A | – | Porter et al. 2003 |
| *xa8* | RM263 | CCC AGG CTA GCT CAT GAA CC | GCT ACG TTT GAG CTA CCA CG | – | Chen et al. 1997 |
| *Xa10* | RM206 | CCC ATG CGT TTA ACT ATT CT | CGT TCC ATC GAT CCG TAT GG | – | Chen et al. 1997 |
| *Xa10* | RM254 | AGC CCC GAA TAA ATC CAC CT | CTGGAG GAG CAT TTG GTA GC | – | Chen et al. 1997 |
| *xa13* | RG136 | TCC CAG AAA GCT ACT ACA GC | GCA GAC TCC AGT TTG ACT TC | *Hinf*I | Zhang et al. 1996a |
| *xa13* | RM230 | GCC AGA CCG TGG ATG TTC | CAC CGC AGT CAC TTT TCA AG | – | Chen et al. 1997 |
| *Xa21* | pTA248 | AGA CGC GGA AGG GTG GTT CCC GGA | AGA CGC GGT AAT CGA AAG GAT GAA A | – | Chunwongse et al. 1993 |
| *Xa27(t)* | M631 | CTG CAT CCA TGC CGG TGG CCG | AAA CGT CAC ATG AAG ACT CCA ATT GT | *Nde*I | Gu et al. 2004 |
| *Xa27(t)* | M1230 | AGG GAT GTC GAG ATG AGA GCT TC | GGT GTC CTT CTT TAC GGG CCT CC | *Eco*RI | Gu et al. 2004 |
| *Pi-1* | Pi-1 | CTC CTT CTC CGA CCG TGC TC | AGC ATA GAA GCA CAT CAT TG | – | Cho et al. 2003 |
| *Pi2* | RG64 | GTT GTT TGA GCT CTC CAA TGC CTG TTC | GGA CCG GCA TGT AAC GTG ACG TC | *Hae*III | Hittalmani et al. 1995 |
| *Pi-5* | JJ80 | TTA TGA GAT TAG GAG TGT AT | ATG TAA AGG CAA AAG CTG AT | – | Cho et al. 2003 |
| *Pi-9* | pB8 | CCC AAT CTC CAA TGA CCC ATA AC | CCG GAC TAA GTA CTG GCT TCG ATA | – | Liu et al. 2002a |
| *Pi-9* | pB14 | TGG TGC ACT CAG AAA GAA | GCA GTG TCA TCT TGT CTC C | – | Liu et al. 2002a |
| *Pi10* | OPF6-1 | GGG AAT TCG GTT TTA CAA CCA CCG | GGG AAT TCG GAT CTC CGG GGG TAG | – | Naqvi and Chattoo 1996 |
| *Pi10* | OPF6-2 | TTT TAC AAC CAC CGG TTT TAT GAC | ATC TCC GGG GGT AGA GCA CTG TTT | – | Naqvi and Chattoo 1996 |
| *Pi-12* | P265.560 | CAG CTG TTC AGT CGT TTG | CAG CTG TTC ATA CAA GAA AT | – | Zheng et al. 1995 |
| *Pi33* | C483 | CTT CCA CCA TAA AAC CGG AG | ACA CCG GTG ATC TTG TAG CC | – | Berruyer et al. 2003 |
| *Pi-b* | Pi-b | ATC AAC TCT GCC ACA AAA TCC | CCC ATA TCA CCA CTT GTT CCC C | – | Cho et al. 2003 |
| *Pi-ta* | Pi-ta$_{440}$ | CAA CAA TTT AAT CAT ACA CG | ATG ACA CCC TGC GAT GCA A | – | Jia et al. 2002 |
| *Pi-ta* | Pi-ta$_{1042}$ | AGC AGG TTA TAA GCT AGG CC | CTA CCA ACA AGT TCA TCA AA | – | Jia et al. 2002 |
| *Pi-z* | MRG5836 | TAT AAG CCG CAG CCA AAT TC | AAA AAC CTA GAA AAT GGG AAA ATG | - | Conaway-Bormans et al. 2003 |
| *Pi-z* | MRG2431 | ATC CAA ATC CAA TGG TGC AG | GTG GCG AAA GGG AAC ATT CT | – | Conaway-Bormans et al. 2003 |
| *Gm2* | PF8 | GGG ATA TCG GGG ATG AAA TGC CAA | GGG ATA TCG GTC ATT GCA GTG GAG | – | Nair et al. 1995 |
| *Gm2* | PF10 | GGA AGC TTG GCT TAT AGT AAC TAG | GGA AGC TTG GAA ATG CAA GAT CTT | – | Nair et al. 1995 |
| *Gm4* | E20 | TTA TTG ATG AGG ACT TAG GG | TGG ATA GGT TAG CAG AGC TG | – | Nair et al. 1996 |
| *Gm6* | RG214 | GGT AGA CAC GCG GGC GAG GTT G | CAC GCT CAA TCC AGG TGG ACA | – | Katiyar et al. 2001a |
| *Gm6* | RG476 | GAT GGC AAG CCA ATC AGA TCG | GAA GTG AGG AAG CCT ACA GTA AGC C | – | Katiyar et al. 2001a |
| *Bph2* | Bph2 | TAA CTG GTG TTA GTG CGA ATG C | AAT TCA CGG CAT GTG AAG CCC TAG | – | Murai et al. 2001 |
| *Bph13* | AJ09 | TCG ACC TAG AAA GGC CTG TGT | CAC TGG AAA TTT GAG CGA GAA | – | Renganayaki et al. 2002 |
| *tms3* | F18 | AGA GAG TGA TCT ATG CCC TG | GCG GAC CGT GGA AGC TGG GG | – | Lang et al. 1999 |

**Table 11.** (continued)

| Gene | Linked marker | Forward primer (5′–3′) | Reverse primer (5′–3′) | Enzyme | Reference |
|---|---|---|---|---|---|
| *tms5* | C365-1 | ATT TTG GTT GCG CAT TAG AGG | GAA ATA TGC CAA GTA CGG AGG AT | – | Wang et al. 2003b |
| *tms5* | G227-1 | ACA CAT CAG CAA CAA TTC ATC TAC | AAC AGC ATT TCC CCC TAC TAC A | – | Wang et al. 2003b |
| *tms (?)* | TS200 | CGG AAT GTA ATT CAC ATG C | CAG AGA AAC ATC AGT TGT GG | – | Reddy et al. 2000 |
| *rtms1* | Rev1 | CAG GTC CCT AAC CCT TAG CAA AG | CAC GAA CAA GAA GGA ATG AG | – | Jia et al. 2001 |
| *Rf1* | S12564 | CTA GTT AGC CGA ATA ACT GAG GTT C | TTT GTG GGT TTG TGG CAT TGA GAA AAT | *Tsp*509I | Komori et al. 2003 |
| *Rf1* | C1361 | AAA GCA ACC GAC TTC AGT GGC ATC ACC | CTG GAC TTC ATT TCC CTG CAG AGC | *Mwo*I | Komori et al. 2003 |
| *wx* | wx | CTC TCT CAC CAT TCC TTC AG | CAC AAG CAG AGA AGT GAA GC | – | Han et al. 2004 |
| *sbe1* | *sbe1* | GAG TTG AGT TGC GTC AGA TC | AAT GAG GTT GCT TGC TGC TG | | Han et al. 2004 |
| *sbe2* | *sbe2* | CCG AGG GAA TGC CAG GAG TAC CAG | GAA CCA CAA CCA AGT CCA AGG CAA | – | Han et al. 2004 |
| *sbe3* | *sbe3* | GTC TTG GAC TCA GAT GCT GGA CTC | ATG TAT AAC TGG CAG TTC GAA CGG | *Spe*I | Han et al. 2004 |
| *fgr* | RG28 | TGC CAA GTA TCC CCT GAT TCC | TTT GTG CCT CCT TTG CAG ATT C | – | Garland et al. 2000 |

eties resistant to a number of biotypes. Allele-specific PCR-based markers from RAPD fragments linked to *Gm2* and *Gm4* have been developed for MAS (Nair et al. 1995, 1996; Sardesai et al. 2001). In each case, RAPD fragments tightly linked to the genes were sequenced and primers were designed. Katiyar et al. (2001a) developed a PCR-based MAS kit containing the primer pairs based on the terminal sequences of the linked markers RG214 and RG476 for transferring the *Gm6(t)* gene into susceptible cultivars in China. Among the mapped BPH resistance genes, *bph2* has been mapped with higher resolution and an AFLP marker KAM4 showing complete cosegregation with the gene has been converted into an STS (Murai et al. 2001).

### 1.5.3 MAS for Grain Quality

Among the grain quality traits, amylose content is an ideal candidate for MAS because these traits can be evaluated only after the reproductive stage. Genetic basis of amylose content has mostly focused on the *Wx* gene encoding the granule bound starch synthase (GBSS). A G-T polymorphism at the 5′ splice site of the first intron of GBSS was earlier identified by Ayres et al. (1997) to determine amylose production, and this was exploited by Bormans et al. (2002) to develop a non-gel-based assay for MAS of grain quality. Larkin and Park (2003) cloned and sequenced GBSS cDNA from a number of cultivars differing in amylose content and found two SNPs in exons 6 and 10 that resulted in amino-acid substitutions, which makes changes in quality characteristics. The association of these point mutations with the functional differences between GBSS alleles could be useful in the development of varieties with superior eating, cooking, and processing characteristics. Besides *Wx* gene, starch branching enzyme 3 (*Sbe3*) played an important role for variation in amylose content. Liu et al. (2004c) developed tags for *Sbe1* and *Sbe3* by exploiting the sequence diversity for MAS of amylose content. In another study, Zhou et al. (2003b) improved eating and cooking quality traits in Zhenshan 97, an elite parent of hybrid rice by introgressing the waxy region from Minghui 63 and by using an SSR *waxy* marker and two flanking RFLP markers, C688 and C952. Garland et al. (2000) tested polymorphism in homologous regions of the marker RG28 linked to the major fragrance gene of rice (*fgr*) and detected a small mononucleotide repeat that was polymorphic between a pair of fragrant and nonfragrant cultivars and converted into a codominant PCR-based marker. Two more SSR markers, RM223 and RM42, were also mapped in the vicinity of *fgr* to distinguish fragrant varieties from nonfragrant varieties.

### 1.5.4 MAS in Hybrid Rice Breeding

Attributes such as fertility restoration, PGMS, TGMS, and reverse TGMS are either difficult to evaluate or can be evaluated only in the progeny of test crosses. In hybrid rice-breeding programs, these genes are often transferred to different genetic backgrounds to develop inbred lines by successive backcrossing. Development of molecular tags for these traits would allow selection at the seedling stage, resulting in considerable savings in both time and effort.

Komori et al. (2003) fine mapped the *Rf-1* gene that restores the pollen fertility in BT-type male sterile cytoplasm by using nine PCR-based markers developed from tightly linked RFLP markers on chromosome 10. Due to the tight linkage of the *Rf-1* to the flanking markers S12564 *Tsp509I* and C1361 *MwoI*, it will now be possible to transfer the *Rf-1* gene more efficiently and precisely. For the same restorer gene, a number of PCR-based markers have been developed and utilized by many other investigators (Akagi et al. 1996; Ichikawa et al. 1997; Mishra et al. 2003). The discovery of the tight linkage of the marker R2349 with the wide-compatibility gene $S_5$ (Liu et al. 1997) provides an opportunity to transfer the $S_5^n$ alleles to different varieties in intersubspecific hybrid breeding.

Introgression of the TGMS or reverse TGMS gene through conventional breeding is cumbersome because it involves identification of TGMS plants in the segregating generation followed by induction of fertility by rationing at appropriate temperatures. Lang et al. (1999) developed both dominant and codominant STS markers from the RAPD markers linked to the *tms3* gene and reported an accuracy of 85% in MAS at the vegetative stage. For *tms4(t)* on chromosome 2, Dong et al. (2000) converted an AFLP marker E5/M12-600 mapped at a distance of 3.3 cM into an STS for marker-assisted transfer of this gene to different genetic backgrounds. Wang et al. (2003b) developed one STS marker, C-365-1, and another CAPS marker, G227-2, that flanked the *tms*5 gene at a distance of 1.04 and 2.08 cM, respectively. Lopez et al. (2003) reported a successful transfer of *tms2* from Norin PL12 to an aromatic Thai cultivar KDML 105 using linked

SSR markers RM2 and RM11 on chromosome 7. The accuracy of selecting sterile plants during segregating generation was more than 90%. Jia et al. (2001) sequenced and converted a closely linked AFLP marker, *rev1,* 4.2 cM from the *rtms1* gene into a SCAR marker that could facilitate MAS of the *rtms1* gene.

### 1.5.5 Gene Pyramiding

Because different resistance genes provide resistance to different races or isolates, gene pyramiding is often considered a viable approach to improve durability of resistance in crop cultivars. Pyramiding of both major and minor genes may lead to durable resistance. The process of stacking of genes in a single cultivar can now be achieved more efficiently by performing MAS. It expedites the variety development process by offering the opportunity to select for all desirable genes simultaneously as well as eliminating the time-consuming process of inoculation for different races or isolates at different time intervals. Additionally, it allows the identification of individuals with desirable attributes in the segregating generation at the early vegetative stage well ahead of flowering to facilitate further crossing and/or backcrossing. A number of reports have demonstrated successful pyramiding of blast or BLB resistance genes (Huang et al. 1997a; Hittalmani et al. 2000; Sanchez et al. 2000; Singh et al. 2001).

Huang et al. (1997a) pyramided four bacterial blight (BB) resistance genes, *Xa-4, xa-5, xa-13,* and *Xa-21*, in different combinations. Breeding lines with two, three, and four resistance genes were developed, and these pyramid lines showed a wider spectrum and a higher level of resistance than lines with only a single gene. Sanchez et al. (2000) later transferred three BB resistance genes, *xa5*, *xa13*, and *Xa21*, to three promising but susceptible new plant type (NPT) lines, IR65598-112, IR65600-42, and IR65600-96, by employing STS markers. The $BC_3F_3$ NILs having more than one BB resistance gene showed a wider resistance spectrum and manifested increased levels of resistance to the Xoo races. The accuracy of selection in identifying homozygous resistant plants for *xa5* and *xa13* in two populations was 95% and 96%, respectively. Another marker-aided pyramiding experiment involving the above three BB genes into PR106, a widely grown cultivar in India, was conducted by Singh et al. (2001). Davierwala et al. (2001) used 11 STMS and 6 STS markers to identify lines with the resistance genes *xa5* and *Xa4* in an $F_3$ population of a cross between IR-64 and IET-14444.

Hittalmani et al. (2000) pyramided three major genes, *Pi1, Piz-5,* and *Pi-ta*, for blast resistance located on chromosomes 11, 6, and 12, respectively, using DNA markers. For *Piz-5*, a PCR-based SAP marker was used, whereas flanking markers were used for the other two. Field testing of the pyramided lines in the Philippines and India showed enhanced resistance against leaf blast in comparison with the lines with a single gene.

Effort has been made in a number of cases to combine both molecular breeding and genetic transformation to improve elite rice lines. Narayanan et al. (2002) stacked three major genes, *Pi-1*, *Piz-5,* and *Xa21,* in line Co 39 and two major genes, *Piz-5* and *Xa21,* in line IR50 by using both MAS and genetic transformation for resistance against blast and bacterial blight. In the first stage blast-resistant isolines were developed by four rounds of backcrossing in conjunction with MAS and, in the second stage, the resistant isolines were transformed with *Xa21*, which is known to confer resistance to all races of *Xanthomonas oryzae pv. oryzae.* In another study, Datta et al. (2002) reported the development of transgene-pyramided rice cv. IR72 lines using MAS that showed durable and broad-spectrum resistance against disease and insect pests by conventional crossing of two independently developed transgenic lines with different genes such as *Xa21* (for BB resistance), a *Bt* fusion gene (for yellow stem borer resistance), and chitinase gene (for tolerance of sheath blight). In this study the transgenes were used as the STS markers for rapid development of homozygous pyramided lines. Jiang et al. (2004) pyramided *Xa21* gene for resistance to BB and a fused *Bt* gene (*cry*1Ab/*cry*1Ac) conferring resistance to lepidopteran insects into a restorer line "Minghui 63." Results from the field trials indicated that hybrids of the pyramided line with the CMS lines "Zhenshan 97A" and "Maxie A" maintained similar yield levels under conditions without chemical spray. With the help of STS and SSR markers for both *Xa21* and *waxy* genes, Ramalingam et al. (2002) isolated 20 true-breeding lines with high amylose content and *Xa21* from four crosses.

### 1.5.6 MAS for Other Traits and QTL

Any trait that has been tagged with a molecular marker is amenable for MAS. Using a microsatellite

marker RM219 and a codominant PCR-based marker RM464A (derived from a microsatellite marker RM464) that are linked to *Sub1* by 3.4 and 0.7 cM, respectively, Xu et al. (2004) developed several NILs from the submergence tolerance source IR40931-26 in temperate japonica cultivar M-202 background. These two markers were tested in 55 diverse indica and japonica rice cultivars and breeding lines, and RM219 showed 14 different alleles, whereas none of the 55 cultivars had the same allele as the tolerant source. But RM464A showed three different alleles in the 55 cultivars. Thus, RM219 will be useful in breeding programs to select for the *Sub1* gene in a wide range of backgrounds, whereas RM464A will be helpful in selection for the *Sub1* gene in japonica rice background. Siangliw et al. (2003) successfully transferred *Sub1* from three submergence-tolerant lines to Thai Jasmine rice cultivar KDML105 by marker-assisted backcross breeding.

To improve drought tolerance, a marker-assisted backcross program was implemented to transfer the Azucena alleles at four QTLs for deeper roots (on chromosomes 1, 2, 7, and 9) from selected DH lines into IR64 (Shen et al. 2001). After evaluating 29 selected $BC_3F_3$ NILs in replicated experiments it was concluded that introgression of those QTLs in some of these NILs improved target root traits compared to IR64. For example, in the case of three tested NILs carrying target 1, one had significantly improved root traits over IR64. Three of the seven NILs carrying target 7 alone, as well as three of the eight NILs carrying targets 1 and 7, showed significantly improved root mass at depth. Four of the six NILs carrying target 9 had significantly improved maximum root length. But, because of likely cointrogression of linked QTLs, some NILs were taller than IR64 and all of them had a decreased tiller number.

Cho et al. (1994) established the order of DNA (RG220-RG109-RG381), morphological [anthocyanin activator (A), purple node (*Pn*), purple auricle (*Pau*)], and isozyme markers (*EstI-2*) of the semidwarf gene (*sd-1*) region on chromosome 1 and, after selfing of marker-aided selected individuals for four generations, demonstrated that three markers, *EstI-2*, RG220, and RG109, were tightly linked with *sd-1* locus and that genotypic selection for this recessive trait was effective at the seedling stage.

Anther culturability of rice is a quantitative trait controlled by nuclear-encoded genes. To increase the efficiency of green plant regeneration from microspores of 43 rice cultivars and two $F_2$ populations, "MG RI036"/"Milyang 23" and "MG RI036"/"IR 36", Kwon et al. (2002b,c) used three markers, RG323, RG241, and RZ400, that are tightly linked to the QTL on chromosome 10. They reported that marker RZ400 was effective in identifying genotypes with good and poor regenerability and will be helpful in introgressing this trait into elite lines.

Ahmadi et al. (2001) introgressed the rice yellow mottle virus (RYMV)-resistant allele of two QTLs from an upland resistant japonica variety, Azucena, into a lowland susceptible indica variety IR64 by using RFLP and microsatellite markers in backcross breeding. The efficient introgression was reflected from the improved performance of the introgressed lines.

### 1.5.7 MAS for Introgression of Alien Genes

Wild and exotic accessions provide a useful resource for a large number of useful genes, particularly biotic and abiotic stress tolerance. Using molecular markers, introgression of such useful genes is now possible with minimum linkage drag in backcrossing programs. In rice, genes from wild species of rice have been identified in the advanced backcross progenies from crosses involving wild species, *O. australiensis* and *O. brachyantha,* using molecular markers (Ishii et al. 1994; Brar et al. 1996). Substitution lines have been developed with chromosome segments of *O. glaberrima* in *O. sativa* background using RFLP markers during the backcrossing process and should constitute useful resources for rice improvement (Doi et al. 1997).

## 1.6 Map-Based Cloning of Rice Genes and QTL

One of the important triumphs of molecular genetics and related genomics is the direct application of genetic map information to isolate a gene corresponding to a phenotype. The identification of a gene behind a phenotype has been a major goal in genetics since Mendel's discovery of the laws of inheritance. Many novel discoveries in the 21st century, including the principle of recombination by T.H. Morgan, the reality of inheritance by Avery et al., the structure of DNA by F.H.C. Crick and J.D. Watson, molecular cloning by P. Berg in conjunction with new technology of DNA analysis and manipulation such as nucleotide

sequencing by F. Sanger, and polymerase chain reaction by K. Mullis led to the success of identification of genes corresponding to phenotypes. The most well-known case is the competition of gene hunting for Huntington disease, a serious human-inherited disease (Allitto et al. 1991). Through this competition, tagging of phenotype by DNA markers provides an efficient strategy for gene cloning. The success of this case greatly encouraged the efforts of isolation of a gene behind a phenotype relying on molecular genetics. This idea was also applied subsequently to plants (Tanksley et al. 1995), and the efforts of generating reliable DNA markers were launched as described in previous sections.

Among plants, *Arabidopsis* was chosen as a model system for gene isolation by molecular genetics, such as map-based cloning or positional cloning (Giraudat et al. 1992; Arondel et al. 1992). Although *Arabidopsis* is a weed; it has been the target for full genome sequencing because of its small genome size (130 Mb) (Arabidopsis Genome Initiative 2000). The idea to sequence the *Arabidopsis* genome was also supported by the existence of collection of mutants to apply genomics information to identify the genes responsible for mutation. Many important genes of *Arabidopsis* have been identified by map-based cloning since 1992. Using a similar strategy of map-based cloning, though depending on the degree of preparation of infrastructure such as molecular genetic map, a genomic library with large-sized insert DNA, and genome sequence information, challenges to isolate genes have been made successful in tomato (Mao et al. 2000), wheat (Yan et al. 2003), and barley (Brueggeman et al. 2002) as well as in rice.

For performing an effective map-based or positional cloning strategy, first, the resource of the target phenotype must be genetically pure and must be established as a single Mendelian factor. This is a prerequisite to applying molecular genetic tools effectively in the accurate tagging of a phenotype. In addition, the target phenotype must be preferably distinct to obtain a clear segregation pattern. Second, the DNA markers to tag the phenotype must be codominant and must have high density in the vicinity of plausible position of the gene in the case of PCR-based markers. Third, once a candidate region is fixed within a few hundred kilobase, the region must be narrowed further by increasing the population size to reduce the number of candidate genes. To facilitate the analysis of many plant samples, pooling of five siblings as one sample to check recombination must be adapted. Fourth, genomic libraries with large-size inserted DNA such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), P1-derived artificial chromosome (PAC), and cosmid libraries must be prepared and made available for screening by DNA markers tagged to the phenotype. If a positive genomic clone is identified, the sequence must be determined. And if the candidate gene region still spans more than 100 kb at this stage, genetic narrowing of the candidate region must be done using the sequence information to identify new polymorphisms such as SNP. Even if a plausible gene is found among the predicted genes within a candidate region, the target gene must be carefully confirmed because tandem duplicated similar genes are common in the rice genome. When several allelic variants exist for a common phenotype, Southern hybridization by a candidate DNA fragment should give supporting evidence if different patterns are obtained for each allelic variant. It is preferable to look for expressed genes by screening a cDNA library constructed from a specific tissue where the target phenotype is expressed.

The final step of map-based cloning is the confirmation of the biological function of the candidate gene. This is performed by transformation. The direction of transformation is either gain or loss of function depending on the characteristics of the candidate genome fragment. Transformation of rice plants by introducing *Agrobacterium tumefaciens* as an infectious tool of alien DNA has become easier and more efficient (Hiei et al. 1997). The candidate genomic region is cut out by an appropriate restriction enzyme and ligated to some eukaryotic expression vector carrying a suitable promoter (e.g., CaMV 35S). The japonica rice is considered better than indicas in their response to cell culture, although recently *Agrobacterium*-mediated transformation has been quite efficient in indica rice. Still the efficiency of transformation in the case of rice needs to be improved to obtain a large number of regenerated rice plants to obtain reliable data.

Some of the well-documented examples of map-based cloning include the isolation of disease resistance genes against viruses and bacteria. The identification of a disease resistance gene in plants was first reported in *HM1* of maize that controls the expression of the NADPH-dependent HC toxin reductase for resistance against the fungus *Cochliobolus carbonum* race 1 (Johal and Briggs 1992). However, this was performed in 1992 by transposon-induced mutagenesis, not by map-based genetics. At that time, molecular tools in maize for map-based cloning were not well

established. Then, in 1993, Tanksley's group at Cornell University published the isolation of the *Pto* gene of tomato that confers resistance to races of *Pseudomonas syringae* by map-based cloning (Martin et al. 1993). The success of this pioneering work on map-based cloning can be attributed to the well-developed molecular genetic tools for tomato such as a molecular genetic map, YAC library, cDNA library, and an efficient transformation strategy.

In 1995, the first report of success in isolation of rice disease resistance gene, *Xa21,* was published (Song et al. 1995). *Xa21* gene confers resistance to most races of *Xanthomonas oryzae* pv.*oryzae* (*Xoo*), including race 6, and carries both a leucine-rich repeat motif and a serine-threonine kinase-like domain. The tagging of *Xa21* by DNA markers on rice molecular genetic maps led to the identification of a closely cosegregating marker located on chromosome 11. This DNA marker was derived from rice genomic DNA and showed 20 to 30% identities to diverse proteins carrying leucine-rich repeat (LRR) motif. This LRR motif was identified in several disease-resistant genes from dicotyledonous plants (Staskawicz et al. 1995). The cloning of corresponding cDNA to this marker revealed its whole structure, which is commonly found in resistance (*R*) gene. Finally, the candidate was confirmed by the resistance reaction of the transformant in a susceptible japonica rice variety T-309. After this first success, several disease resistance genes of rice such as *Xa1*, which confers resistance against *Xoo* race 1 (Yoshimura et al. 1998), *Xa26,* which confers resistance against a wide range of *Xoo* races (Sun et al. 2004), *Pib*, which confers resistance against *Magnaporthe grisea* strain ARPC90-18C (Wang ZX et al. 1999), and *Pita,* which confers resistance against *M.grisea* strain O-137 (Bryan et al. 2000) were identified by map-based cloning. These isolated rice disease resistance genes carry common structural characteristics with dicotyledonous plants such as *Arabidopsis,* and this information gives us a hint for understanding the mechanism, evolution and biology of plant-microbe interaction in cereals (Ayliffe and Lagudah 2004).

Besides disease resistance genes, other agronomically and biologically important genes have been isolated from rice by map-based cloning. This includes the genes responsible for signal transduction of the plant hormone gibberellin, which controls plant growth and height. This trait is very important in rice and other cereal crops, particularly in increasing the capacity of light reception, and thereby increasing yield efficiency. Breeding efforts in the 1960s focusing on this trait led to the so-called "Green Revolution" (Conway 1997). The genes used in this program for rice and wheat are *sd1* and *Rht1*, respectively, which have been identified by map-based cloning and revealed to be involved in signal transduction pathway of gibberellin biosynthesis. The rice *sd1* (Ashikari et al. 2002; Sasaki et al. 2002a; Monna et al. 2002a; Spielmeyer et al. 2002) and wheat *Rht1* (Peng et al. 1999) are controlled by gibberellin $(GA)_{20}$ oxidase and a nuclear transcription factor, respectively. Later, orthologs of *Rht1* were isolated from rice as *slender 1 (slr1)* (Ikeda et al. 2001). Although the amino-acid sequences of both proteins are homologous, the mutated point of each gene corresponds to a different functional motif regarding the activity under gibberellin. This causes dwarfness in the case of *Rht1* and the slender phenotype in the case of *slr1*. Other important genes involved in gibberellin signal transduction, *d*1 (Ashikari et al. 1999; Fujisawa et al. 1999) and *gid2* (Sasaki et al. 2003), were also isolated by map-based cloning. The *d*1 and *gid2* are thought to play roles at the early and late steps of signal transduction, respectively.

The above-mentioned cases of gene isolation are applicable if the corresponding phenotype is coded by a specific gene. However, many important traits used for crop production are controlled not by a single gene, but by a gene network. This network can be analyzed as a genetically defined character known as QTL. Some well-known QTLs in rice include those for grain weight, number of panicles, flowering time, and culm length. Recent development in genetic mapping with many DNA markers enables accurate identification of QTLs by an interval mapping method (Lander and Botstein 1986). Also, DNA markers make it accurate to judge the genotype of any genomic region among the siblings. Therefore, a QTL identified in an $F_2$ population can be separated into each locus by repeated backcrossing and genotyping of each sibling. The resultant chromosome segment substitution lines (CSSL) can be used for making a segregating population by focusing on one of the loci of the target QTL to isolate as a single Mendelian factor (Tanksley 1993). This strategy was first developed to identify genes of QTL conferring fruit size (Frary et al. 2000) and also has been used to identify QTLs of rice-flowering time (Yano et al. 2001). Crossing japonica rice variety Nipponbare and indica rice variety Kasalath, so far 15 QTLs of flowering time have been identified with a significant LOD score (Fig. 4). They were found

without clustering at a specific locus. By inspecting the response to photoperiod for each locus using corresponding CSSL, about half of the QTLs of flowering time were revealed to be photoperiod sensitive. Detection of flowering under a controlled photoperiod condition is very clear and the data obtained are reliable. This condition satisfies the prerequisite for a successful map-based cloning strategy as described above.

Among photoperiod-sensitive QTLs of rice-flowering time, six loci have been fine mapped, and four of them have been succesfully identified. The first gene identified is called *Hd1* (Yano et al. 2000) located at chromosome 6 and mainly contributing to flowering time (LOD = 44.2) of the cross of Nipponbare and Kasalath. The *Hd1* gene was revealed to be an ortholog of *CONSTANS* (*CO*) gene of *Arabidopsis* that was identified also as a gene involved in flowering time (Putterill et al. 1995) and characterized by transcription factors carrying zinc-finger domain and CCT motif. The difference in sequence between Nipponbare and Kasalath is recognized in many positions, and the most crucial factor that affects the activity of gene products is the 2-bp deletion in the second exon of the Kasalath allele (Yano et al. 2000). The other isolated genes were *Hd3a* (Kojima et al. 2002) and *Hd6* (Takahashi et al. 2001), orthologs of *Arabidopsis* flowering time (*FT*), and casein kinase 2 alpha (*CK2α*), respectively. These three rice genes have orthologs in *Arabidopsis*, but the fourth one, called *Ehd1* (Doi et al. 2004), which might be the same as *Hd14* and carries a homologous sequence with B-type response regulator, does not have any ortholog in *Arabidopsis*. The identified genes of rice QTL of flowering time must be further analyzed for their biochemical and physiological function with regard to their interactive gene network as QTL (Sasaki et al. 2003). In addition to these studies, CSSL for each locus of QTL is used in the genetic analysis of interaction of each locus by crossing each locus.

Besides flowering time, other rice QTLs such as grain size and weight (Thomson et al. 2003), seed dormancy (Takeuchi et al. 2003), durable resistance to blast (Liu et al. 2004a), resistance to UV-B (Ueda et al. 2004), and eating and cooking quality (Li et al. 2003b) have been targeted to clarify genes involved in their expression. Examples of isolated rice genes by map-based cloning are listed in Table 12. The candidate genes for some of them have already been tagged closely, and some are still being analyzed by genetic mapping for tagging. The map-based cloning strategy must be complemented by other methods such as transposon tagging by *Tos17* (Hirochika 2001) or T-DNA insertional mutagenesis (An et al. 2003; Sallaud et al. 2004). The latter one, in principle, can identify only one gene by each tagged line, but in the case of transposon insertion affecting the phenotype relating to a target QTL, the information must be helpful to accelerate the identification by combining genetic and reverse-genetic analysis.

## 1.7 Advanced Works

### 1.7.1 Rice Physical Maps

Physical maps expedite positional cloning, whole genome sequencing, and thorough analysis of genome organization. Several cloning vectors such as yeast artificial chromosome (YAC) (Burke et al. 1987), bacterial artificial chromosome (BAC) (Shizuya et al. 1992), and P1-derived artificial chromosome (PAC) (Ioannou et al. 1994) have been developed to clone large chromosome fragments for facilitating the construction of physical maps. Umehara et al. (1995) constructed a YAC library, which was used later for developing a physical map of rice (Saji et al. 2001). Wu et al. (2002) screened this YAC library using specific primers designed from 6,731 unique expressed sequence tags (ESTs) from 19 cDNA libraries and placed 6,591 EST sites on this YAC-based physical map that covered 80.8% of the rice genome. Expressed sequence tags are partial nucleotide sequences of expressed genes and are obtained by random sequencing of many cDNA clones. ESTs provide the cheapest and fastest means to catalog all genes by comparing the homology of the DNA sequences and its inferred protein sequences with those of other organisms deposited in various databases. Uchimiya et al. (1992) reported the first set of rice ESTs. Later, the Japanese Rice Genome Program undertook extensive EST sequencing in rice (Yamamoto and Sasaki 1997). As of 16 June 2005, about 1,184,706 ESTs of rice have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html).

BAC and PAC libraries were constructed to develop an accurate sequence ready physical map for obtaining a reliable genome sequence (Baba et al. 2000; Chen et al. 2002). Fingerprinting was applied

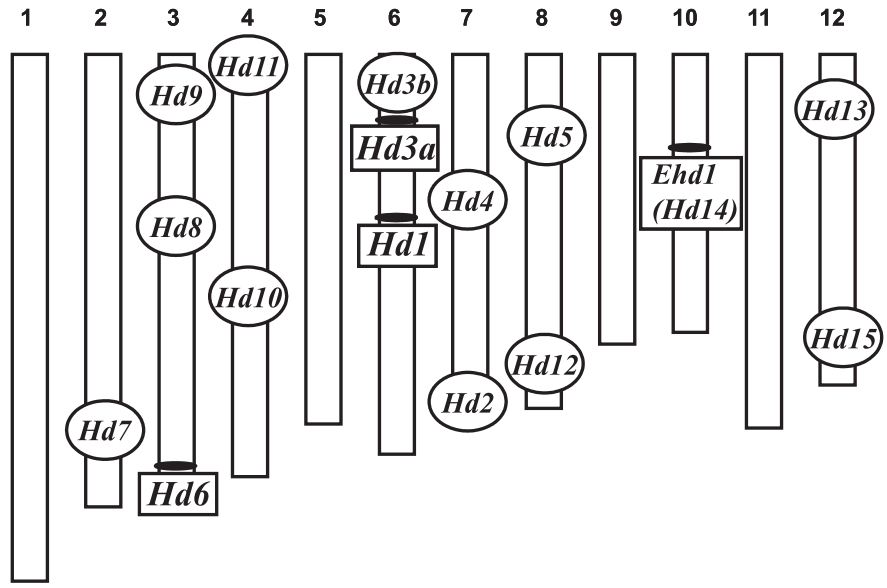


**Fig. 4.** Chromosomal location of each gene involved in rice flowering time QTL. The position of four genes, Hd1, Hd3a, Hd6 and Ehd1(Hd14) are shown as *bold lines* because they were identified

to determine the contiguous BAC clones and a physical map of the whole rice genome was established and integrated with the rice genetic map (Chen et al. 2002). This integrated genetic and physical map provides an essential tool for efficient and rapid isolation of agriculturally important genes in rice and comparative genome analysis among grass relatives.

### 1.7.2 Tools for Rice Functional Genomics

Genome mapping and sequencing are two important tools of structural genomics and have been the major focus of plant genomics for the past two decades. Both whole genome and EST sequencing in rice has generated enormous amounts of sequence data to provide the platform for functional genomics investigations. The greatest challenge now is to elucidate the function of each individual gene sequence in the growth and development of the rice plant. Quantifying the spatial and temporal expression patterns at mRNA and protein levels helps clarify the role of each annotated gene. A genomewide approach is being pursued rather than a traditional gene-by-gene approach to realize this important goal. Two major tools, DNA microarrays and insertional mutagenesis, have become essential components of rice functional genomics studies.

### 1.7.3 DNA Microarray

DNA microarray is a powerful tool for functional genomics studies because it allows quantification of gene expression on a global scale (Deyholos and Galbraith 2001). Application of this technology to the recently completed whole rice genome sequence data will be beneficial in terms of assigning a function to those annotated sequences. Schena et al. (1995) used this technology for the first time to compare the expression pattern of 48 EST clones between roots and leaves in *Arabidopsis*. Microarrays are available in two formats: cDNA array (Schena et al. 1995) and oligonucleotide array (Lockhart et al. 1996).

The cDNA microarrays are largely preferred by plant scientists for transcription profiling because a large number of cDNA and EST clones can be easily generated. Rice cDNA microarrays have been used to study the role of phytohormones, brassinosteroids (BRs), gibberellins (GAs), and abscisic acid (ABA) in growth and development (Yazaki et al. 2003; Yang et al. 2004), monitoring gene expression during pollination and fertilization (Lan et al. 2004), metabolic changes under phosphorous stress (Wasaki et al. 2003), and abiotic stress response (Kawasaki et al. 2001; Cooper et al. 2003; Rabbani et al. 2003).

There are only a few studies involving rice oligoarrays. Zhu et al. (2003) used a rice 21,000 gene chip microarray covering half the rice genome to inves-

**Table 12.** Examples of rice genes isolated mainly by map-based cloning

| Gene | Phenotype | Characteristics of predicted gene product | Chromosome | Reference |
|---|---|---|---|---|
| *Xa1* | Resistance to *X.oryzae pv. oryzae* (race 1) | NBS-LRR type of plant R-gene | 4 | Yoshimura et al. 1998 |
| *Xa21* | Resistance ro *X.oryzae pv. oryzae* (race 21) | Receptor-LRR type of plant R-gene | 11 | Song et al. 1995 |
| *Xa26* | Resistance to *X.oryzae pv. oryzae* | NBS-LRR type of plant R-gene | 11 | Sun et al. 2004 |
| *Pib* | Resistance to *M.grisea* (race 003) | NBS-LRR type of pant R-gene | 2 | Wang et al. 1999b |
| *Pita* | Resistance to *M.grisea* | NBS-LRR type of plant R-gene | 12 | Bryan et al. 2000 |
| *Spl7* | Spotted leaf (lesion-mimic) | Heat stress transcription factor | 5 | Yamanouchi et al. 2002 |
| *Spl11* | Spotted leaf (lesion-mimic) | U-box/armadillo repeat protein | 12 | Zeng et al. 2004 |
| *d*1 | Dwarf (Daikoku) | Alpha subunit of heterotrimeric GTP-binding protein | 5 | Spielmeyer et al. 2002; Peng et al. 1999 |
| *ebisu dwarf (d2)* | Dwarf | Cytochrome P450(CYP90D2) | 1 | Hong et al. 2003 |
| *gid2* | Dwarf | F-box protein | 2 | Peng et al. 1999 |
| *sd1* | Semi-dwarf (Dee-geo-woo-gen, IR8) | GA20 oxidase (GA20ox-2) | 1 | Ashikari et al. 2002; Sasaki et al. 2002a; Monna et al. 2002a; Spielmeyer et al. 2002 |
| *moc1* | Abnormal tillering | GRAS family nuclear protein | 6 | Li XY et al. 2003 |
| *LAX* | Lax panicle | Helix-loop-helix transcription factor | 1 | Komatsu et al. 2003 |
| *PLAST-CHRON1* | Timekeeper of leaf initiation | Cytochrome P450(CYP78A11) | 10 | Miyoshi et al. 2004 |
| *slg* | Slender glume | Ubiquitin-related modifier | 7 | Nakazaki et al. 2003 |
| *Rf1* | Fertility restoration | Mitchodrially targeted pentatricopeptide repeat protein | 10 | Komori et al. 2004; Akagi et al. 2004 |
| *Hd1* | QTL of flowering time | Transcription factor, *CONSTANS* family | 6 | Yano et al. 2000 |
| *Hd3a* | QTL of flowering time | *FT* family | 6 | Kojima et al. 2002 |
| *Hd6* | QTL of flowering time | Casein kinase *CK2?* family | 3 | Takahashi et al. 2001 |
| *Ehd1* | QTL of flowering time | B-type response regulator | 10 | Doi et al. 2004 |

tigate nutrient portioning during rice grain filling. This study revealed that different isoforms of different enzymes of the starch biosynthesis pathway are expressed in different tissues and at different developmental stages, suggesting synchronization in the expression of coordinately regulated genes. Yazaki et al. (2004) constructed oligoarrays of 20,500 transcriptional units identified by the rice full length cDNA consortium and identified new ABA and GA responsive genes.

Recently, 28,469 full-length cDNA clones from cv. Nipponbare have been characterized (Full-Length cDNA Consortium 2003). One important conclusion from this study is that there are 19,000 to 20,500 transcriptional units in the rice genome. Seventy-six percent of these clones were assigned tentative functions, and 64% of these are homologous to *Arabidopsis* proteins. Osato et al. (2004) analyzed these sequences and found large numbers of sense-antisense transcript pairs, which suggests gene regulation by the antisense transcripts.

### 1.7.4 Insertional Mutagenesis

The reverse genetics approach offers an efficient strategy to validate the function of most rice genes. Various physical, chemical, and biological methods can be employed to systematically disrupt the genes of rice plant, and several such mutant populations have been

generated in a number of laboratories (Hirochika et al. 2004). Biological agents such as T-DNA of *Agrobacterium* (Zambryski et al. 1980) and transposable element *Ac/Ds* of corn (McClintock 1956) have been used to generate mutant populations for gene discovery in rice (Izawa et al. 1997; Jeon et al. 2000). Since the sequences of these agents are known, it is much easier to simultaneously disrupt and tag the gene from its altered phenotypic expression. T-DNA tagged lines helped in the identification of cold responsive genes (Lee et al. 2004), genes for Mg-chelatase (Jung et al. 2003), Poly (A) binding proteins (Han and An 2003). Hirochika (1997) discovered an endogenous transposon *Tos17* as an efficient tool for insertional mutagenesis compared to *Ac/Ds* system. *Tos17* is activated by tissue culture and is widely distributed over the rice genome and preferentially integrated into low-copy-number genomic regions (Yamazaki et al. 2001). A rice homeobox gene *OSH15* responsible for dwarf phenotype due to alteration in internode architecture was identified by this method (Sato et al. 1999).

Another resource for functional genomics investigation is a collection of deletion mutants of an indica cv. IR64 developed at the International Rice Research Institute (Leung et al. 2000). These mutant populations were generated using fast neutron, gamma irradiation, and diepoxybutane. These mutant populations are being evaluated for alteration in morphology and response to biotic and abiotic stresses to develop a database. Compared with the mutants generated by the T-DNA, *Ac/Ds*, or *Tos17*, IR64 mutants are not amenable for easy identification of genes responsible for phenotypic change due to absence of a tag. But PCR screening of these mutant populations may be employed to identify gene mutations.

## 1.8 Future Scope of Work

The rice genome sequence has been completely and accurately decoded by the International Rice Genome Sequencing Project (IRGSP) (http://rgp.dna.affrc.go.jp/IRGSP/). This map-based precise sequence information of the japonica rice variety Nipponbare will have a significant impact on any further rice research. About 90% of the cultivated and consumed rice belongs to the indica type, not japonica. Aside from some morphological and physiological characteristics, no detailed information on the difference between these two subspecies has been made available so far. Although both belong to a common species, *Oryza sativa*, the progenies derived from crossing the two subspecies have low or no fertility. The Nipponbare genome sequence can be used as a reference to understand the difference between two subspecies and among varieties within each subspecies as well. In this sense, the Nipponbare genome sequence will serve as the gold standard for further genomics research based on comparison of genome sequence and structure. The subspecies japonica is mainly cultivated in Japan, Korea, Taiwan, and Italy, and their genetic diversity is narrow. On the other hand, the subspecies indica is mainly cultivated in China, India, Thailand, and Indonesia, and their genetic diversity is wide (Nakagahra et al. 1997). Comparison of these two subspecies of *O. sativa* will prove the validity of the gold standard sequence to easily and promptly attain genome information of other cereals. The most desirable tool for further work on comparative genomics is a physical map that enables genomewide comparison of the structures and gene repertoire of target species. Once such a reliable physical map is available, the sequence information at the precise corresponding genomic region could be easily obtained by routine sequencing work. Although there are about 120,000 indica varieties worldwide (Khush 1997), it is quite reasonable first to start with the variety Kasalath, which has been used as a parent of the $F_2$ population for construction of a molecular genetic map and, consequently, has been used to identify QTLs such as flowering time (Yano et al. 2001).

A BAC library of variety Kasalath was constructed to facilitate comparison with the variety Nipponbare. End sequencing of the clones of this library was carried out, and 78,427 high-quality BAC end sequences (BESs) were collected. At an average read length of 482 bp, a total length of 37.8 Mb sequence was obtained (Katagiri et al. 2004). After removal of BESs containing repetitive sequences and use of the Nipponbare sequence as a standard, a total of 12,170 clones with paired BESs were mapped *in silico* onto the 12 rice chromosomes. These clones consisted of 450 contigs and showed a total physical length of 308.5 Mb, indicating genome coverage of about 80%. Confirmation of the chromosomal position of the Kasalath BAC clones mapped on chromosome 1 using specific DNA markers revealed that the map accuracy was extremely high, at least 94.8%. A frequency of 0.71% for single nucleotide polymorphisms (SNPs) and 1.23 sites per kilobase for InDels (1-16bp length), respectively, were

observed between Nipponbare and Kasalath (Katagiri et al. 2004). The Chinese indica varieties, Guangluai 4 (Feng et al. 2002) and 93-11 (Yu et al. 2002a), have been sequenced using a map-based strategy and a whole genome shotgun strategy, respectively. A detailed comparison of the 2.3-Mb genome sequence of chromosome 4 of Guangluai 4 to the corresponding chromosome of Nipponbare revealed a 0.37% frequency of SNPs (Feng et al. 2002). The genomewide frequency of SNPs and InDels between Nipponbare and 93-11 were calculated as 0.37% and 1.05 sites per kilobase, respectively (Yu et al. 2002a). Unfortunately, mapping of genomic clones of these Chinese indica varieties to Nipponbare sequences has not yet been performed. However, these results clearly showed the utility of the Nipponbare sequence as a powerful resource to perform comparative genomics of a wide range of indica subspecies. Such minute information on polymorphism is directly linked to the identification of new alleles regarding the difference of phenotype in a quantitative manner. Genetic analysis of a quantitative trait and extensive data of SNPs among many rice varieties including wild relatives of *O.sativa* are two main indispensable points for improvement of rice.

Cultivated rice, *O. sativa,* has many wild relatives (Table 2) (http://www.knowledgebank.irri.org/wildRiceTaxonomy/default.htm). Some of them are not diploid but tetraploid and some have a genome size two to three times that of *O. sativa* (400 Mb). Although not yet clearly demonstrated by molecular genetic data, *O. sativa* is said to have evolved from *O. rufipogon* via *O. nivara* aided by efforts of domestication by humans (Khush 1997). During this domestication process, some genome arrangements occurred and the rice species suitable to an existing environmental condition and with high yield have been selected to breed. On the other hand, traits without any agricultural value were not incorporated into the breeding program. However, it is well known that genes associated with tolerance to biotic and abiotic stresses still remain in wild relatives of *O. sativa*. In addition, recent achievement of the genome sequence of *O. sativa* can reveal the distinct tandem repeat of many genes and partly polyploidy nature of rice chromosomes. The genome size of each wild *Oryza* species is reported to be larger than *O. sativa*. For example, the genome size of *O. glumepatula* is 475 Mb (Uozu et al. 1997). So far, there is no molecular information on what happened to both japonica and indica genomes during the domestication process such as whether the deletion of a genic or intergenic region occurred or whether segmental or tandem duplication occurred before or after this genome size reduction. Molecular interpretation on such points based on a comparison of genomes is needed to understand the history of domestication and on how to improve the current cultivated species with more favorable traits.

Using DNA markers derived from expressed genes, it is clear that the rice genome carries colinearity of gene alignment with other cereals such as millet, sorghum, barley, maize, and wheat (Gale and Devos 1998). This syntenic relationship is undoubtedly the major driving force that establishes rice as the model or reference plant for cereal genomics. The *Rht1* gene of wheat and *D*8 of maize have been identified using this information (Peng et al. 1999). However, success in identifying targeted genes is so far limited. This is because of the unexpected complex structure of the cereal genomes revealed first by mapping of disease resistance genes by common markers and second by genome sequencing. Although current cereals diverged from their common ancestor about 60 million to 70 million years ago (Kellog 2001) and carry its footprint in each diverged species, each must have its specific genome structure to assert itself to survive under biotic and abiotic stresses. If it has received selection pressure by breeding, it must also alter its genome structure to accept selection. As a result, the linearity of each gene within ancestral species must be shuffled and rearranged during the evolution of each species. The remnant of the ancestral structure is observed as broken colinearity in rice, sorghum, maize, and barley, which was recently identified by detailed sequence comparison (Bennetzen and Ma 2003). This breakage was observed even for a commonly existing housekeeping gene locus like alcohol dehydrogenase I (Tikhonov et al. 1999; Tarchini et al. 2000; Bennetzen and Ramakrishna 2002).

So far, our knowledge of the validity of synteny based on sequence information is very limited. This is also true in the case of dicotyledonous plants using *Arabidopsis* as a model species with revealed genome sequence (Rossberg et al. 2001; Boivin et al. 2004). Synteny itself is a very important concept to be verified to understand the evolutionary history of each genus. Detailed sequence comparison of a specific gene locus could clarify what happened by diversification like duplication, insertion of a transposable element, or insertion of a species-specific new gene. Extensive collection of sequence information on more

gene loci and preferably on genome of related species is required for further understanding of plant. This, of course, must be carefully performed based on a close interaction of genetics and biology of the target genus.

The ultimate goal of rice genomics researchers today is to apply rice genome information to breed rice varieties with improved yield, superior quality, and resistance to biotic and abiotic stresses. This can only be achieved once we assign a function to each and every annotated sequence and discover the complex interactions among them. The recent release of the rice genome sequence coupled with technological advances in microarray technology and reverse genetics tools will expedite such activities by providing a global perspective on response of rice genes at different growth and developmental stages.

The most important benefit that has accrued from rice genomics studies is the application of MAS in rice-breeding programs. It has become routine in most rice-breeding programs all over the world because molecular markers have demonstrated their utility in making the selection process much easier and more efficient. Most of the success stories of MAS have been limited to simply inherited traits (reviewed earlier). The role of MAS in improving complex agronomic traits, however, has been minimal. This is because of the complexity resulting from pleiotropy, epistasis, and genotype × environment interaction associated with the quantitative traits (Tanksley 1993). Despite the complexity associated with many economically important traits, there has been successful cloning of QTLs controlling many useful traits. Thanks to advances in genomics, we are now in a better position than ever before to enhance our understanding of the molecular basis of these complex traits. Complex genetic traits can be studied in detail by integrating QTL mapping with microarrays. Generation and utilization of novel genetic stocks such as mutants, near-isogenic lines, substitution lines, deletion lines, and transgenic lines for gene expression studies will further facilitate the genetic analysis of complex agronomic traits. With advances in gene chip technology and our understanding of complex traits, it may be possible to select the desirable rice lines from the unique expression pattern of several genes associated with a number of agronomic traits.

To derive benefit from the accurate rice genome sequence it is imperative to catalog the allelic variation in the available germplasm for important agronomic traits at the nucleotide level. Both wild and cultivated rice germplasms are reservoirs of genes for yield, biotic, and abiotic stresses (Tanksley and McCouch 1997). Rice genome information should be exploited to unlock useful variations for rice improvement. Correlating these nucleotide sequence variations with the phenotypic variation will help select desirable alleles for incorporation by genetic engineering or MAS. Since there is no control over the number of transgenes or the sites of integration associated with transformation by *Agrobacterium* or particle bombardment, Terada et al. (2002) developed a method to insert genes in targeted sites using homologous recombination. With the knowledge of the rice genome sequence this method can further be refined for precise incorporation of useful genes for rice improvement.

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