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CHAPTER 3

Molecular Approaches for Controlling Blast Disease in Rice

DEEPTI SRIVASTAVA[1], PRAMILA PANDEY[2], N. A. KHAN[2], and K. N. SINGH[2]

[1]Department of Plant Molecular Biology and Genetic Engineering, N. D. University of Agriculture and Technology, Kumarganj, Faizabad 224229, Uttar Pradesh, India

[2]CSIR-National Botanical Research Institute, 226 001 Lucknow, Uttar Pradesh, India.

[1]Corresponding author: E-mail: deeptifzd@gmail.com

ABSTRACT

Blast disease caused by the fungus pathogen *Magnaporthe oryzae* is one of the most severe diseases of rice. Many control procedures are used for the management of rice blast disease, but due to the instability of blast fungus, rice production remains threatened by blast disease. This chapter outlines the application of traditional and molecular approaches for the control of blast disease. Traditionally, chemical control method is most effective; however, the use of chemicals is not generally desired due to the serious environmental threat it poses in rice. Although biocontrol agents for blast have been successfully deployed to combat the disease in the laboratory, greenhouse, and fields, the feasibility of such strategies on a commercial scale still remains to be tested in the natural fields condition. For effective management of blast disease, breeding program should be focused on utilizing the broad spectrum of resistance genes and pyramiding of genes and quantitative trait loci. The availability of rice and *M. oryzae* genome sequence data is facilitating blast resistance management program to new paradigms which includes isolation and characterization of R and Avr genes. With the identification, isolation and characterization of blast resistance genes in rice, it is now possible to dissect the actual allelic variants of these genes within an array of rice cultivars via allele mining. In present chapter, role of molecular breeding, transgenics, and few new methods like miRNA in controlling rice blast disease is also discussed. All these update information will be helpful guidance for rice breeders to develop durable blast resistant rice variety through advanced molecular techniques.

3.1 INTRODUCTION

Rice (*Oryza sativa*) is one of the major food crops that constitute the staple diet all over the world. It is cultivated everywhere in the world except Antarctica and has tremendous economic importance. More than 23% of the calories consumed by the world population comes from rice. Of the total area under rice cultivation, 92% of the rice is grown in Asia, which is the home to more than half of the world population. Nowadays, yield of rice reduces significantly due several phytopathogens. Among several phytopathogens, rice blast is the most destructive disease broadly prevalent in rice fields, leading to significant grain yield and quality reduction. Losses caused by the blast disease are dependent on the growth stage of the plant at which infection occurs, level of resistance, and existing environmental conditions. It occurs more frequently in rain-fed areas in wet season due to favorable

environmental conditions for disease development. In India, blast was first recorded in 1913 and the first devastating epidemic was reported in 1919 in the Tanjore delta of erstwhile Madras state. A 4% reduction in yield due to blast was estimated for the first time in India. During 1960–1961, the total loss due to blast was 265,000 t. Thirty percent of rice yields are lost due to blast disease caused by fungal pathogen *Magnaporthe oryzae* (Spence et al., 2014), so rice production will be required to increase by more than 30% to meet the staple food requirements by 2030 due to rapidly increasing world population, limitations to increase cultivated land and nonavailability of water for irrigation. Reducing the loss due to blast can prove to be a critical component toward mitigating the world food security. Although many efforts are given on the study of genetics of rice, it continues to be the most destructive disease of rice. Therefore, strategies for the reduction of yield losses in an environmentally sustainable and economical manner need to be implemented urgently. The present chapter describes blast pathogen and its management with various traditional and molecular approaches.

3.2 THE BLAST PATHOGEN

The fungus that causes rice blast is called anamorph form *M. oryzae* (formerly, *Magnaporthe grisea*). It is a haploid filamentous *Ascomycete* with a relatively small genome of ~40 Mb divided into seven chromosomes (Dean et al., 2005). It is an ascomycete because it produces sexual spores (ascospores) in structures called asci and is classified in the family Magnaporthaceae. The asci are found within specialized structures called perithecia. Pyriform macroconidia are produced on conidiophores which protrude from lesions on plants. The name *Pyricularia* refers to the pyriform shape of the conidia. The conidiogenous cells of *Pyricularia* are polyblastic; integrated on the conidiophores; and are sympodial, cylindrical, geniculate, and denticulate. These germinate and develop an appressorium at the tip of the germ tube, which attaches to the surface of plant tissues; an infection-peg from the appersorium penetrates into plant tissues. The wall of conidiophores and appersorium are pigmented by melanin.

3.3 HOST RANGE OF THE PATHOGEN

M. oryzae is capable of infecting many grass species but individual isolate exhibits a limited host range, infecting one, or at most a few, grass species

not show water-soaking symptoms. The highly variable-specific virulence of the fungus and its genetic plasticity make its control and management difficult. Thus, *M. oryzae* is one of the most devastating threats to food security worldwide.

1a 1b

FIGURE 3.1 (a) Blast-infected rice plant in field and (b) typical symptom of blast disease on leaves of rice. (Reprinted from Adeyemi, M. M. Hassan, The Potential of Secondary Metabolites in Plant Material as Deterents against Insect Pests: A Review. Afr. J. Pure Appl. Chem. 2010, 4(11), 243–246. https://creativecommons.org/licenses/by/4.0/)

3.3.2 DISEASE CYCLE

A disease cycle begins when a blast spore infects and produces a lesion on the rice plant and ends when the fungus sporulates repeatedly for about 20 days and disperses many new airborne spores. Under favorable moisture and temperature conditions (long periods of plant surface wetness, high humidity, little or no wind at night and night temperatures (between 12 and 32°C) the infection cycle can continue. In the canopy of rice plants, newly developed leaves act as receptors for the spores. The maximum number of spores produced was 20,000 on 1 lesion on leaves and 60,000 on 1 spikelet in 1 night. Lesions on leaves become an inoculums source for panicles (Royal Society of Chemistry).

3.4 RICE BLAST MANAGEMENT

Many of the control practices useful in reducing plant diseases are of limited use to control rice blast. Since blast is present in most rice growing areas,

and it has such a wide host range, eradication and crop rotation are of little value. Lots of work on developing effective rice blast management strategies has been done over a century. The control measures found effective and utilized in the fields are described below (Fig. 3.2). The following procedures broadly used for the control of blast disease as follows.

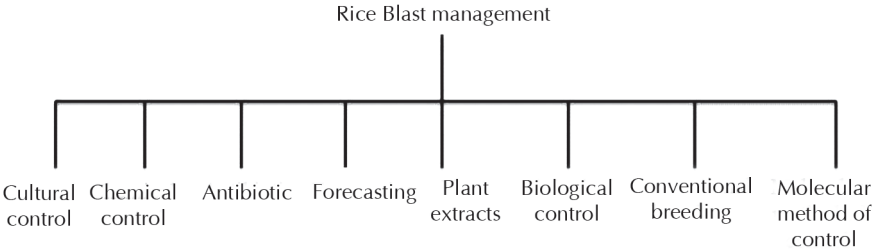

FIGURE 3.2 Various methods for controlling rice blast disease.

3.4.1 CULTURAL CONTROL

When there were no methods of disease management in the past, cultivation practices were the only means to control the diseases. These include nutrient management, water management, time of planting, spacing, etc.

3.4.1.1 NUTRIENT MANAGEMENT

In case of rice blast, two nutrients, namely, nitrogen and silicon have been found to affect the disease occurrence and development significantly. Since a long time back, studies have shown that high N supply always induces heavy incidence of rice blast (Hori, 1898). Delayed or large top dressings are often responsible for severe disease (Ikeda, 1933; Murata et al., 1933). A limit of 15 kg N/ha is recommended for upland rice in Brazil, specifically to reduce vulnerability to blast (Prabhu and Morais, 1986). Plant receiving large amount of N are found to have fewer silicated epidermal cells and thus have lower resistance (Miyake and Ikeda, 1932). The correlation between silica content and disease incidence was also studied on different cultivars of rice, and it was observed that plants with high silica content or large number of silicated epidermal cells had slight damage from blast disease (Onodera, 1917). So it is suggested that resistance of rice to blast can be increased by applying silica slag in the field (Kawashima, 1927). Studies conducted at

University of Florida USA, showed that reduction in the rice blast with the application of silica (calcium silicate slag) was comparable to that of fungicide (Benomyl) and now silicon fertilization has become a routine practice in Florida rice production (Datnoff et al., 1997). Singh and Singh (1980) reported that application of water hyacinth compost to soil reduces the rice blast disease. Positive effects of Si application in controlling blast disease have also been reported in many studies (Prabhu et al., 2003; Seebold, 1988; Seebold et al., 2000). In Malaysia, Ashtiani et al. (2012) investigated the effects of different rates and sources of Si on rice variety MR219 toward controlling the blast disease. Silicon was applied to the soil prior to planting using two different Si sources which were silica gel (0, 60, 120, 180 g/5 kg soil) and liquid sodium silicate (0, 1, 2, 3 mL/L), respectively. Results revealed that there was a significant decline on disease severity and incidence for all rice plants that were treated with Si compared to the nontreated rice plants. The Si depositions were more intensive on the dumbbell-shaped cells in the Si-treated plants compared to the nontreated plants. Highest reduction (75%) of disease severity was observed for plants receiving silica gel application at the rate of 120 g. This result confirmed that Si displays a significant role in controlling rice blast fungus.

3.4.1.2 WATER MANAGEMENT

The availability of water also affects the susceptibility of host plant to *P. oryzae*. Rice grown under upland conditions is more susceptible than rice grown in flooded soil (Kahn and Libby, 1958). Under upland conditions, susceptibility is increased further with increasing drought stress. Hence, flooding the field in upland rice can reduce the severity of blast.

3.4.1.3 TIME OF PLANTING

Planting time also has a noticeable effect on the development of blast within a rice crop. For rice blast control, early planting is recommended. In tropical upland rice, crops sown early during the rainy season generally have a higher probability of escaping blast infection than late-sown crops, which are often blasted severely. In upland areas of Brazil, farmers are advised to sow early to escape inoculums produced on neighboring farms (Prabhu and Morais, 1986).

found than the parent (Kaur et al., 1971). Blast resistance mutant R917 was derived from the F_1 progeny radiated by 10 krad 60Co c-ray (Zhang et al., 2003). The Mtu 17 mutants possessing desirable agronomic characters through chemomutagenesis with dES; some of the mutants had blast resistance, whereas the parent Mtu 17 was susceptible (Gangadharan and Mathur, 1976). Through mutation breeding, several mutant lines, such as Mahsuri Mutant SPM 129, SPM 130, and SPM 142 (Azlan et al., 2004) have produced successfully for blast resistance in Malaysia (Mohamad et al., 2006). In China, the mutant rice variety "Zhefu 802" deriving from var. "Simei No. 2," induced by Gamma rays, has a high resistance to rice blast (Ahloowalia et al., 2004; Shu et al., 1997). Today, the technique became part of the tools kit breeders have to enhance specific rice characteristics in well-adapted varieties. Through conventional breeding programs major genes *Pib*, *Pita*, *Pia*, *Pi1*, *Pikh*, *Pi2*, and *Pi4* have been introduced into rice varieties for blast resistance (Kiyosawa, 1982). Identifying key genomic regions associated with blast resistance against a broad spectrum of isolates in backcross introgression lines have been developed through conventional breeding program (Korinsaka et al., 2011).

Some components of breeding strategies suggest prolong durability of resistance which generally can be adopted for stabilization and control of blast disease in rice are discussed below:

3.5.4.1 BACKCROSSING FOR CONCENTRATION OF SLOW-BLASTING (MINOR GENES) COMPONENTS

Breakdown of varietal resistance to rice blast disease attributed to the failure of varieties to capture the entire complement of genetic factors for disease resistance from the respective parent sources in their parentage (Nottegham, 1993). Existence of slow-blasting characters, originally presented as horizontal resistance (van der Plank, 1975), mainly found only in tall, upland rice varieties (Bidaux, 1976; Villareal, 1980) and identified several slow blasting components in several varieties (Villareal, 1980). Identification of slow blasting sergeants in segregating populations is difficult particularly in bulk breeding systems. It might be somewhat easier in a pedigree system of breeding where discrete progeny rows can be evaluated for identification of lines with slow blasting components.

3.5.4.2 COMBINATION OF MAJOR GENES WITH SLOW-BLASTING (MINOR GENES) COMPONENTS

The combination of major genes (vertical resistance) with slow-blasting components (minor genes) is believed to provide increased stability of the resistance mechanism to blast, because the genes for vertical and horizontal resistance in combination increase the effectiveness of each other. Centre International de Agricultural Tropical (CIAT) rice breeding program (CIAT, 1982) attempted adopting this strategy, but found difficult to detect the combinations of the two types of resistance in a given pedigree line, as the lower level of horizontal resistance is masked by the presence of vertical genes. Under such circumstances, it is proposed to select for vertical resistance and hope for the best. Therefore, the practical outcome of using this strategy in a breeding program is not predictable.

3.5.4.3 MIXTURES OF VARIETY

Varietal mixtures are the way of reducing the development of blast races consisting of 80–90% resistant plants and 10–20% susceptible plants of similar varietal background. This strategy is easier to introduce but need to ensure their agronomical uniformity. In Yunan province of Southeast China, highly susceptible glutinous plants were mixed and planted with non-glutinous hybrid indica rice reduced the development of blast in glutinous rice (Leung et al., 2003; Zhu et al., 2005). But measuring panicle blast resistance is difficult because the panicle infection is influenced by weather, and even small differences in maturity period between lines can result inaccurate assessment of their level of resistance. Several researchers mentioned that minor genes that play an important role in maintaining an acceptable level of disease under field conditions. Such genes (minor) are difficult to identify and characterize in the presence of major genes due to epistatic interactions among themselves. Their presence could also affect the accuracy of classification of lines for complete resistance to blast (Wang et al., 1994).

3.5.4.4 MULTIPLE LINES

The durability resistance of multiline varieties depends upon the rate of blast races develop, the number of lines component in a mixture, and the extent of planted area. Development of multiline varieties using blast resistant isogenic

lines had been attempted for "Nipponbare" (Higashi et al., 1981; Horisue et al., 1984) "Toyonishiki" (Nakajima, 1994) and "Sasanishiki" (Matsunaga, 1996). Blast control effects by the use of these multiple line varieties have been confirmed (Ise, 1990; Koizumi and Fuji, 1994; Koizumi et al., 1996; Nakajima et al., 1996). New isogenic lines have been bred and elaborated variation analysis in the distribution of the races of the blast pathogen, which is essential for stable utilization (Ashizawa et al., 2001; Tsuji et al., 1999). A cross combination of Koshihikari blast-resistant isogenic lines (BLs) was made by (Ishizaki et al., 2005). The BLs were bred by crossing with Sasanishiki (*Pia*), Todorokiwase (*Pii*), *Pi4 (Pita-2*), Niigatawase *(Piz*), Koshiminori (*Pik*), Tsuyuake (*Pik-m*), Toride 1 (*Piz-t*), and BL1 (*Pib*) as the donor parent, respectively, and then repeated backcrossings with "Koshihikari" as the pollen parent were performed. Individuals used for backcrossing after the first filial generation of the first backcross generation (BC_1F_1) were sprayinoculated with race 001 of the blast pathogen for the nursery test. About 30 heterozygotes showing resistance were selected for cultivation in the field. Individuals that resembled "Koshihikari" were selected prior to the backcrossing procedure. Selected individuals were transplanted to 1/5000 a Wagner pots and about 50 seeds were obtained per stump. Backcrossing was performed six times for BL No. 4 and five times for the other lines, following examples of BLs for Toyonishiki, Nipponbare and Sasanishiki, which have been bred so far. Seedlings of B_5F_2 or B_6F_2 after selfed generation were spray-inoculated with race 001 for selecting homozygotes with true resistance as well as for fixation. All the Koshihikari BLs was found to be identical with the original "Koshihikari" in terms of practical agronomic traits and clearly superior in suppressing blast.

3.5.4.5 DEPLOYMENT OF GENE

This strategy involves the use of the distinct type of blast resistance mechanism in each variety and use of varieties in a predetermined pattern (temporal or spatial). Based on rice cultivation practices, seasonal and regional preferences for different location specific varieties are used. As a result, distinct varieties could be developed using diverse sources of blast resistance. Even among varieties used for a particular season, variety with different maturity period should consist of distinct sources of blast resistance. This situation will slow down the development of new virulent races, and improve the durability of blast resistance in present varieties. Among many strategies, distinct gene deployment in different maturity groups may help to improve

the durability of blast resistance in newly developing rice varieties. Nevertheless, the conventional resistance breeding has apparent weakness, such as long breeding cycle, low selection efficiency, and difficulty in distant crossing, leading to the lag between the development of new resistant cultivars and the emergence of virulent pathotypes of the causal pathogen.

3.6 BIOTECHNOLOGICAL AND MOLECULAR APPROACHES FOR BLAST RESISTANCE

3.6.1 BLAST-RESISTANT GENE

Conventional genetic analysis identified donors with resistance, availability of pure isolates of the blast pathogen and use of advanced molecular analysis techniques, These have been designated as *Pi1*, *Pi2*, *Pi3*, *Pi4*, *Pi5*, *Pi6*, *Pi7*, *Pi9*, *Pi10*, and *Pi11* (Causse et al., 1994; Wang et al., 1994), *Pia*, *Pib*, *Pik*, *Pit*, *Pita*, *Pita 2*, *Pi12*, *Pi17*, *Pi18*, *Pi19*, *Pi20*, *Pi23*, *Pi57*, *Pi62* (Nagato and Yoshimura, 1998), *Pii* and *Pi15* (Pan et al., 2003), *pi21* (Fukuoka and Okuno, 2001), *Pi25* (Yang et al., 2001), *Pi27* (Zhou et al., 2004), *Pi24*, *Pi25*, *Pi26*, *Pi27*, *Pi28*, *Pi29*, *Pi30*, *Pi31* and *Pi32* (Sallaud et al., 2003), *Pi33* (Berruyer et al., 2003), *Pish* (Fukuta et al., 2004), *Pi35* (Nguyen et al., 2006), *Pi36* (Liu et al., 2005), *Pi37* (Chen et al., 2006), *Pi38* (Gowda et al., 2006), *Pi39* (Lin et al., 2007), and *Pi40* (Jeung et al., 2007). Out of 100, 5 blast resistance gene, that is, *Pi-kh* or *Pi54* (Sharma et al., 2005), *Pitp(t)* (Barman et al., 2004), *Pi38* (Gowda et al., 2006), *Pi42(t)* (Kumar et al., 2010) and, *Pi-Wn(t)* (Rathore et al., 2012) have been mapped in India. Nine blast resistance genes—*Pi-b* (Wu et al., 2004), *Pi-ta* (Bryan et al., 2000) and *Pi-kh* (Sharma et al., 2005), *Pi37* (Lin et al., 2007), *Piz-5* and *Piz-t* (Zhou et al., 2005), *Pi9* (Qu et al., 2006), *Pid2* (Chen et al., 2006), and *Pi36* (Liu et al., 2007) have been cloned. The wide genetic variation available in blast fungus may be the main reason why many resistance genes in rice have evolved. Majority of the quantitative trait loci (QTLs) are associated with qualitative genes. Quantitative resistance is generally considered as partial resistance in a particular cultivar (Parlevliet, 1979) which is controlled by multiple loci, known as QTLs. Approximately, 350 leaf blast resistances QTLs have been mapped. These QTLs were identified in 15 different populations, most of which are derived from indica japonica crosses (Chen et al., 2003; Lopez-Gerena, 2006; Sallaud et al., 2003; Sato et al., 2006; Wu et al., 2004; Talukder et al., 2005). Partial resistance is characterized by compatibility between the pathogen and the plant with reduced development of disease compared to

plants with no partial resistance (Bonman and Ahn, 1990; Parlevliet, 1998). Four partial-resistance genes have been identified and described as specific, *Pif* (Yunoki et al., 1970), *Pi21* (Fukuoka and Okuno, 1997), *Pb1* (Fujii et al., 1995), and *Pi34* (Zenbayashi-Sawata et al., 2005).

3.6.2 HOST RESISTANCE

Exploitation of host resistance is the most cost-effective and reliable method of disease management. In some instances, resistant varieties have provided effective and durable disease control. But in the case of rice blast, success is short-lived or not easily achieved. It is because of the presence of lineages (that may consist of different physiologic races) overcoming host resistance (IRRI, 2010). Early studies on host resistance were more concentrated on nature of resistance. Miyake and Ikeda (1932) reported that the cultivar Bozu, resistant to rice blast contains a large amount of silicon than the susceptible cultivar. Further studies showed that degree of resistance increases in proportion to the amount of silica applied and also to the amount of silicon accumulated in the plant. Ito and Sakamoto (1939) found that resistance to mechanical puncture of the leaf epidermis was positively related with resistance to blast. They found that puncture resistance was reduced by application of nitrogen fertilizer and by low soil moisture, but was increased as the plant become older. Hori et al. (1960) reported that distribution of starch in the leaf sheath is related to resistance that is, longer accumulation indicates more resistance. It is known that resistance to penetration of fungus is obviously less important than resistance to its spread within the host plant after penetration. A hypersensitive reaction is common in resistant cultivars. Kawamura and Ono (1948) were able to isolate *P. oryzae* from hypersensitive lesion 2 days after inoculation but not after 4 days. Low toxins pyricularin and α-picolinic acid produced by *P. oryzae* are toxic to rice plant and cause stunting of seedlings, leaf spotting and other injurious effects. Earlier, Tamari and Kaji (1955) found that when combined with chlorogenic acid or ferulic acid, both present in the rice plant, they (pyricularin and α-picolinic acid) become nontoxic to rice plant. So they believed that ability of rice plant to biosynthesize chlorogenic acid is related to resistance. All these findings not only generated the knowledge of host pathogen interaction but also contributed in searching resistance sources and setting the strategies of breeding for blast resistance. First, most important step in resistance breeding is the evaluation of germplasm for disease resistance sources. In 1969, Link and Ou (1969) proposed a system of standardization of race numbers of

P. oryzae. IRRI also stepped forward and planted uniform blast nurseries in 50 testing stations in 22 different countries for pathogen race evaluation and till 1975 more than 260 physiologic races of *P. oryzae* were reported from the different parts of world. Resistance to *P. oryzae* in rice is usually dominant and controlled by one or few pairs of genes (Thurston, 1998). At IRRI, in 1979, almost 100,000 lines and accessions were tested and not a single one was found to be completely resistant to all races. Host plant resistance can be broadly categorized as follows.

3.6.2.1 VERTICAL RESISTANCE

Vertical Resistance (also known as Complete resistance, specific resistance or true resistance), in which the pathogen fails to produce sporulating lesions, can be manipulated easily by breeders. But it also has been known to break down, sometimes with serious economic consequences. In Korea, the resistance of the Tongil varieties was effective for 5 years before a virulent race appeared in 1976 (Lee et al., 1976). The variety Reiho had complete resistance to Japanese races upon its release in Japan in 1969. Its area of cultivation increased until 1973, when it was damaged severely by blast (Matsumoto, 1974). In Japan, the longevity of complete resistance seems to be about 3 years. Similarly, with var. Reiho was later released in Egypt as a blast resistant variety in 1984, it occupied about 25% of the rice crop area within a year but resistance was overcome in the first year, resulting in a blast epidemic of some consequence (Bonman and Rush, 1985). In Colombia, a series of resistant varieties was released from 1969 to 1986, but their resistance lasted only a year or two before being overcome by previously unidentified virulent races (Ahn and Mukelar, 1986).

3.6.2.2 HORIZONTAL RESISTANCE

Assuming the gene-for-gene relationship (Flor, 1956) and given the variability of the pathogen, it is not difficult to understand why the effectiveness of complete blast resistance is short-lived. It has been observed that when complete resistance was overcome by the pathogen, usually some level of residual resistance remains. This residual resistance has been referred to variously as, horizontal resistance (HR), general resistance, field resistance, slow-blasting, and partial resistance, among others. The general, HR to *P. oryzae* was reported in 1971 (Ou et al., 1971). Efforts to identify,

characterize, and exploit this type of resistance which is effective against all races of pathogen were undertaken by IRRI. But the 1978 epidemic of *P. oryzae* in Korea altered the attitude of IRRI breeders and pathologists toward HR. The improved indica–japonica hybrid rice cultivars grown in Korea were possessing vertical (monogenic) as well as horizontal (polygenic) resistance suddenly became susceptible to *P. oryzae* in 1978 (Crill et al., 1982). Korean pathologists had defined the HR as varieties with disease ratings 4–5. Since HR studies in Korea (Crill et al., 1982) may have been defined qualitatively and not quantitatively, the Korean experience should not be used as a reason to discontinue the search for rice cultivars with HR (Thurston, 1998). There are many examples of partial resistance that appear to be effective and durable under field conditions. The varieties IR36 and IR50 are susceptible to the same races of *P. oryzae* (Bonman et al., 1986), but when inoculated with the same isolates, IR36 produces fewer and smaller lesions than does IR50 (Yeh and Bonman, 1986). Frequently, the main strategy of breeder and pathologist, given the choice, is to save only the most resistant-appearing lines in a screening nursery, and usually these are lines with complete resistance to the races present in the nursery. Because complete resistance masks partial resistance, there is no way to evaluate such lines without either challenging them with isolates of *P. oryzae* that are virulent (i.e., the complete resistance is overcome), or by progeny-testing of a cross with a highly susceptible variety. Using the IRRI 1975 blast rating scale, lines with ratings of 3–6 probably represent those with usable levels of partial resistance that are not masked by complete resistance. So by introducing such lines into a breeding program and avoiding lines with little partial resistance, a strong pool of genes that contribute to race-nonspecific partial resistance could be gradually accumulated in the breeding population. Effective resistance can be achieved by combining into the same cultivar, different race-specific genes and genes conferring quantitative resistance. Another method is by deploying resistance genes in mixed plant populations. Recent studies indicated that use of cultivar mixture is an effective tool in blast management. IRRI scientists introduced the practice of inter-planting glutinous rice varieties with blast-resistant hybrid varieties in Yunnan province, China. Blast caused great yield loss on traditional glutinous rice varieties in China and farmers were spraying fungicides for up to seven times. Interplanting has prevented the fungus from continuous build-up of inoculum that had previously occurred in the monoculture fields of the glutinous varieties.

3.6.3 MARKER-ASSISTED SELECTION

Conventional breeding for disease resistance is laborious, time consuming, and highly dependent on environmental conditions in comparison to molecular breeding particularly Marker assisted selection (MAS), which is simpler, highly efficient, and precise (Koizumi, 2007). In addition, conventional breeding generally depend upon the phenotype of artificial identification, and the performance of field resistance is too long to catch up with the frequent emergences of new virulent races of the pathogen and the release of improved varieties cannot be guaranteed (Werner et al., 2005; Zhang et al., 2006). MAS is extremely powerful in blast resistance breeding because resistance phenotypes are often simple or encoded by single or few genes (Young, 1994). MAS have the advantage for the blast control by governing definite interaction of a particular resistance (R) gene with a particular avirulence gene in the pathogen (Silue et al., 1992). Molecular markers have greater opportunity to improve the efficiency of conventional breeding by carrying out selection not directly on the trait of interest but also on linked molecular markers of that particular trait. Some SSR markers (RM168, RM8225, RM1233, RM6836, RM5961, and RM413) have been found by Ashkani et al. (2011) that could be used in MAS programs (Table 3.1). Availability of molecular markers along with MAS strategies are essential to develop durable blast-resistant variety against different races of *M. oryzae* (Ashkani et al., 2012). The high cost of MAS will be a major obstacle for it adoption in the developing countries in the nearest future. The initial cost of using markers is more expensive compared to conventional breeding; though time savings could lead to expedite variety release which could lead to greater profit. The low reliability of markers to determine phenotype is another important factor obstructing the successful application of markers for line development.

3.6.4 MARKER-ASSISTED BACKCROSS BREEDING

Marker-assisted backcross breeding (MABC) as another technique recently has been given attention in rice breeding for the introgression of blast-resistance genes (one or a few genes) into the susceptible or in an adapted or elite varieties. MABC is the process of using markers to select for target loci, minimize the length of the donor segment containing a target locus, and accelerate the recovery of the recurrent parent (RP) genome during backcrossing (Charcosset, 1994; Hasan et al., 2015; Hospital, 2001). The

TABLE 3.1 List of Available Blast-resistance Genes and Tightly Linked Markers in Rice.

Chromosome	Gene	Tightly linked marker		Map position (cM)	Donor rice	Resistance type
		Marker type	Marker name			
1	*Pit*	SNP	t311, t256, t8042	12.2	Tjahaja	Complete
	Pi27(t)	Microsatellite	RM151.RN259	28.4–38.8	Q14	Complete
	Pi24(t)	–	–	64.4	Azucena	–
	Pitp(t)	Microsatellite	RM246	114.1	Tetep	–
	Pi35(t)	Microsatellite	RM1216, RM 1003	132.0–136.6	Hokkai 188	Partial
	Pi37	Microsatellite	RM302, RM212, FPSM1, FPSM2, FPSM4	136.1	St. No. 1	Japonica
		STS	S15628, FSTS1, FSTS2, FSTS3, FSTS4			
2	*Pi(t)*	–	–	–	–	–
	Pid1(t)	Microsatellite	RM262	87.5–89.9	Digu	–
	Pig(t)	Microsatellite	RM166, RM208	142.0–154.1	Guangchangzhan	–
	Pitq5	–	–	150.5–157.9	Teqing	Complete
	Piy1(t)	Microsatellite	RM3248, RM20	153.2–154.1	Yanxian No. 1	–
	Piy2(t)	Microsatellite	RM3248, RM20	153.2–154.1	Yanxian No. 1	
	Pib	SNP	b213, b28, b2, b3989, Pibdom	154.1	Tohoku IL9	Complete
		Micosatellite	RM138, RM166, RM208, RM266, RM138, RM166, RM 208, RM 266			
	Pi25(t)	–	–	157.9	IR64	–
	Pi14(t)	–	–	–	Maowang	Complete
	Pi16(t)	–	–	–	AUS373	Complete
4	*Pi21*	STS	P702D03–79	58.6	Owarihatamochi	Partial
	Pikur1	–	–	86	Kuroka	–

TABLE 3.1 *(Continued)*

Chromosome	Gene	Tightly linked marker		Map position (cM)	Donor rice	Resistance type
		Marker type	Marker name			
	Pi39(t)	Microsatellite	RM3843, RM5473	107.4–108.2	Chubu 111 (Haonaihuan)	–
	Pi(t)	–	–	–	–	–
5	*Pi26(t)*	–	–	22.5–24.7	Azucena	–
	Pi23(t)	–	–	59.3–99.5	Sweon 3655	–
	Pi10	InDel	OPF62700	88.5–102.8	Tongil	Complete
6	*Pi22(t)*	–	–	38.7–41.9	Sweon 365	–
	Pi26(t)	–	–	51.0–63.2	Gumei 2	–
	Pi27(t)	–	–	51.9	IR64	–
	Pi40(t)	Microsatellite	RM3330, RM527	54.1–61.6	IR65482-4-136-2-2	–
		CAPS	S2539			
	Piz-5	–		58.7	Tadukan	Complete
	Piz	InDel	z4794	58.7	Zenith	Complete
		SNP	Z60510, z5765, z56592, z565962			
	Piz-t	InDel	Z4794	58.7	Torde1	Complete
	Pi9	–	–	58.7	75-1-127 (101141)	Complete
	Pi25(t)	–	–	63.2–64.6	Gumei2	–
	Pid2	–	–	65.8	Digu	complete
	Pigm(t)	CAPS	C26348	65.8	Gumei4	–
	Pitq1	–	–	103.0–124.4	Teqing	Complete
	Pi8	–	–	–	Kasalath	Complete
	Pi13(t)	–	–	–	Mawong	Complete

main purpose of MABC is to transfer the desired character/or targeted gene along with recovering the RP characters/or genes. MABC is now playing an important role for the development of blast-resistant cultivars (Sundaram et al., 2009) and is superior to conventional backcrossing in precision and efficiency and time saving. Molecular markers which are tightly linked with important traits are used in MABC. Therefore, molecular markers are the tools that can be used to detect the presence of desire character in backcrossing and greatly increases the efficiency of selection (Table 3.2). The methods and potential application of MAS and MABC for the improvement of rice have been reviewed (Collard and Mackill, 2008; Hasan et al., 2015).

3.6.5 GENE PYRAMIDING

Pyramiding is the accumulation of genes into a single line or cultivar. In a gene pyramiding, strategy is to cumulate genes identified in multiple parents into a single genotype. The end product of a gene-pyramiding program is a genotype with all of the target genes. Pyramiding multiple-resistance genes provides durable stress-resistance expression in crops. Gene pyramiding technique generally used for combining multiple disease or pest-resistance genes for specific races of a pathogen or insect to develop long-lasting resistance. It helps in crop improvement program by decreasing the breeding duration. Different R-genes often have resistance to different isolates, races, or biotypes. So, by combining these different R genes will expand the resistance spectrum against the different number of races or isolates in a variety at the same time.

Molecular markers help in the identification and selection of desired traits in early developmental stages of the plant. Such molecular markers greatly enhances the gene pyramiding efficiency by reducing the time of selection of plants and savings of resources such as greenhouse or field space, water, and fertilizer Therefore, MAS-based gene pyramiding will help in the pyramiding of different genes into a single genetic background (Joshi and Nayak, 2010; Koide et al., 2009; Shinoda et al., 1971). Hittalmani et al. (2000) have successfully pyramided three genes, *Pi1*, *Piz5*, and *Pita* in a susceptible rice variety, Co39. Chen et al. (2004) successfully adopted MAS and pyramided three blast-resistance genes—*Pi-d(t)1*, *Pi-b*, and *Pi-ta2*—in rice G46B from Digu, BL-1, and Pi-4, respectively. Similarly Singh et al. (2011) also pyramided blast-resistance genes *Piz 5* and *Pi54* into "PRR78" basmati restorer line of rice hybrid Pusa RH10 by Marker-assisted simultaneous

TABLE 3.2 Examples for Application of Marker-assisted Selection (MAS) and Marker-assisted Backcrossing (MABB) in Rice.

Trait	Gene(s)/QTLs	Marker(s) used	Technique used	Application	Reference
Blast resistance	*Pi1*, *Piz-5*, *Pita*	RFLP	MAS	Pyramiding of three near isogenic lines (C101LAC, C101A51 and C101PKT) for blast resistance in into a single cultivar Co-39, each carrying the major genes, *Pi1*, *Piz-5*, and *Pita*, respectively	Hittalmani et al. (2000)
Blast resistance	*Pi1*	SSR and ISSR	MAS	Applied for backcross breeding of variety	Liu et al. (2002)
Bacterial blight resistance + blast resistance	*Xa21*, *Piz*	SSR	MAS	MAS functional for pyramiding of target traits	Narayanan et al. (2002)
Blast resistance	*Pid1*, *Pib*, *Pita*, and *Pi2*	SSR	MAS	*Pid1*, *Pib*, and *Pita* genes were introduced into G46B cultivar, while *Pi2* Zhenshan97B cultivars of rice	Chen et al. (2004)
Blast resistance	*Pi-z*	SSR	MAS	Closely linked with *Pi-z* locus has been successfully used for selection of blast resistance in a wide array of rice germplasm	Fjellstrom et al. (2006)
Blast resistance + bacterial blight resistance + sheath blight resistance	*Xa13*, *Xa21*, *Pi54*, qSBR11	SSR [for blast resistance (*Xa13* and *Xa21)*, for bacterial blight resistance (*Pi54*), and sheath blight resistance (qSBR11)]	MAS	MAS-assisted transfer of genes conferring the resistance toward three different diseases in rice	Singh et al. (2012a)
Blast resistance + Bacterial blight resistance	*Pi*-genes, *Xa5*	SSR	MAS	SSR MAS Near-isogeniclines (NILs) derived from two blast resistant crosses (RD6 × P0489 and RD6 × JaoHomNin) were pyramided with IR62266 (*xa5*), to transfer bacterial leaf blight resistance to RD6 lines	Pinta et al. (2013)

TABLE 3.2 *(Continued)*

Trait	Gene(s)/QTLs	Marker(s) used	Technique used	Application	Reference
Blast resistance	Pita	Gene specific marker	MAS	Existence of the *Pi-ta* gene in 141 rice germplasm has been successfully determined, but the results were more articulated when *Pi-ta* gene was introduced through advanced breeding lines	Wang et al. (2007)
Submergence tolerance + brown plant hopper resistance + blast resistance + bacterial blight resistance	chr9 QTL, *Xa21*, Bph and QTLs blast, and quality loci	SSR and STS	MABB	MABB confirmed the transfer of gene and QTL for into cultivar KDML105	Toojinda et al. (2005)
Blast resistance	*Pi1*, *Pi2*, *Pi33*	SSR	MABB	Introgressed into Jin23B cultivar through MABB	Chen et al. (2008)
Blast resistance + bacterial blight	*Pi1*, *Pi2*, *Xa23*	SSR for blast resistance (*Pi1*, *Pi2*), for bacterial blight resistance (Xa23)	MABB	Successfully applied for breeding the variety (Rongfeng B)	Fu et al. (2012)
Blast resistance	*Piz-5*, *Pi54*	SSR	MABB	Combination of blast resistance gene from donor lines (C101A51 and Tetep) into cultivar PRR78 to develop Pusa1602 (PRR78 + *Piz5*) and Pusa1603 (PRR78 + *Pi54*), respectively	Singh et al. (2012b)
Blast resistance	*Pi-9(t)*	pB8		MABB applied to introgress the cultivar Luhui17	Wen and Gao (2011)
Blast resistance	*Pi-1*, *Pi-z*	SSR	MABB	Pyramiding of *Pi-1* and *Piz-5* genes into introduced PRR78 cultivars	Gouda et al. (2013)

but stepwise backcross breeding (MASS-BB). *Piz5* and *Pi54*, taken from non-Basmati donors, C101A51 and Tetep, respectively, and transferred into PRR78. Marker-assisted foreground selection coupled with stringent phenotypic selection and background analysis was carried out for recovery of RP phenome and genome (RPG) in two separate backcross series to produce BC_2F_1 plants with individual blast-resistance gene. Background analysis revealed that the RPG recovery was up to 91.6% (Table 3.3).

Pyramiding is the strategy for the accumulation of different genes into a single line or cultivar. The developed end product of a gene-pyramiding program is a genotype with all of the target accumulated genes. Gene pyramiding of multiple resistance genes provides durable resistance in crops for the multiple diseases/stress. Presently, gene pyramiding technique broadly employed for combining multiple disease, pest resistance of different abiotic resistance genes for specific races of a pathogen or insect or stresses to develop durable resistance. Gene pyramiding helps in the crop improvement program rapidly and reduces breeding duration. Different R-genes confer resistance to different isolates, races, or biotypes in the plants, so combining these resistance gene broadens the number of races or isolates that a more than one character in a variety at the same time.

For the development of new elite breeding lines and varieties, plant breeders will combine desirable traits from the multiple parental lines. Nowadays, gene pyramiding can be accelerated by using molecular markers for the identification and selection of plants that contain the desired allele combination in very early stage. The above advantage resulting in obvious savings of resources including greenhouse or field space, water, and fertilizer. Molecular marker can help in the existing plant breeding programs which allows plant breeders to admittance, transfer, and combine different genes with precision manners. MAS-based gene pyramiding makes possible in pyramiding of different genes effectively into a single genetic background of a crop (Joshi and Nayak, 2010). There are several factors that is, number of genes to be transferred, distance between the target genes and flanking markers, number of genotype selected in each breeding generation, and nature of germplasm used is decisive for successful gene pyramiding program. Presently, gene pyramiding programs are considered as one of the most valuable strategies for attaining durable resistance against blast disease in rice (Hittalmani et al., 2000; Koide et al., 2009; Shinoda et al., 1971). There are different successful stories of the accumulation of different blast resistance genes in elite rice cultivars by different researchers (Table 3.3).

TABLE 3.3 Examples for Application of Gene Pyramiding in Controlling Blast Disease in Rice.

Traits	Parental lines	Pyramided genes	DNA (markers) used	Reference
Blast resistance	C101LAC, , C101A51	*Pi1*, *Pi2*, and *Pi33*	SSR	Chen et al. (2008)
Blast resistance	IR5, IR8, IR20, IR22, IR24, IR26, IR28, IR29, IR30, IR32, IR34, IR36, IR38, IR40, IR42, IR43, IR44, IR45, IR46, IR48, IR50 IR52, IR54, IR56, IR58, IR60, IR62, IR64, IR65, IR66, IR68, IR70, IR72, IR74	*Pib* and *Pita*	SSR	Fujita et al. (2009)
Blast resistance	CO39	*Pish* and *Pib*	SSR	Koide et al. (2009)
Blast resistant	IR64, JHN	Multiple resistance QTLs	Multiple resistance QTLs	Sreewongchai et al. (2010)
Blast resistant	Rongfeng B	*Pi1*, *Pi2 Xa23*		Fu et al. (2012)
Blast resistant	C101LAC, C101A51	*Pi-1* and *Pi-2*	RG64 and C481	Mahdian and Shahsavari (2013)
Blast and bacterial leaf blight resistance	RD6 × P0489; RD6 × JHN	Four QTLs for blast resistance and one gene for bacterial leaf blight (xa5)	SSR	Pinta et al. (2013)
Blast resistant	C101A51, Tetep	*Piz5* and *Pi54*	SSR	Singh et al. (2011)
Blast resistance	Carnaroli, Baldo, Arborio	*Piz* and *Pi5*	SSR	Urso et al. (2013)
Leaf blast resistance	Koshihikari	*Pi21*, *Pi34*, and *Pi35*	SSR	Yasuda et al. (2014)
Blast resistance	GZ63-4S	*Pi2* and *Xa23*	SSR (*M-Xa23)*	Jiang et al. (2012)

useful for the identification of multiple loci controlling complete resistance in a lofty resistant cultivar as well as in estimating the number, location and effect of genomic region involved in partial blast resistance against the pathogens (Sallaud et al., 2003). Different rice improvement programs now aspire to include quantitative or polygenic resistance into elite rice varieties. Several previous studies have confirmed that genetic linkage maps constructed with different molecular markers are very helpful for the analysis and recognition for the qualitative trait loci (Bao et al., 2000; Price et al., 2000).

3.6.7 ASSOCIATION MAPPING (GENETICS)

Association mapping also known as "linkage disequilibrium mapping", is a method of mapping QTLs that takes advantage of historic linkage disequilibrium to link phenotypes (observable characteristics) to genotypes (the genetic constitution of organisms). Association mapping is based on the idea that traits that have entered a population only recently will still be linked to the surrounding genetic sequence of the original evolutionary ancestor, or in other words, will more often be found within a given haplotype, than outside of it. It is most often performed by scanning the entire genome for significant associations between a panel of SNPs (which, in many cases are spotted onto glass slides to create "SNP chips") and a particular phenotype. These associations must then be independently verified in order to show that they either (a) contribute to the trait of interest directly, or (b) are linked to/in linkage disequilibrium with a QTL that contributes to the trait of interest (Gibson & Muse, 2009). Association mapping also requires extensive knowledge of SNPs within the genome of the organism of interest, and, therefore, difficult to perform in species that have not been well studied or do not have well-annotated genomes (Yu et al., 2008). Association mapping for blast resistance was performed on 226 *japonica* rice cultivars with 118 pairs of SSR markers. The blast resistance was evaluated by inoculating with two isolates, DB22 and DB77, at the tillering stage in 2013 and 2014, separately. A total of 31 associations with 17 different SSRs were significantly ($P < 0.05$) associated with blast resistance based on the mixed linear model, of which nine markers could be detected in both 2013 and 2014, including two markers that were simultaneously associated with the two isolates. Five of the nine stable markers were consistent with the genome regions identified by linkage mapping in previous reports. Phenotypic effects of each allele of the nine stable markers were compared, and 18 favorable alleles were identified. Five elite parental combinations were designed for improving

blast resistance in rice. Results demonstrate that association mapping can complement and enhance previous QTL information for MAS and breeding by design (Guo et al., 2015).

3.7 OTHER BIOTECHNOLOGICAL APPROACHES

Breeding work utilizing both phenotypic and genotypic markers are more reliable and fast. Conventional breeding are based on gene expression due to which many limitations, for example, epistatic effect exist. Conventional breeding methods may create a resistance variety which is time consuming and intensive task. Biotechnological approaches are important in introducing genes which provide resistance against *M. grisea*. Rice breeders are looking at basic bioscience and biotechnology for solving some important problems that conventional breeding methods have not satisfactorily solved. Therefore, future breeding strategies should focus at broadening the genetic and cytoplasmic background of new varieties that are being developed not only for blast resistance but also for other important pests and diseases as well. These information greatly advance our understanding of molecular mechanisms that govern race specificity. The application of advanced molecular technologies could speed up crop improvement. There are some biotechnological approaches that can be used for the development of blast resistance rice.

3.7.1 TISSUE CULTURE

The various tissue-culture methods and gene-transfer techniques now available could significantly shorten the breeding process and overcome some of the substantial agronomic and environmental problems that have not been solved using conventional methods (Zapata et al., 1995). Tissue culture is one of the fundamental tools of crop science research. Cell culture is one of the alternative methods of inducing resistance to diseases in susceptible cultivars which are well adapted to local soil and climatic conditions. The genetic variation produced in tissue culture, termed somaclonal variation, has been reported in many crop species (Larkin and Scowcroft, 1981). Fortunately during the last 30 years, extensive work has been done on selecting the disease resistant plants against different pathogens. The cell-free culture filtrate (CF) or the pure toxins of the pathogens and direct infection by the pathogen or all of them together could be used for the selection of novel disease-resistant plants (El-Kazzaz, 2001; El-Kazzaz and Ashour, 2004). However, the information

on somaclonal variation for blast resistance is very little. According to Pachon (1989), there was no variation for blast resistance in somaclones. On the other hand, Bouharmon et al. (1991) obtained R_2 lines resistant to blast from the calli derived from mature embryos. In Brazil, a high degree of partial resistance has been reported in progenies of regenerated plants derived from immature panicles of a susceptible upland rice cultivar IAC47 (Araujo et al., 1997). These discrepancies may be partly attributed to the test conditions and disease pressure under which the somaclones were assessed, and the nature of resistance. Also various other factors may affect somaclonal variation such as genotype, explants source, composition of the culture medium, and time of cultivation (Evans et al., 1984). The alterations in the genome have been attributed to expression of mutant cells in explants, meiotic crossing over, and cytological changes (Evans et al., 1984). The studies on somaclonal variation may permit accomplishment of breeding objectives in relation to rapid development of blast-resistant lines from existing commercial susceptible rice cultivars. An experiment was conducted by (Araujo et al., 2000) on Basmati-370 rice which was susceptible to some pathotypes of *P. oryzae* in Brazil, to assess the degree of blast resistance and some agronomic characteristics in the advanced generations of its somaclones. These evaluations were carried out in R5–R9 generations, in field trials, in rice blast nursery, and greenhouse. Significant variations in grain quality and other agronomic characteristics were not observed. However, some of the somaclones showed higher degree of blast resistance. Two somaclones, SCBAS04 and SBAS16 exhibited higher degree of partial resistance to blast. The degree of blast resistance of upland rice (*Oryza sativa* L.) cultivar Araguaia has decreased significantly due to yield loss obtained from resistant somaclones, adaptation to greenhouse and field selection procedures (Araujo et al., 2002). Greenhouse selection with two specific physiologic races yielded 44 somaclones with slow-blasting resistance, similar plant type and yield potential as that of Aragaia. A step-by-step protocol followed for resistant calli selection via a tissue culture technique under stress of *P. oryzae* CFs (El-Kazzaz et al., 2009). The results reveal that the resistance in regenerated rice plantlets to *P. oryzae* pathogen segregated as 1: resistant, 2: moderate resistant, 1: susceptible to *P. oryzae* may be controlled by one pair of genes.

3.7.2 GENE TRANSFORMATION

Transgenic plants can acquire a single desired trait without any alteration of the original genetic make-up and can overcome the limitation of traditional

was increased compared with the control. This provided another evidence for the feasibility of rice blast crude toxin as measures for resistance to rice blast. Recently, chloroplast-expressed MSI-99 in tobacco improves disease resistance and displays inhibitory effect against rice blast fungus The antimicrobial peptide MSI-99 has been suggested as an antimicrobial peptide conferring resistance to bacterial and fungal diseases. A vector harboring the MSI-99 gene was constructed and introduced into the tobacco chloroplast genome via particle bombardment. Transformed plants were obtained and verified to be homoplastomic by PCR and southern hybridization. In plants, assays demonstrated that the transgenic tobacco plants displayed an enhanced resistance to the fungal disease. The 23-amino-acid long antimicrobial peptide MSI-99 is a derivative of Magainin II, with a His7 to Lys substitution that prevents the gene from recognition and digestion by plant endonucleases, while maintaining its normal functions. The evaluation of the antimicrobial activity revealed that the crude protein extracts from the transgenic plants manifested an antimicrobial activity against *E. coli*, even after incubation at 120°C for 20 min, indicating significant heat stability of MSI-99. The MSI-99-containing protein extracts were first proved in vitro and in vivo to display significant suppressive effects on two rice-blast isolates. These findings provide a strong basis for the development of new biopesticides to combat rice blast (Wang et al., 2015).

3.8 ALLELE MINING

Allele mining is used to identify novel alleles or allelic variants of a gene/or candidate genes of interest, based on the available information about the genes, from a wide range of germplasm. Genetic material used for allele mining should be diverse and gene sequence information of a particular crop species should be known. For allele mining generally wild relatives. Wild relatives and local landraces are reservoirs of various beneficial alleles that are hidden in their phenotype (Tanksley et al., 1996). Therefore, wild relatives and local landraces are generally used for allele mining. Nowadays, rice genome is completely sequenced that helps in the mining of allelic diversity throughout the rice germplasm with the help of several bioinformatics tools. Allele mining can be divided into approaches that is EcoTilling and sequence-based allele mining where sequence-based allele mining is simple as well as cost-effective (Ashkani et al., 2014; Ramkumar et al., 2012).

To date, many works on identification of novel blast resistance by allele mining are done by many researchers. Allele mining of genes from wild

and cultivated rice species aims to detect superior alleles for blast resistance (Kumari et al., 2013). Through allele mining techniques, functional marker to discriminate the resistance and susceptible alleles of *Pi54* has been developed (Ramkumar et al., 2012). Costanzo and Jia (2010) analyzed the sequence level similarity for *Pikm* alleles, derived from 15 different rice cultivars. *M. oryzae* has also been differentiated from *M. grisea* by using allele mining (Couch and Kohn, 2002). Vasudevan et al. (2015) identified novel alleles of the rice blast-resistance gene *Pi54.* They explored the allelic diversity of the *Pi54* gene among 885 Indian rice genotypes that were found resistant in screening against field mixture of naturally existing *M. oryzae* strains as well as against five unique strains. Sequence-based allele mining was used to amplify and clone the *Pi54* allelic variants. Nine new alleles of *Pi54* were identified based on the nucleotide sequence comparison to the *Pi54* reference sequence as well as to already known *Pi54* alleles. DNA sequence analysis of the newly identified *Pi54* alleles revealed several single polymorphic sites, three double deletions, and an eight base pair deletion. A SNP-rich region was found between a tyrosine kinase phosphorylation site and the nucleotide binding site domain. Newly identified *Pi54* alleles will expand the allelic series and are candidates for rice blast-resistance breeding programs (Table 3.5).

3.9 miRNA IN BLAST DISEASE MANAGEMENT

miRNAs are small regulatory RNAs of 20–22 nt that are encoded by endogenous MIR genes. Their primary transcripts form precursor RNAs, which have a partially double-stranded stem-loop structure and which are processed by DCL proteins to release mature miRNAs (Voinnet et al., 2009). In the miRNA biogenesis pathway, primary miRNAs (pri-miRNAs) are transcribed from nuclear-encoded MIR genes by RNA polymerase II (Pol II) (Lee et al., 2004) leading to precursor transcripts with a characteristic hairpin structure (Fig. 3.3). In plants, the processing of these pri-miRNAs into pre-miRNAs is catalyzed by DCL1 and assisted by HYPONASTIC LEAVES 1 (HYL1) and SERRATE (SE) proteins (Voinnet et al., 2009). The pre-miRNA hairpin precursor is finally converted into 20–22-ntmiRNA/miRNA duplexes by DCL1, HYL1, and SE. The duplex is then methylated at the 3′ terminus by HUA ENHANCER 1 (HEN1) and exported into the cytoplasm by HASTY (HST1), an exportin protein (Kim et al., 2008). In the cytoplasm, one strand of the duplex (the miRNA) is incorporated into an AGO protein, the catalytic component of RISC, and guides RISC to bind to cognate target transcripts by

TABLE 3.5 Example of Allele Mining for Blast Resistance in Rice.

R-Genes/Locus	Chromosome	Rice germplasm	Reference
Pi-ta	12	From wild rice species [*O. rufipogon* (Griff) and from *O. rufipogon* (ETOR)]	Yang et al. (2007), Geng et al. (2008)
Pi-ta	12	From *O. rufipogon*	Huang et al. (2008)
Pi-ta	12	From cultivated (AA) and wild species and invasive weedy rice	Lee et al. (2009, 2011)
Pi-ta	12	In 26 accessions, consisting of wild rice (*O. rufipogon*), cultivated rice (*O. sativa*) and related wild rice species (*O. meridionalis* and *O. officinalis*) collected from 10 different countries of the world	Yoshida and Miyashita (2009)
Pi-ta	12	From land races and wild *Oryza* species	Ramkumar et al. (2012)
Pi-ta	12	In Indian land races of rice	Sharma et al. (2010)
Pi-ta	12	From Indian landraces of rice collected from different ecogeographical regions including the northwestern Himalayan region of India	Thakur et al. (2013a)
Pi-kh (Pi54)	11	From wild and cultivated species of rice	Rai et al. (2011)
Pi-kh (Pi54)	11	From the blast-resistant wild species of rice, *O. rhizomatis*	Das et al. (2012)
Pi-kh (Pi54)	11	From six cultivated rice lines and eight wild rice species	Kumari et al. (2013)
Pi-kh (Pi54)	11	In Indian land races of rice	Sharma et al. (2010)
Pi-z(t)	06	In Indian landraces of rice	Sharma et al. (2010)
Pi-z(t)	06	In 529 land races of rice collected at three different geographical locations of India	Thakur et al. (2013b)
Pid3	06	From 36 accessions of wild rice *O. rufipogon*	Shang et al. (2009), Xu et al. (2014)
Pid3-A4	06	From wild rice A4 (*O. rufipogon*)	Lv et al. (2013)
Pi9		Indifferent rice species, five AA genome *Oryza* species including two cultivated rice species (*O. sativa* and *O. glaberrima*) and three wild rice species (*O. nivara*, *O. rufipogon*, and *O. barthii*)	Liu et al. (2011)
AC134922	11	Rice lines from various sources	Wang et al. (2014)

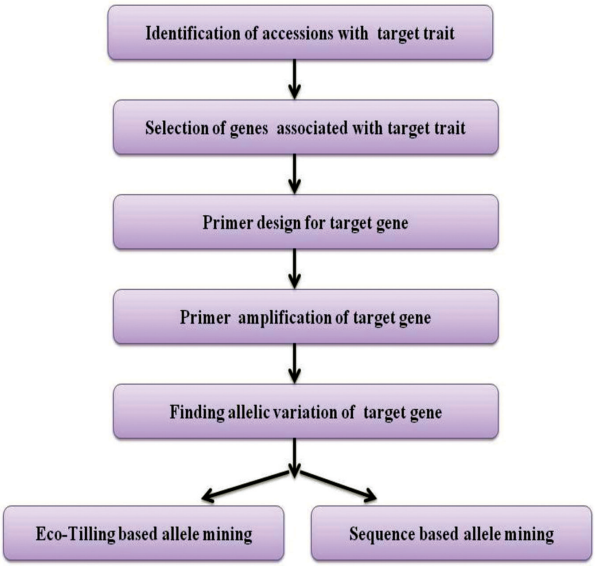

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FIGURE 3.3 Diagrammatic step involved in allele mining. (Reprinted with permission from Macmillan Publishers Ltd. Waterhouse, P. M.; Helliwell, W. C. Exploring Plant Genomes by RNA-Induced Gene Silencing. Nature Rev. Genet. **2003**,)4 (1), 29–38. © 2003 Elsevier.

sequence complementarity (Fig. 3.4). In addition to the control of targets at the posttranscriptional level, miRNAs regulate gene expression by causing epigenetic changes such as DNA and histone methylation (Khraiwesh et al., 2010; Bao et al., 2004).

A number of miRNAs have been linked to biotic stress responses in plants. Role of miRNAs in plants after infection by pathogenic bacteria, viruses, nematodes, and fungi has been reported (Katiyar-Agarwal & Jin, 2010; Ruiz-Ferrer & Voinnet, 2009). Sequenced small RNA libraries from rice tissues (leaves, roots) that had been treated with elicitors obtained from the rice blast fungus *M. oryzae*, the causal agent of the rice blast disease (Talbot, 2003) has been studied. Functional characterization of a novel miRNA from rice, osa-miR7695, which negatively regulates an alternatively spliced transcript of the OsNramp6 (Natural resistance-associated macrophage protein 6) metal transporter gene. Overexpression of the newly identified miRNA results in enhanced resistance to pathogen infection in rice plants.

MicroRNAs (miRNAs) are indispensable regulators for development and defense in eukaryotes. However, the miRNA species have not been explored

for rice (*Oryza sativa*) immunity against the blast fungus *M. oryzae*. Deep sequencing small RNA libraries from susceptible and resistant lines in normal conditions and upon *M. oryzae* infection, Li et al. (2014) identified a group of known rice miRNAs that were differentially expressed upon *M. oryzae* infection. They were further classified into three classes based on their expression patterns in the susceptible japonica line Lijiangxin Tuan Hegu and in the resistant line International Rice Blast Line Pyricularia-Kanto51-m-Tsuyuake that contains a single-resistance gene locus, *Pyricularia-Kanto 51-m* (*Pikm)*, within the Lijiangxin Tuan Hegu background. RNA-blot assay of nine of them confirmed sequencing results. Real-time reverse transcription-polymerase chain reaction assay showed that the expression of some target genes was negatively correlated with the expression of miRNAs. Moreover, transgenic rice plants over expressing miR160a and miR398b displayed enhanced resistance to *M. oryzae*, as demonstrated by decreased fungal growth, increased hydrogen peroxide accumulation at the infection site, and upregulated expression of defense-related genes. Taken together, their data indicate that miRNAs are involved in rice immunity against *M. oryzae* and that overexpression of miR160a or miR398b can enhance rice resistance to the disease.

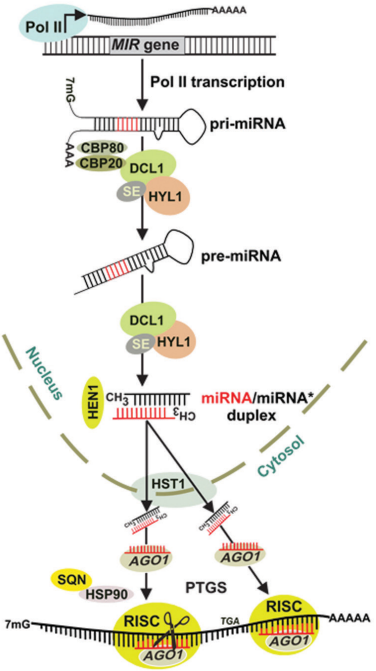

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FIGURE 3.4 miRNA biogenesis (Reprinted with permission from Khraiwesh, B.; Zhu, J. K.; Zhu, J. Role of miRNAs and siRNAs in Biotic and Abiotic Stress Responses of Plants. *Biochim. Biophys. Acta* **2012,** *1819*, 137–148. © 2012 Elsevier.)

3.10 FUTURE PROSPECTS

Molecular biology and biotechnological tools have totally changed the research on rice blast management. The availability of genome sequences of both, the host rice (Dean et al., 2005) and the pathogen has opened many doors for further research. Introduction of new sciences like nanotechnology in agricultural research and management could be proved very beneficial in future as a nanotech-based company *viz*., NANO GREEN has reported the control of rice blast using nanomolecules. Cloning of R and Avr genes and study of their gene products will add to the knowledge of host pathogen interaction. Enhancing the host plant resistance is being considered as the best approach to handle the rice blast disease. Rice cultivars containing only a single *R* gene to a specific pathogen race often become susceptible over time due to the emergence of new virulent races. Understanding of genetic identity of contemporary *M. oryzae* is important for accurate deployment of rice cultivars with different *R* genes. Stacking *R* genes with overlapped resistance spectra can lead to long-lasting resistance. For combinations of different blast-resistance genes in host plant in the rice blast breeding programs, superior alleles of the targeted genes should be considered. During the evolution and artificial selection processes, a significant portion of beneficial alleles have been left behind in the land races and wild species (McCouch et al., 2007), which can be used for the development of better rice varieties. Effective blast management also requires international cooperation. The knowledge gained by collaborative effort ought to lead to more effective methods to reduce crop loss due to blast disease worldwide. Although considerable progress has been made toward understanding the nature of disease-resistance genes, defense responses, and the signal transduction leading to activation of defense responses in rice, the whole story is still far from clear. The development of genetically engineered bioagents will supplement the environment friendly ways of management of rice blast. There is still a need for the further development of noble fungicides and fungistats with longer residual effect which can be better assisted by biotechnology in future. For resistance management, strategies like gene rotation, gene pyramiding, spatial and temporal gene deployment, and use of varietal mixtures will be the best mean to reduce the epidemics. The development and use of transgenic rice would be the best way of rice blast management in the future. Allele mining possesses good potential to be applied in molecular plant breeding. With the ever-increasing sequence data in GenBank and ever-expanding crop gene banks, it is highly essential to develop novel and efficient mining strategies to screen GenBank collections more efficiently

for DNA sequence variation and the management of genome resources. The success of allele mining mainly depends on the type of genetic materials used for screening and should be as diverse as possible. To this end, wild relatives and local landraces are used because they are reservoirs of useful alleles hidden in their phenotype (Tanksley et al., 1996). The challenge to build the association between sequence polymorphisms and putative function is of importance for the application of allele mining for R (resistant) genes. GWAS (genome-wide association study) analysis and high-throughput functional validation, like RNAi approaches, could facilitate the identification of new R alleles. Overall, to improve food security, to promote fundamental research and practical breeding new technologies must be developed and advanced methods for identification of novel alleles for functional genomics and crop improvement may become an effective tool for helping in feeding of the twenty-first-century world.

3.11 CONCLUSION

Due to the increase in population there is urgent need to increase rice production globally. To increase rice production, development of high-yielding rice varieties with tolerance to biotic and abiotic stresses is required. Among the biotic stresses blast disease is the most harmful threat to high productivity of rice. Yet the lots of management tools and practices are available for the disease but the effectiveness is dependent on their integrated use. Sanitation measures, fertilizer practices, and other aspects of rice culture, as they relate to the onset and development of blast needs further research. Durable resistance is influenced by environmental factors, so other means of disease management must be applied to assist host–plant resistance. The use of resistant rice cultivars is a powerful tool to reduce the use of environmentally destructive pesticides. Using classical plant breeding techniques, plant breeders have developed a number of blast-resistant cultivars adapted to different rice growing region worldwide. Recent advances in rice genomics provide additional tools for plant breeders to develop rice production systems that could be sustainable and environmentally favorable. The breeding strategies such as pyramiding of genes, gene rotation, and multiline varieties have been found effective in resistance management. Biotechnological tools and techniques, assisting the development of control measures have very bright future. Due to the highly variable nature of pathogen, need for the continuous research on development of durably resistant cultivars will always be there. Present chapter outlines the different traditional

methods and as well as molecular approaches for the control of rice blast disease and by which rice yield will meet up the demand in the coming years and decades globally. Finally, the knowledge gained through research must be communicated and demonstrated to the farmers so that they can use it.

KEYWORDS

- **lesions**
- **resistance gene**
- **alleles**
- **quantitative trait**
- **transgenic**

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