Chapter 1

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Rice (Oryzae sativa L), which has been documented as a source of food as far back as 2500 BC in history books, has fed more people over a longer period of time than any other crop in the world (Rost, 1997). It provides the main source of food for approximately half of the world’s population and, hence, may be the most important plant on this earth (Shimamoto, 1995; Goff, 1999) (Fig. 1.1 Rice Plant– the major food source for 50% of the World’s population). India is the second largest producer of rice after China with a production of 87.80 Million tones covering 44.62 M ha of area (Agricultural Statistics Division, 2005). Rice is one of the three cereals produced annually world wide at levels of approximately half a billion tones. However, unlike other major cereals, more than 90% of the rice is milled almost exclusively as food for human consumption (Goff, 1999) and forms three-fourth of the total diet for millions of people. This member of the grass family (Gramineae) is abundant in carbohydrates and is a major source of protein for the masses of Asia (Chang, 1984). Rice is the only cereal that can withstand flooding and produces more calories to sustain a larger number of persons per unit of land than any other cereal in monsoonal areas (Chang, 1984).

Considering the growth in human population every year all over the World, the demand of rice is ever increasing. However, the total area under rice cultivation has remained stable since 1980 (Khush, 1985), and hence, increasing yield is the only possible alternative to meet the anticipated higher demand of rice. Since conventional breeding tools which were responsible for
the first 'Green Revolution', were by themselves unable to meet the growing demand for rice, the Rockefeller Foundation launched its rice initiative in the early 1980s, focusing on integrating biotechnology, chiefly genetic engineering, into rice research in order to boost its productivity, improved nutritional quality and pest and diseases resistance.

Despite substantial advances in plant disease control strategies, our global food supply is still threatened by a multitude of pathogens and pests Moffat, A.S. (2001) Garelik, G. (2002). Plant diseases can dramatically reduce crop yield and the impact of disease outbreaks is particularly acute in developing nations. Pesticides provide effective protection but their applicability can be compromised by adverse environmental effects and by the emergence of resistant pathogen strains.

Chemical controls are often beyond the means of farmers in developing nations. For these reasons, much effort has been invested towards understanding innate resistance mechanisms in plants. Plants can activate a very effective arsenal of inducible defense responses, comprised of genetically programmed suicide of infected cells (the hypersensitive response, HR), as well as tissue reinforcement and antibiotic production at the site of infection Hammond-Kosack, K.E. and Jones, J.D.G. (1996). These local responses can, in turn, trigger a long lasting systemic response (systemic acquired resistance, SAR) that primes the plant for resistance against a broad spectrum of pathogens Dong, X. (2001), Me’traux, J.P. (2001).

This multi-component response requires a substantial commitment of cellular resources, including extensive genetic reprogramming and metabolic re-allocation Somssich, I.E. and Hahlbrock, K. (1998). Thus, defenses are kept under tight genetic control and are activated only if the plant detects a
prospective invader. Plants do not have the benefit of a circulating antibody system so plant cells autonomously maintain constant vigilance against pathogens by expressing large arrays of ‘R genes’ (R, resistance) Dangl, J.L. and Jones, J.D. (2001), Holub, E.B. (2001) Jones, J.D. (2001). R genes encode putative receptors that respond to the products of ‘Avr genes’ (Avr, avirulence) expressed by the pathogen during infection. In many cases, a single R gene can provide complete resistance to one or more strains of particular pathogen, when transferred to a previously susceptible plant of the same species. For this reason, R genes have been used in conventional resistance breeding programs for decades Pink, D.A.C. (2002). The strong phenotypes and natural variability at R loci have also been exploited by molecular geneticists to clone the R genes and investigate their molecular modes of action.

Figure: 1.1 Rice Plant– the major food source for 50% of the World’s population
Figure 1.2: Bacterial Blight (*Xanthomonas oryzae* pv. *oryzae*) affected rice field

Figure 1.3: Blast (*Pyricularia grisea*) on Rice Plant
R gene-mediated resistance has several attractive features for disease control. When induced in a timely manner, the concerted responses can efficiently halt pathogen growth with minimal collateral damage to the plant. No input is required from the farmer and there are no adverse environmental effects. Unfortunately, R genes are often quickly defeated by co-evolving pathogens Pink, D.A.C. (2002). Many R-genes recognize only a limited number of pathogen strains and therefore do not provide broad-spectrum resistance. Furthermore, introgression of R genes into elite cultivars by conventional breeding is a lengthy process. However, recent molecular-level insights into the function of R proteins and downstream signal transduction pathways might provide strategies to remedy these deficiencies.

Bacterial blight (Fig. 1.2) and Blast (Fig. 1.3) has been managed primarily by host resistance but virulent races or pathotypes have emerged to overcome the deployed resistance (Mew et al 1992). Therefore, an effective and long term disease management strategy through utilization of host plant resistance demand a comprehensive understanding of the population structure and virulence characteristics of the pathogen. Knowledge of pathogen variation and the mechanism that derives the genetic changes in the pathogen population is also necessary for formulating an effective disease management strategy. Understanding the distribution of genetic diversity in the pathogen and its virulence pattern on a set of differential lines is important for the successful identification of resistant germplasm and its deployment (Shanti et al 2001).

Traditionally, pathogen races are used as markers to assess population diversity (Mew et al 1993). Diversity of Xoo has been analyzed on the basis of virulence typing (Gupta et al 1986, Mew et al 1992, Noda et al 2001) and DNA fingerprinting by restriction fragment length polymorphism (RFLP)

In the absence of effective chemical or other control agents against BB pathogen, host plant resistance is the most effective, economical and eco-friendly strategy to combat the disease. Several genes for bacterial blight and blast have been tagged with molecular markers. Twenty-five major genes (Xa1 to Xa25) conferring resistance to BB pathogen have been identified (Chen et al 2002) and mapped on different rice chromosomes (Nos. 4, 5, 6, 7, 8, 11 and 12). Durable resistance to Pyricularia grisea is confirmed by both major and minor genes. Extensive genetic studies and QTL analysis using DNA markers have identified more than 30 major genes and 10 QTLs in rice. Large scale and long-term cultivation of varieties with resistance governed by a single gene enables the pathogen to overcome resistance. However, this can be delayed by pyramiding resistance genes into suitable rice cultivars. The probability of simultaneous pathogen mutation for virulence to two or more effective genes is much lower than for a single gene. Moreover, none of the single resistance gene is effective against all the races of the pathogen. Furthermore, combining major-gene and minor-gene resistance may lead to increased durability (Wang et al 1994).

Gene pyramiding is difficult by conventional breeding methods due to the dominance and epistasis effects of genes governing disease resistance (Huang et al 1997). However, molecular markers closely linked with each resistance gene make the identification of plants with two or more genes possible. Thus, marker-assisted selection (MAS) offers unique advantages to generate pyramided lines with durable disease resistance in a straight manner by overcoming the limitations of conventional breeding.
Most of the present popular rice cultivars are suffering from either bacterial blight or blast or from both the diseases. This is because these cultivars either have no resistance genes or only one or two major genes which limits their ability to resist diseases. A systematic approach has been taken in the present study to develop a resistance gene pyramid and diversified rice cultivars. A total of four genetically diverse donor parents (AOSB-3, AOSB-15, AOSBB-7 and AOSBB-10) were used to pyramid BB genes (xa-5, xa-13, Xa-21) and Blast genes (Pi-1 and Pi-2).

The specific objectives of the present study were:
1. Pyramiding major bacterial blight and blast resistance genes in a single cultivar.
2. Development of diversified and diseases resistant rice cultivars.
3. Molecular characterization of Xoo and *M. grisea* isolate collection
4. Establishing a high throughput MAS breeding strategy.

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