LITERATURE REVIEWS

Blast : *Pyricularia grisea* Sacc.

Rice blast disease is considered to be the most important rice disease world widely. The causing agent of disease is the fungus called *Pyricularia grisea* Sacc. Saccardo was named as the type species of the genus in honour to his first report of the *Pyricularia* on Trichothecium griseum grass in 1880. The other popular synonym of the fungus is *Pyricularia oryzae* named by Cavara who first found it on rice in Italy (Holliday, 1989). Apart from these two names for the genus *Pyricularia*, there were other names such as *Dactylaria grisea*, *D. oryzae* and *Pyricularia* but not as popular. Although *Pyricularia oryzae* have been originally found in two different hosts, these two species were morphologically indistinguishable and interfertile. In addition, as the fact that *Pyricularia grisea* was the first name given to this group (Rossman *et al*., 1990).

Pyricularia oryzae has the teleomorph called *Magnaporthe grisea* (Hebert) Barr. The perfect stage of the fungus was first discovered and described as *Ceratophaeria grisea* by Hert (1971) which had been changed later to the genus *Magnaporthe* by Barr (1977). As the telemorph was rarely found in the nature but the asexual stage was known, Rosman *et al*. (1990) stated that it was acceptable to call this fungus as either *Pyricularia grisea* Sacc. Or *Magnaporthe grisea* (Hebert) Barr.

Blast symptoms and Infection

Rice pathogenic isolate of the blast pathogen produces lesions on all parts of the rice plant; leaves, neck node, and panicles. The rice blast disease is characterized by examining these parts of the plant. When lesions appear on leaves, they are often white to gray-green with dark green or brown border. Their shape varies but lesions are characteristically spindle shape. There are several types of lesions based on the resistance and plant age. Leaf reactions vary from pinpoint infections to large elliptical lesions up to 1.5 cm long, 0.3-0.5 cm broad. Large lesions without signs of

plant resistance are referred to sometimes as acute lesions, while small lesions indicate a degree of resistance.

Blast infection in early plant stages can cause stunting or death, depending on the severity of the attack and on the humidity and temperature during the infection period. Neck or panicle usually causes more severe damage to the crop than leaf blast. Neck blast is seen as a gray brown lesion round the upper most nodes just below the panicle, causing the panicle to fall over ("rotten neck"). Early attack at this point will result in no or very poor gain filling and high harvest losses. A later attack can be less damaging, although the grains will not completely develop.

Morphology

Conidiophores produced in clusters from each stoma, are rarely solitary and have 2-4 septa. Conidias are pyriform with three cells on basal appendage. The average size of conidia was 19-23 x 7-9 μm. Conidia germinate from the apical or basal cell and less frequently from the middle cell. A conidium forms an apressorium at the tip of the germ tube when it germinates on the host plant. At this stage, it is called a resting spore or chlamydospore. It is produced at an early stage of infection and attaches to the host tissue by secreting a mucilaginous substance. Conidia growth on media culture at optimum temperature 28 ^{o}C, and the sporulations were produced rapidly but the production decrease after 9 days. Perithecium of the perfect stage is nonstromatic, with a spherical to subspherical base that is embedded in the host tissue and has a long neck. The asci are eight-spores, cylindrical to clavate and mostly 60-90 x 5-7 μm. Ascospre was fusiform, curved, and rounded at the end. At maturity, the ascospores have extruded from the ostiole in a gelatinuos mass (Ou, 1985).

Life cycle

The life cycle of the fungus can start after conidia are deposited on the aerial part of the rice plant. Under suitable conditions, germination occurs and an infection peg is formed which penetrates the epidermis by both hydrostatic pressure in the appressorium and enzymatic action. After successful penetration, the fungus colonizes

the intracellular zone, epidermal cells and mesophyll cells. After a latent period determined by temperature, conidiophores are produced upon which the conidia are borne. The complete cycle requires a minimum of 3-4 days. The sexual stage of the fungus is isolated from rice. Mature perithecia are produced after mating with hermaphrodite strains from *Eleusine caracana, Phalaris arundinaceae and Hordeum vulgaris*. Subsequently, mature perithecia form with mating of rice isolates from many countries (Leung *et al*., 1988).

Mechanism of *P. grisea* Infection

Germination of the fungal germ tube is depended upon additional signal; infection signal and vegetative growth signal. Additional signal such as the contact of conidia to a solid surface would help the germination of germ tube (Lee and Dean, 1993). Sensing the infection signal such as hydrophobic leaf surface would cause the swelling of hyphal tip into appressorium which will penetrate into the leaf surface. After penetration, enzymes will take action and life cycle will be completed within 3-4 days.

Genetic of Blast Resistance

Resistant cultivar is one of the solution to prevent or reduce yield loss due to rice blast epidemics. Blast resistance was classified into two types according to gene expression induced by the attack of the pathogen. One type is called qualitative or complete resistance while the other is called quantitative or incomplete resistance (Ou, 1979).

Qualitative or complete resistance shows reaction indicating the absence of compatible type lesion being controlled by major gene(s) (Ahn, 1994) having race specificity (Matchetti, 1983) and expressing hypersensitivity to the pathogen. The first study on blast resistance gene was reported by Sasaki (1923) who found a single dominant blast resistance gene in Japanese rice variety Tsurugi had initiated a light at the end of the tunnel. Forty-two years laterp, tworestant genes designated *Pi-1* and *Pi-6* were identified in the United states of America (Atkins and Johnson, 1965). Four

dominant genes i.e., *Pi4, Pi13, Pi22* and *Pi25* were also identified (Hsieh *et al*., 1967). The total of 11 major genes designated *Pi-k, Pi-k5, Pi-kh, Pi-ta, Pi-z, Pi-a, Pi-b, Pi-f, Pi-i and Pi-lm* had been reported fourteen years later (Kiyoawa, 1981). The work on gene mapping had revealed that *Pi5(t)* and *Pi7(t)* mapped on chromosome4 and 11 were linked to marker RG778 and RG103, correspondingly (Wang *et al*., 1994). Based on these information, near isogenic lines (NILs) of rice with single resistant gene for each line were developed by backcrossing four donor cultivars to the recurrent parent CO39 (Mackill and Bonman, 1992).

Quantitative (Incomplete) resistance has been called field resistance or partial resistance, in general. It is characterized by lesions typically spindle-shaped, fewer in number, reduce in size, slower to develop and shorter-lived (Tabien *et al*., 2002). Partial resistance is more difficult to use than complete resistance due to its quantitative inheritance which usually polygenic and sensitive to environmental factors such as temperature, leaf wetness duration, nitrogen-fertilization, soil type and water stress (Ou, 1985; Rouomen, 1994). It has also been stated that quantitative or partial resistance is usually controlled by polygenes that are minor genes (Bonman *et al*., 1992). Examples of partial resistance varieties have been reported as IRAT13, IAC24, IAC27 and Dourado Precose studied by Nottegham (1985). Quantitative resistance could be separated into two components. First, is the efficiency of quantitative resistance in eliminating an avirulent portion of any available inoculum. Second, is the ability to lower the infection efficiency of a virulent portion (Ahn and Koch, 1988).

Recombinant Inbred Line (RIL)

The basis for detection of resistant genes starts with the cross between resistant and susceptible parents. Segregation of alleles will appear at meiosis stage leading to equal frequency of alleles in the gametes. The progeny having independent segregation alleles from both parents is Recombinant Inbred Line (RIL). The segregation ratio for parents and recombinants according to Mendelian's law is 1:1. Therefore, the percentage of parents and progenies has equal frequencies. Since

crossing over will occur at meiosis stage, target loci could have been moved over to another chromosome. Thus, a genetic distance can be calculated from a recombinant frequency. RILs population had been used by several researchers recently. Wu and Tanksley, (1993) use RILs derived from Thong156 x Gumei2 population to locate blast resistant genes under field conditions in China. Sirithunya *et al*., (2002) detected QTLs associated with leaf and neck blast resistance on chromosome7 and 9 using RILs derived from KDML105 x CT9993-5-10-M. Tabien *et al*., (2002) also mapped blast resistant genes from RILs of Lemont and Teqing population.

RIL is generated by single seed descent (SSD). SSD is designed to maintain the total range of variation in a population by precluding loss of noncompetitive plants by taking a single seed from each individual of the population, starting from F2, to propagate the next generation by bulking. Selection is not practiced until F5 or F6, as individuals in the population are reasonably homozygous. It can be said that SSD is a modification of the bulk method of breeding.

Computer simulation studies revealed that at high heritability the pedigree method is more effective while at low heritability SSD is more effective. It is also been reported that SSD was more effective in situation in which competition effects are important.

Step for preparation of RILs

First year: Screening the parents and make crosses between each pair of them. The parents may be varieties, single or multiple crosses.

Second year: Grow F1 plants and parents for comparison. F1 are hybrid of self fertilization. Harvest the F1 plants in bulk for each cross.

Third year: Grow F2 generation of each cross. Harvest the plants of each cross in bulk. Take single or equal number of seeds from each plant and composite them for raising the next generation.

Fourth to seventh year: In F3, F4, F5, and F6 generations, grow a single seed per line for each generation. Individual line is harvested in bulk.

The SSD method, as a modification of the bulk-population method, has features that overcome the problem of natural selection and inadequate sampling in the conventional bulk-population method. This method minimizes natural selection without eliminating it. Thus, if population size is limiting. It is expected that the SSD method will maintain more genetic variability.

The SSD method has the obvious advantage in that gene frequencies are stabilized. The other advantage is that the segregating generations can be advanced with the maximum possible seed, wherever facilities such as greenhouse and off-season nurseries are available. This can be extremely rapid in low nutrient, continuous-light environments. Depending on the crop plant, the breeding cycle can be reduced from about 8 years with mass selection to about 4 years with SSD. Thus, the SSD saves the time and labor and offers good possibilities in isolating superior genotypes. In crops such as lentils, where poor growth habit lacks of synchronized maturity make it difficult to practice the pedigree method, SSD should be preferred.

Rice Diseases

Rice diseases are among the most important limiting factors that affect rice production causing annual yield loss conservative estimated at 5% (Song and Goodman 2001). Two types of rice diseases are recognized: infectious and noninfectious diseases. The infectious diseases are caused by pathogens or biological vectors, such as fungi, bacteria, viruses, mycoplasmas and nematodes, Noninfectious diseases result from unfavorable environmental of nutritional conditions such as deficiencies or excesses of nutrients, temperature extremes, toxins, etc (Webster 1992). At every growth stage, the plant is subject to diseases that reduce both yield quality and quantity. The severity of disease depends on the presence of a virulent pathogen, a disease-conductive growth condition and the susceptibility of the cultivar. The actual number of distinct rice diseases is not clear. Over 80 biotic and abiotic

diseases were characterized. However, not all rice diseases are economically important (Ou 1985).

Control of rice blast disease

Several means to control rice disease have been used such as manipulating the time of planting, fertilizer, water management, the use of fungicides, biological control and cultivation of resistant cultivars.

Manipulating time of planting

In tropical upland rice, crops sown early after the onset of the rainy season are more likely to escape blast infection than the late-sown crops. It was observed in Bangladesh that blast is most severe during seasonal periods of low night temperatures and long dew duration. Sowing early could help to limit the exposure time of the crop to these blast conductive conditions (Bonman 1992). This technique was also applied in Brazil (Prabhu and Morais, 1986).

Fertilizer application

High doses of nitrogen fertilizer increase the susceptibility of rice to blast disease. The form of N source also influences the severity of blast disease. It is reported that rice plants fertilized with NO^{3-} are more susceptible than those given NH^{+4}. It is also observed that splitting N application can reduce blast disease compared with a single application. Another plant nutrient element, phosphorus, was reported to increase the susceptibility of rice to blast in certain soil (Bonman 1992).

Water management

This method is based on the observation that drought stress increases blast susceptibility. Providing water to minimize drought stress could help to reduce blast disease (Bonman 1992).

The use of fungicides

Fungicides currently in use are highly effective. However, less toxic and less expensive chemicals with fewer applications offer important advantages in the current state of heightened environmental and economic awareness. Nowadays, 11 blasticides are registered for blast control in Japan: Blasticidin S, Kasugamycin, Edifenphos, Ferimzone, Fthalide, IBP, Isoprothiolane, Probenazole, Pyroquilon, Tricyclazole, and Carpropamid, These blasticides, excep Blasticidin S, provide systemic resistance in rice against blast disease (Yoshino 1988, JPPA 1992). Among these blasticides, Probenazole, which is a resistance inducer, has the most used one, and strong effects with high activity and results in long term control of blast.

A rang of microorganisms was screened for promising candidates to be used as biological control agents for rice blast disease (Sy *et al*., 1990). Avirulent isolates of *M.grisea* and the non-rice pathogen Bipolaris sorokiniana were found to reduce blast disease when sprayed on plants. Recently, it was found that a specific pheromone produced by Saccharomyces cerevisiae could minimize infection by inhibiting production of the appressorium. However, not much field application has been done.

Cultivation of resistant rice cultivars

Growing resistant cultivars is the most effective and economical way to control blast disease. The farmers do not have to purchase fungicides and does not contaminate the environment like with the use of fungicides. In some areas where the environment is not highly conductive to blast, the disease can be controlled easily by growing resistant cultivars. However in some tropical area where the environment is favorable for blast disease, resistance can be broken down shortly after a resistant cultivar is released. Therefore, development of durable resistant cultivars attracted the attention of many breeders.

Integrated pest management

To achieve a satisfactory control in the environment with a high potential for blast epidemics, it is necessary to combine different methods of blast control with cultivation of resistant cultivars and including manipulating time of planting, fertilizer, and water management as well as the use of fungicides.

Rice Genome and Genome Size

Haploid rice genome consists of 12 chromosomes containing a complete set of genetic information for rice growth and development. Using the flow cytometry, the estimation rice nuclear DNA content of rice haploid genome is 4.3×10^5 which is approximately six times smaller than maize genome and 40 times smaller than wheat genome making it attractive for genome structure study.

Linkage Map Construction

In genetic mapping construction, DNA marker is used to determine locations of targeted genes in the chromosome (Paterson *et al*., 1988; Lander and Botstein, 1989). DNA marker is a molecular or genetic marker which is a tool used to establish linkages between a marker and a gene or to enhance the establishment of genes controlling a targeted trait more precisely and rapidly than can be achieved by conventional breeding. Important agronomic traits such as plant height and heading (Li *et al*., 1995), grain yield components (Lin *et al*., 1996), seedling vigor (Redora and Mackill, 1996) and root morphological character related to drought avoidance (Champoux *et al*., 1995) had been used for linkage mapping construction. Most of these traits are controlled by quantitative trait loci (Hallaner and Miranda, 1998) which simply involved with polygenes (Geldermann, 1975). Population ideal for mapping construction can be either segregated progenies of F2 population (Burr and Burr, 1998), back cross population (Paterson *et al*., 1988), double haploid population (Guiderdoni, 1989; Chen *et al*., 1997) or recombinant inbred population (Burr and Burr, 1989; Wang *et al*., Tabian *et al*., 2002). Mapping distance can be calculated

from recombination percentage as 1 percent recombinant is equivalent to 1 centimogan (cM) which is approximately 206 kb (Wu and Tanksley, 1993).

Once the test is carried out on used population, data on molecular marker and phenotype will be analyzed statistically. Primarily, scientists used single molecular marker analysis with polygene and the relationship between marker and phenotype was analyzed using linear regression (Thoday, 1961; Soller and Brody, 1976). The additive effect associated with the marker locus can be estimated by linear regression of marker and genotype while the relationship between a marker and quantitative trait locus was analyzed using one-way ANOVA (Stuber *et al*., 1992). However, this method can only detect a QTL near the marker. Thus, interval mapping using the likelihood approach (LOD score) (Lander and Botstein, 1989) and the use of a set of linkage markers with regard to effects on the quantitative trait loci were introduced to improve the results and therefore the program for data analysis was developed and commonly known as Mapmarker/QTL (Lincoln *et al*., 1992).

Mapping Populations

Different populations used in mapping study are differed in advantage and disadvantage. F2 population can be achieved quickly but a quantitative estimation on the variation in the replicated progenies tested is not adequately accurate. Furthermore, there is a limitation in availability of tissues for DNA extraction. Double haploid (DH) line derived from anther culture has the advantage that it can be homozygous in only one step as diploid plants from anther culture are obtained from haploid cells. Among populations used for mapping study, recombinant inbred lines (RILs) take longer time to reach than others as they need 6-7 generations in breeding program and undergo multiple rounds of meiosis before homozygosity is obtained. These result in high recombination between closely linked loci which is approximately twice than that of DH (Haldane and Woddington, 1949). The other major advantage is that RILs are no longer segregated. Thus, they can be propagated easily. The use of DH and RILs was reported to be well studied to QTL analysis (Burr and Burr, 1991) and that the combination of a set of molecular marker and RILs make

a perfect match for researchers to easily exchange the information on rice genes at the molecular level (Glenn, 1997).

Molecular

In agriculture, molecular marker is the tool for generating genetic linkage maps and has provided a major contribution to the genetic knowledge of many cultivated plant species useful for crop improvement and increase breeding efficiency. In addition to being of basis importance to genetic and evolutionary studies, molecular marker is useful to localize monogenic and polygenic traits allowing the efficient introgression and selection of individuals with specific characteristics. Basically, any DNA sequence used to distinguish between individuals, lines, varies or to localize agriculturally important genes and construct genetic linkage map can be considered as a molecular marker. Molecular marker is more specific and accurate than other markers i.e., morphological or biochemical markers. Other advantages are direct measurement on genetic materials, numerous markers in a single population and measurement not subjected to environmental or developmental effect. They could localize any positions on the chromosomes, which can be detected and inherited to progenies.