Rice Blast Management Through Host-Plant Resistance: Retrospect and Prospects

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Received: 27 September 2011 / Accepted: 27 November 2011 / Published online: 19 January 2012
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Abstract Rice (*Oryza sativa*) plays a significant role in achieving global food security. However, it suffers from several biotic and abiotic stresses that seriously affect its production. Rice blast caused by hemibiotrophic fungal pathogen *Magnaporthe oryzae* is one of the most widespread and devastating diseases of rice. The crop rice is vulnerable to this pathogen from seedlings to adult plant stages affecting leaves, nodes, collar, panicles and roots. This disease can be effectively managed through host resistance. Of the 100 blast resistance genes, identified and mapped in different genotypes of rice, 19 genes have been cloned and characterized at the molecular level. Most of these genes belong to nucleotide binding sites and leucine rich repeats. Besides more than 350 quantitative trait loci (QTLs) have also been identified in the rice genome. These blast resistance genes and QTLs have been successfully mobilized in the commercial cultivars by using standard plant breeding techniques and also by marker assisted backcross breeding. With the advent of latest molecular biology techniques and our understanding of the basic mechanisms of *Magnaporthe*-rice pathosystem, the strategies for broad-spectrum resistance to *M. oryzae* can be designed in future.

Keywords *Magnaporthe oryzae* · Blast resistance genes · Pi genes · Avirulence gene · NBS–LRR · Pathogen inducible promoter

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 come from rice. Of the total area under rice cultivation 92%, of the rice is grown in Asia, which is home to more than half of the world population. Rice blast caused by *Magnaporthe oryzae* poses a serious threat to the world food security as rice is the

staple food for more than 60% of the world populace. Occurrence of new races of the pathogen in Japan have resulted in frequent breakdown of resistance causing 20–100% of crop losses despite utilization of many blast resistance genes in local varieties [62]. 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 2, 65,000 T [90]. Seven epidemics of blast happened between 1980 and 1987 in the states of Himachal Pradesh, Andhra Pradesh, Tamilnadu and Haryana resulting in huge yield losses. Despite repeated epidemics and huge potential to influence the yields, concrete information on rice yield loss data due to blast disease during the last 30 years is not available. The amount of rice destroyed by blast annually is sufficient to give food to 60 million people world over [98]. Blast is a major contributor to the yield gap

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caused by biotic stresses. Rice production will be required to increase by more than 30% to meet the staple food requirements by 2030. In this era of rapidly increasing world population, limitations to increase cultivated land and non-availability of water for irrigation, reducing the loss due to blast can prove to be a critical component towards mitigating the world food security. Despite almost 100 years of dedicated efforts into the study of its genetics, rice blast 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. In the past decade, focus has been on utilizing resistance genes in rice cultivars rather than using fungicides for the control of rice blast.

The most effective way of management of this pathogen is to use blast resistant cultivars. Hence, there is lot of pressure on rice breeders to develop durably resistant rice cultivars. Resistance genes offer the most lucrative and environmentally safe option for the management of this pathogen. These genes can be utilized in combination of breeding and transgenic programmes to introgress high degree of resistance in otherwise successful and well performing commercial cultivars which are susceptible to *M. oryzae*. The immediate challenge in front of the rice blast community is to build up a repertoire of resistance genes which could be used against continuously evolving and geographically diverse strains of *M. oryzae*. Current status of research on rice blast resistance and their future prospects have been discussed in this article.

Sources of Blast Resistance Genes

The genus *Oryza* includes two cultivated and 21 wild species. The Asian rice, *O. sativa*, is cultivated all over the world whereas the African cultivated rice *O. glaberrima*, is grown on a small scale in western Africa. Based on the transferability of genes, two cultivated species, *O. sativa* and *O. glaberrima*, and six wild species, *O. rufipogon*, *O. nivara*, *O. glumaepatula*, *O. meridionalis*, *O. breviligulata*, and *O. longistaminata* have been grouped into a primary gene pool [63]. The Asian rice, *O. sativa*, is considered to be one of the world's ancient crop species domesticated by human beings dating back to almost 9000 years. The recent study explains that rice was domesticated around 8,200–13,500 years ago and rice was first cultivated in the Yangtze Valley of China [81]. Over the period rice plant has encountered many biotic and abiotic stresses which might have influenced its growth and development. During the course of domestication, rice plant has been subjected to selection both by nature and man which led to reduction of diversity in the present rice species. The domesticated rice genotypes which were subjected to mass cultivation

occupied major area under rice crop compared to the less cultivated wild rice. Since human beings are growing rice for food purpose, the selection process naturally favoured the agronomically more suitable characters over those of less cultivated species and wild relatives. During long period of cultivation this selection process lead to more uniformity in the cultivated rice lines than wild relatives and land races. The more uniformity in the cultivated rice lines narrowed down the genetic base which indeed favoured plant pathogens for better survival. Meanwhile the large source of genetic pool was left unexplored from wild rice, land races and some cultivated rice germplasm.

Breeders have been successfully tapping available wild sources for many genes in rice breeding for useful traits such as blast resistance genes *Pi9* from *Oryza minuta* [116, 2], *Pi-40(t)* from *Oryza australiensis* [56] and *Pirf2-1(t)* from *O. rufipogon* [20]. The wild rice, *O. rufipogon* has been reported to be a potential source for blast resistance genes [103]. The introgression of broad-spectrum blast resistance gene(s) from *Oryza rufipogon* into *indica* rice cultivar has also been reported [102].

Even though during the course of rice improvement many genes and their alleles from available land races, cultivars, elite rice lines and wild rice species have been explored, still there is great potential to tap the rice germplasm for the improvement of important traits of rice. So far 100 rice blast resistance (R) genes have been identified (Table 1). Of the 100 blast resistance genes identified, 45% are from *japonica* cultivars, and 51% from *Indica* cultivars and the rest 4% from wild species of rice. Contributions of important cultivars having been reported to contain two or more than two blast resistance genes have been compared (Fig. 1). Since only a few such genes have been isolated from wild species of rice, there still remains a lot of unexplored genes among these species which can be a rich source of more useful resistance genes.

Identification and Mapping of Rice Blast Resistance Genes

Rice blast disease resistance genes were first described in 1923 by Sasaki in Japan. Since the identification of the first rice blast resistance gene *Pi-a* by Kiyosawa in 1967, from *japonica* variety Aichi Asahi, around 100 rice blast resistance genes have been identified (Table 1). Different approaches used for the identification and mapping of the rice blast resistance genes are explained briefly in the following paragraph.

Molecular Map Based Approach

This is the most directed approach for the identification of resistance genes. Availability of complete molecular maps

Table 1 Blast resistance genes and their genetic location in different rice cultivars

S.No.	Gene name	Location		Source cultivar	Country	Reference
		Chr No.	Position (bp)			
1	<i>Mpiz</i>	11	4073024–16730739	Zenith (J)	Japan	[31]
2	<i>Pb1</i>	11	21711437–21361768	Modan (I)	Japan	[25, 40]
3	<i>PBR</i>	11	–	St- No 1 (J)	Japan	[26]
4	<i>Pi(t)</i>	4	–	P167 (I)	–	[9]
5	<i>Pi1</i>	11	26498854–28374448	LAC23 (J)	Philippines	[133]
6	<i>Pi10</i>	5	14521809–18854305	Tongil (I)	India	[83]
7	<i>Pi11</i>	8	–	Zhai-Ya-Quing8 (I)	China	[9]
8	<i>Pi12</i>	12	6988220–15120464	K80-R-Hang Jiao-Zhan (J), Moroberekan (J)	Japan	[52, 137]
9	<i>Pi13(t)</i>	6	12456009–16303608	<i>O. minuta</i> (W), Kasalath (I), Maowangu	Philippines	[2, 91, 95]
10	<i>Pi14(t)</i>	2	1–6725831	Maowangu	Japan	[96]
11	<i>Pi15</i>	9	9641358–9685993	GA25 (J)	China	[92]
12	<i>Pi157</i>	12	8826555–18050447	Moroberekan (J)	India	[83]
13	<i>Pi16(t)</i>	2	1–6725831	Aus373 (I)	Japan	[94]
14	<i>Pi17</i>	7	22250443–24995083	DJ123 (I)	Philippines	[54, 93]
15	<i>Pi18(t)</i>	11	26796917–28376959	Suweon365 (J)	Korea	[142]
16	<i>Pi19(t)</i>	12	8826555–13417087	Aichi Asahi (J)	Japan	[41]
17	<i>Pi20</i>	12	6988220–10603823	IR24 (I)	Philippines	[51]
18	<i>pi21</i>	4	5242654–5556378	Owarihatamochi (J)	Japan	[27]
19	<i>Pi22(t)</i>	6	4897048–6023472	Suweon365 (J)	Korea	[1]
20	<i>Pi23</i>	5	10755867–19175845	Suweon365 (J)	Korea	[1]
21	<i>Pi24(t)</i>	1	5242654–5556378	Azuena (J)	France	[107]
22	<i>Pi25</i>	6	18080056–19257588	Gumei 2 (I)	China	[141]
23	<i>Pi25(t)</i>	2	34360810–37725160	IR64 (I)	France	[107]
24	<i>Pi26</i>	6	8751256–11676579	Gumei 2 (I)	China	[126]
25	<i>Pi26(t)</i>	5	2069318–2760202	Azuena (J)	France	[107]
26	<i>Pi27</i>	1	5556378–744329	Q14 (I)	France	[107]
27	<i>Pi27(t)</i>	6	6230045–6976491	IR64 (I)	France	[107]
28	<i>Pi28(t)</i>	10	19565132–22667948	IR64 (I)	France	[107]
29	<i>Pi29(t)</i>	8	9664057–16241105	IR64 (I)	France	[107]
30	<i>Pi3(t)</i>	6	–	Pai-kan-tao (J)	Philippines	[78]
31	<i>Pi30(t)</i>	11	441392–6578785	IR64 (I)	France	[107]
32	<i>Pi31(t)</i>	12	7731471–11915469	IR64 (I)	France	[107]
33	<i>Pi32(t)</i>	12	13103039–18867450	IR64 (I)	France	[107]
34	<i>Pi33</i>	8	5915858–6152906	IR64 (I)	France	[107]
35	<i>Pi34</i>	11	19423000–19490000	Chubu32 (J)	Japan	[135]
36	<i>Pi35(t)</i>	1	–	Hokkai 188 (J)	Japan	[86]
37	<i>Pi36</i>	8	2870061–2884353	Q61 (I)	China	[76]
38	<i>Pi37</i>	1	33110281–33489931	St- No 1 (J)	China	[12, 73]
39	<i>Pi38</i>	11	19137900–21979485	Tadukan (I)	India	[34]
40	<i>Pi39(t)</i>	4, 12	–	Chubu 111 (J), Q15 (I)	China	[77]
41	<i>Pi40(t)</i>	6	16274830–17531111	<i>O. australiensis</i> (W)	Philippines	[56]
42	<i>Pi41</i>	12	33110281–34005652	93-11 (I)	China	[129]
43	<i>Pi42(t)</i>	12	19565132–22667948	DHR9 (I)	India	[68]
44	<i>Pi44</i>	11	20549800–26004823	Moroberekan (J)	USA	[11]
45	<i>Pi47</i>	11	–	Xiangzi 3150 (I)	China	[48]

Table 1 continued

S.No.	Gene name	Location		Source cultivar	Country	Reference
		Chr No.	Position (bp)			
46	<i>Pi48</i>	12	–	Xiangzi 3150 (I)	China	[48]
47	<i>Pi5(t)</i>	9	–	Moroberekan (J)	Philippines	[55]
48	<i>Pi6(t)</i>	12	4053339–18867450	Apura (I)	USA	[79]
49	<i>Pi62(t)</i>	12	2426648–18050026	Yashiro-mochi (J)	Japan	[127]
50	<i>Pi67</i>	–	–	Tsuyuake	Philippines	[127]
51	<i>Pi8</i>	6	6230045–8751256	Kasalath (I)	Japan	[91, 95]
52	<i>Pi9</i>	6	10386510–10389466	<i>O. minuta</i> (W)	China	[2, 99]
53	<i>Pia</i>	11	4073024–8078510	Aichi Asahi (J)	Japan	[33, 87]
54	<i>Pib</i>	2	35107768–35112900	Tohoku IL9 (J)	Japan	[125, 130]
55	<i>Pib2</i>	11	26796917–28376959	Lemont (J)	Philippines	[119]
56	<i>PiCO39(t)</i>	11	6304007–6888870	CO39 (I)	USA	[10]
57	<i>Pid(t)1</i>	2	20143072–22595831	Digu (I)	China	[14]
58	<i>Pid2</i>	6	17159337–17163868	Digu (I)	China	[15]
59	<i>Pif</i>	11	24695583–28462103	Chugoku 31-1 (J)	Japan	[114]
60	<i>Pig(t)</i>	2	34346727–35135783	Guangchangzhan (I)	China	[139]
61	<i>PiGD1</i>	8	–	Sanhuangzhan 2 (I)	China	[74]
62	<i>PiGD-2</i>	10	–	Sanhuangzhan 2 (I)	China	[74]
63	<i>PiGD3</i>	12	–	Sanhuangzhan 2 (I)	China	[74]
64	<i>Pigm(t)</i>	6	10367751–10421545	Gumei4 (I)	China	[19]
65	<i>Pii</i>	9	2291804–28431560	Ishikari Shiroke (J), Fujisaka 5 (J)	Japan	[53, 114]
66	<i>Pii1</i>	6	2291804–28431560	Fujisaka 5 (J)	Japan	[91, 95]
67	<i>Pii2</i>	9	1022662–7222779	Ishikari Shiroke (J)	Japan	[64]
68	<i>Piis1</i>	11	2840211–19029573	Imochi Shirazu (J)	Japan	[30]
69	<i>Piis2</i>	–	–	Imochi Shirazu (J)	Japan	[30]
70	<i>Piis3</i>	–	–	Imochi Shirazu (J)	–	[30]
71	<i>Pik</i>	11	27314916–27532928	Kusabue (I)	China	[37, 136, 124]
72	<i>Pikg</i>	11	27314916–27532928	GA20 (J)	Japan	[91]
73	<i>Pikh (Pi54)</i>	11	24761902–24762922	Tetep (I)	India	[111, 113]
74	<i>Pikm</i>	11	27314916–27532928	Tsuyuake (J)	China	[60, 3]
75	<i>Pikp</i>	11	27314916–27532928	HR22 (I)	China	[39]
76	<i>Piks</i>	11	27314916–27532928	Shin 2 (J)	Japan	[23]
77	<i>Pikur1</i>	4	24611955–33558479	Kuroka (J)	Japan	[30]
78	<i>Pikur2</i>	11	2840211–18372685	Kuroka (J)	Japan	[32]
79	<i>Pilm2</i>	11	13635033–28377565	Lemont (J)	USA	[118]
80	<i>Pir2-3(t)</i>	2	–	IR64 (I)	Indonesia	[20]
81	<i>Pirf2-1(t)</i>	2	–	<i>O. rufipogon</i> (W)	Indonesia	[20]
82	<i>Pise</i>	11	5740642–16730739	Sensho (J)	Japan	[30]
83	<i>Pise2</i>	–	–	Sensho (J)	Japan	[30]
84	<i>Pise3</i>	–	–	Sensho (J)	Japan	[30]
85	<i>Pish</i>	1	33381385–35283446	Shin 2 (J)	Japan	[50]
86	<i>Pish</i>	11	33381385–35283446	Nipponbare (J)	Japan	[50]
87	<i>Pit</i>	1	2270216–3043185	Tjahaja (I), K59 (I)	Japan	[39, 38]
88	<i>Pita</i>	12	10603772–10609330	Tadukan (I), Yashiro-mochi (J)	USA	[7]
89	<i>Pita2</i>	12	10078620–13211331	Shimokita (J)	Japan	[66, 82]
90	<i>Pitp(t)</i>	1	25135400–28667306	Tetep (I)	India	[5]

Table 1 continued

S.No.	Gene name	Location		Source cultivar	Country	Reference
		Chr No.	Position (bp)			
91	<i>Pitq1</i>	6	28599181–30327854	Tequing (I)	USA	[118]
92	<i>Pitq2</i>	2	–	Teqing (I)	USA	[119]
93	<i>Pitq3</i>	3	–	Teqing (I)	USA	[119]
94	<i>Pitq4</i>	4	–	Teqing (I)	USA	[119]
95	<i>Pi-tq5</i>	2	34614264–35662091	Tequing (I)	USA	[118]
96	<i>Pitq6</i>	12	5758663–7731471	Tequing (I)	USA	[118]
97	<i>Piy1(t)</i>	2	–	Yanxian No 1 (I)	China	[71]
98	<i>Piy2(t)</i>	2	–	Yanxian No 1 (I)	China	[71]
99	<i>Piz</i>	6	10155975–10517612	Zenith (J), Fukunishiki (J), Toride 1 (J), Tadukan (I)	Japan	[31, 138]
100	<i>Pizh</i>	8	4372113–21012219	Zhai-Ya-Quing8 (I)	China	[9]

J japonica; *I indica*; – not known

Fig. 1 Cultivar-wise distribution of blast resistance genes identified in rice. Rice Cultivars which have been reported to contain more than two blast resistance genes have been compared. I and J given in parenthesis indicate that the cultivar belongs to either *indica* or *japonica* type of rice

of all the chromosomes of rice helps in utilizing available marker information for the identification and localization of the resistance genes. This strategy has been used for identification of 30 blast resistance genes like *Pit*, *Pi27(t)*, *Pish*, *Pid1(t)*, *Pig(t)*, *Piy(t)*, *Piy2(t)*, *Pi39(t)*, *Pi10*, *Pi40(t)*, *Piz*, *Pigm(t)*, *Pi33*, *Pi5(t)*, *Pi15*, *PiCO39(t)*, *Pi38*, *PBR*, *Pb1*, *Pi-k^h*, *Pi1*, *Pik-m*, *Pik*, *Pik-p*, *Pik-s*, *Pi62(t)*, *Pi157*, *Pita-2*, *Pi39(t)*, and *Pi20(t)*. Identification and cloning of blast resistance genes in India began in 2002 when rice line Tetep was found to be highly resistant for most of the strains of *M. oryzae* [109]. Since then, four blast resistance genes have been identified in India. Owing to the huge potential of Tetep in resistance breeding for the effective management of rice blast in the North-Western region of India, the *Pi-k^h* (*Pi54*) gene was mapped in the same cultivar Tetep using different types of DNA markers [111]. Earlier, blast resistance gene *Pitp(t)* has been mapped in cultivar Tetep by using simple sequence length polymorphism markers [5]. Besides, *Pi38* was identified in *indica*

rice Tadukan [34] and *Pi-42(t)* from a *indica* cultivar DHR9 by Kumar et al. [68]. Because of its effectiveness against many strains of *M. oryzae* and availability of closely linked and also gene based markers, the *Pi-k^h* (*Pi54*) gene is now being introgressed in Indian cultivars of rice using marker assisted back cross breeding [115].

The In Silico Approach

This approach uses computational methods for identification of the suitable candidate genes. Here the available sequence of two or more genomes are used for genome wide comparison [108]. Candidate genes are identified in silico by gene prediction programs like, FGENESH and RiceGAAS using rice genome sequence of the prescribed size of fragment. For the verification of the true candidate, PCR based markers are developed and used as co-

segregation markers to screen blast resistant and susceptible varieties. Shang et al. [108] identified blast resistance gene *Pid3* by genome-wide comparison of paired NBS–LRR genes and their pseudogene/alleles between the genome sequence of *indica* rice cultivar 93-11 and *japonica* line Nipponbare available in the public domain.

QTL Mapping Approach

Quantitative traits are the traits that are regulated by multiple genes in a cumulative effect for yield, drought tolerance and disease resistance. The genomic locations of the genes required for these traits are known as quantitative trait loci (QTL). Basically there are three approaches for QTL mapping such as single marker analysis (SMA), standard interval mapping (SIM) and composite interval mapping (CIM). Typical single-marker-analysis method divides the mapping population into classes based on the genotype at each marker locus, and demarcates declares a QTL if there is a significant difference in the mean phenotypic score for each of the groups. This method has been employed for the first time for the identification of QTL for rice blast resistance in cv. Moroberekan, a *japonica* rice cultivar cultivated in Africa [123]. The SIM uses the flanking molecular markers of a locus and maps the QTL

between two marker intervals. This method is more precise in locating QTL than SMA. The major problem with SIM is that linked and unlinked QTLs affect the result of the analysis and may result in identification of false QTLs. The method was used for QTL analysis and mapping of blast resistance gene *pi21* in Japanese upland rice cv. Owari-hatamochi [27]. The composite interval mapping uses the subset of markers at linked as well as unlinked QTLs. It helps in the detection of QTL interaction and also information from these markers increases the power of QTL detection. Using above mentioned three methods 350 QTLs for blast resistance have so far been identified [4]. Besides, 23 blast resistance loci such as *Pi24(t)*, *Pi35(t)*, *Pitq5*, *Pi25(t)*, *pi21*, *Pi26(t)*, *Pi27(t)*, *Pi25(t)*, *Pitq1*, *Pizh*, *Pi29(t)*, *PiGD-1(t)*, *Pi28(t)*, *PiGD-2(t)*, *Pilm2*, *Pi30(t)*, *Pi7(t)*, *Pi34*, *Pi24(t)*, *Pitq6*, *Pi31(t)*, *Pi32(t)*, *PiGD-3(t)* have also been identified within these QTL regions (Table 1).

Molecular Cloning of Blast Resistance Genes

Once genes are fine mapped with closely linked DNA markers, map based cloning approach can be effectively used for molecular cloning and characterization of blast resistance genes in rice. Although, 100 blast resistance genes have been identified and mapped both in *indica* and

Table 2 List of cloned and characterized blast resistance genes in rice

S.No.	Gene designation	Chromosome No.	Marker	Cloning strategy	Domain combination
1	<i>Pib</i>	2	Os02g57310, b213, b28, b2, b3989, RM208, S1916, G7031	MB	NBS–LRR
2	<i>Pita</i>	12	Os12g18360, SP4B9, SP9F3, ta642, ta801, ta3, ta577, <i>Pi-ta</i> 440, <i>Pi-ta</i> 1042, <i>Pi-ta</i> 403	MB	NBS–LRR
3	<i>Pi54 (Pi-kh)</i>	11	TRS26, TRS33, RM206	MB	NBS–LRR
4	<i>Pid-2</i>	6	CAPS1, CAPS 8, Os06g29810	MB	Lectin receptor
5	<i>Pi9</i>	6	Os06g17900	MB	NBS–LRR
6	<i>Pi-2</i>	6	Z4792	MB	NBS–LRR
7	<i>Piz-t</i>	6	Z4792	MB	NBS–LRR
8	<i>Pi36</i>	8	Os08g05440, CRG3	MB	CC–NBS–LRR
9	<i>Pi37</i>	1	RM543, FPSM1, RM302, RM212	MB In Silico	NBS–LRR
10	<i>Pikm</i>	11	K2167, K4731, K6441, 85H07554, k3952	MB	NBS–LRR
11	<i>Pi5</i>	9	JJ113-T3, JJ817	MB	CC–NBS–LRR
12	<i>Pit</i>	1	t311, t256, t8042	MB	CC–NBS–LRR
13	<i>Pid3</i>	6	–	In Silico homology based	NBS–LRR
14	<i>pi21</i>	4	RM16913, RM1359	MB	NBS–LRR
15	<i>Pis-h</i>	1	–	Mutant Screening	CC–NBS–LRR
16	<i>Pb1</i>	11	RM206, S723-Pb3810	MB	CC–NBS–LRR
17	<i>Pi-k</i>	11	RM5766, K33, 34, 28	MB	CC–NBS–LRR
18	<i>Pik-p</i>	11	K37-K22	MB In silico	CC–NBS–LRR
19	<i>Pia</i>	11	–	Multifaceted genomics approach	CC–NBS–LRR

– Not known; MB map based

Fig. 2 Time line in the cloning of blast resistance genes in rice. Each block represents a year. Name of the cloned genes is given on the top of each block

japonica types of rice, only 19 genes have been cloned (Table 2). Cloning of resistance genes is an important first step towards understanding R-gene structure and its function, which is of prime importance in understanding the basis of disease resistance. The first breakthrough in cloning blast resistance gene came almost 90 years after the start of blast genetics study, when *Pib* was cloned in Japan in 1999 [125]. *Pita*, another important gene for blast resistance was cloned in 2000 in USA [7]. After a gap of 5 years, *Pi-k^h* (*Pi54*) was cloned in India from an *indica* cv. Tetep [113]. Structural organization analysis of the 100 kb region around *Pi-k^h* locus in both *indica* and *japonica* rice genotypes found variation in number and distribution of motifs involved in phosphorylation which results in the resistance phenotype in Tetep [69]. The *Pi-k^h* gene from Tetep was re-designated as *Pi54* after the gene was further relocated to a slightly distant position from *Pi-kh* locus [112]. Through complementation test, the *Pi54* gene was found to confer resistance to four different isolates of the blast fungus in transgenic lines TP-*Pi54*-2 and TP-*Pi54*-15, among others. The gene was found to induce the synthesis of callose (β -1,3 glucan) in response to pathogen challenge, indicating its possible role in the initiation of a defense response cascade in the blast resistant plants [100]. The microarray and enzymatic analyses showed that *Pi54* gene activates a cascade of defense response genes in a transgenic line up to T6 generation [35]. After sequencing of the rice genome, more and more blast resistance genes are being cloned by different groups. Within a span of 12 years, 19 blast resistance genes have already been cloned (Fig. 2). A maximum of four genes namely *Pid2*, *Pi9*, *Pi2* and *Piz-t* and *Pi5*, *Pit*, *Pid3* and *pi21* were cloned, each in 2006 and 2009, respectively (Table 2).

Though R-genes like sequences are distributed throughout the rice genome, chromosome 11 has been reported to contain maximum number of resistance genes [104]. Genetic and molecular analysis has revealed that out of 100 blast resistance genes identified and mapped, nearly half of these are localized on chromosomes 11, 12 and 6 (Fig. 3). About 24% of the mapped genes are located on chromosome 11 followed by 15 and 14% of the genes mapped on chromosomes 12 and 6, respectively.

Fig. 3 Chromosome-wise distribution of blast resistance genes in rice. Chromosome number has been underlined. NI no information about the chromosome

All the R- genes cloned till date have been divided into five classes based on their predicted protein structure [36]. The largest class of R-genes encodes nucleotide binding-leucine rich repeats (NBS–LRR) protein. The function of the central NBS domain is concerned with ATP binding and/or hydrolysis, and the C-terminal LRR participates in protein–protein interactions of R- and Avr-genes [120]. The NBS–LRR proteins are sub-classified into Toll/interleukin1 receptor (TIR) and coiled-coil (CC) groups on the basis of their N-terminal sequence [132]. NBS–LRR genes are clustered in the genomes [80] and sequences of many clusters are highly homologous to one another. It is believed that the individual genes have evolved through a process of duplication of these conserved regions [80]. Out of 19 cloned and characterized proteins of blast resistance genes, ten proteins belong to the NBS–LRR type while eight proteins are of CC–NBS–LRR class. The *Pid-2* protein is a unique type of B-lectin receptor having serine threonine kinase type

Fig. 4 Different types of domain combinations present in proteins of cloned blast resistance genes. (a) CC-NBS-LRR class contain a CC domain (black hexagon) in addition to NBS (grey rectangle) and LRR (red circles), (b) NBS-LRR class, (c) Serine threonine kinase (purple rectangle is the B-lectin domain while brown one represents kinase domain)

domain. Typical protein structures of three types of these rice blast resistance genes are shown in Fig. 4.

Allele Mining for Rice Blast Resistance Genes

Plant breeding for superior agronomic traits largely depends on the amount of variation found in the plant germplasm. Like all living organisms, plants did accumulate many useful alleles for various agronomic characters. The natural mutation is a great contributor for evolution of new alleles. Mutations like transitions, transversions and InDels are the basis for this evolution. Though during the course of evolution, plant breeders have tapped many useful alleles, still there is a huge potential for gainful exploration of many other useful alleles. The process of identification of alleles of the gene responsible for a given trait and their variants in other genotypes is known as allele mining. Different approaches used for allele mining are briefly explained in the following paragraph.

Tilling (targeting induced local lesions in genomes) is a molecular biology technique that helps in direct identification of induced point mutations in the gene by heteroduplex analysis [122]. In this technique artificially induced mutations are subjected to phenotypic and genotypic analysis. The application of above approach to look for natural mutations in germplasm is called EcoTILLING [16]. Both techniques use mismatches produced by heteroduplexes of alleles of a gene. These sites are subjected to single strand nuclease treatment of end labeled heteroduplexes. The fragments produced in above treatment are separated and site of mutation can be identified by fragment size analysis. Besides, sequence and sequencing-based allele mining approach uses PCR based amplification of the alleles of a gene in different genotypes followed by sequencing of those alleles. Then the sequences are analyzed for the presence of SNPs, and InDels which are used to construct haplotypes, to understand the effect of mutations on gene structure and organization.

These above mentioned approaches were used to identify alleles of important blast resistance genes from wild rice species like *Pi-ta+* from *O. rufipogon* (Griff) [28] and from *O. rufipogon* (ETOR) [128], *Pi-rh* from *O. rhizomatis* (Das 2008), *Pi-ta* from *O. rufipogon* [47] and *Pid3* from 36 accessions of wild rice *O. rufipogon* [108]. In the latter two examples, Huang et al. [47] and Shang et al. [108] used PCR based approach to amplify and sequence alleles of 36 accessions of wild rice. Allele mining is also reported in many cultivated rice plants. Kiyosawa and his colleagues identified 14 resistance alleles at eight loci: *Pi-a*, *Pi-i*, *Pi-k* (alleles: *Pi-k*, *Pi-k^s*, *Pi-k^m*, *Pi-k^h* and *Pi-k^p*), *Pi-z* {*Pi-z* and *Pi-z'*}, *Pi-ta* {*Pi-ta* and *Pi-ta²*}, *Pi-b*, *Pi-t* and *Pi-s^h* [65], *Pi-2(t)* {*Pi-z*, *Pi-z-5*, *Pi-z'*, *Pi-9(t)*}, *Pi-4* {*Pi-4^a(t)*, *Pi-4^b(t)*, *Pi-ta*}, *Pi-5(t)* {*Pi-3(t)*, *Pi-i(t)*}, *Pi-14(t)* {*Pi-16(t)*, *Pi-d(t)*}, *Pi-k* *Pi-k^m*, *Pi-k^s* or 5, *Pi-k^k*, *Pi-k^p*, *Pi-k^h*}, *Pi-ta* {*Pi-ta²*}, *Pi-b* {*Pi-s*} [58]. Sharma et al. [110] reported allele mining for important blast resistance genes like, *Pi-ta*, *Pi-k^h* and *Pi-z(t)* in Indian land races of rice. They found that *Pi-k^h* and *Pi-z(t)* alleles are more variable than *Pi-ta* alleles. Similarly, allele mining for blast resistance gene *Pi-k^h* (*Pi54*) in seven wild species and five land races of rice has been reported [101]. Still application of these novel alleles in rice improvement programmes is yet to be exploited by the plant breeders and molecular biologists.

Introgression of Blast Resistance Genes in Commercial Cultivars

Since the beginning of agriculture, plant breeding has been considered as the most popular method for crop improvement. To develop crop plants for higher yield and other qualitative traits, it has been the method used from time immemorial. Selection of plant varieties for biotic and abiotic stresses is a major objective of plant breeding. Traditionally, breeding techniques like pure line selection, mass selection, recurrent selection and backcross selection have been followed for breeding crops for stress resistance. Bordeos et al. [2] used backcross breeding followed by embryo rescue approach to transfer bacterial blight and blast resistance genes from the tetraploid wild rice *Oryza minuta* to cultivated rice, *O. sativa* cv. IR31917-45-3-2. Some genes have also been introgressed directly from the wild rice [102]. A detailed review on the current status of rice blast resistance is also available [62].

The advent of molecular biology tools has led to the emergence of new methods like marker assisted selection (MAS) which can facilitate gene pyramiding in plants. Gene pyramiding is a strategy which uses either traditional breeding methods or modern molecular biology approaches to introgress more than one gene for specific trait into single genetic background. Deployment of single dominant gene is

a common method to breed crop plants for biotic and abiotic stress resistance. The deployment of single gene for biotic stress is generally subjected to breakdown of resistance as biotic agents always try to overcome plant defense mechanisms. Availability of various molecular markers like simple sequence repeat (SSR), restriction fragment length polymorphisms (RFLP) and single nucleotide polymorphism (SNP) etc. have fastened the procedure for gene introgression and gene pyramiding. The R-genes like *Pi1*, *Pi5*, *Piz-5* and *Pita* [43, 70, 75, 84] have been introgressed in various elite rice genotypes using MAS.

Gene pyramiding helps in the development of broad spectrum, durable resistance to rice blast. The approaches need careful characterization of the resistance spectrum of the genes to be used and combining them in an effective ‘pyramid’ against the target pathogen population [134]. Hittalmani et al. [43] used closely linked RFLPs and polymerase chain reaction (PCR)-based markers to put three blast resistance genes *Pi1*, *Piz-5* and *Pita* into a susceptible cultivar CO39. They found that the plants carrying the two- and three-gene combinations including *Piz-5* showed enhanced resistance to blast compared to the plants with *Piz-5* alone. Recently, Koide et al. [67] developed pyramided genotypes of *Pish* and *Pib* in genetic background of CO39. Singh et al. [115] developed improved Pusa 6A, Pusa 6B and PRR78, the parental genotypes of rice hybrid Pusa RH 10 by transferring *Pi54* + *Piz5* genes for blast resistance. Pyramiding of resistance genes for more than two pathogens is of great significance for plant breeding. This approach was used to pyramid three major R-genes *Pi-1*, *Piz-5* and *Xa21* into rice using MAS and genetic transformation and the two-gene pyramids showed more enhanced resistance than the parental genotypes [85]. Three genes such as *Pi-d(t)1*, *Pi-b*, and *Pi-ta²* have been stacked into a promising donor line of rice, G46B [13] and two genes *Pi1* and *Pi2* into cv. Zhenshan 97 [42]. These findings show that the pyramiding of rice blast resistance genes alone or in combination with other disease resistance genes will be of great significance in preventing huge losses being incurred by ever evolving *M. oryzae* pathogen.

Future Prospects

Pathogen-Responsive Promoters of Rice Blast Resistance Genes

Even though it is possible to reduce the crop losses to some extent through the deployment of R-genes, durable resistance in most crops still remains an elusive dream. The question therefore is, what is to be done now that can lead to a significant step towards the desired goal of minimizing the crop losses due to biotic stresses. In fact, considerable effort has been directed for the development of durable resistance

by gene pyramiding. However, pyramiding of several R genes with constitutive promoter by transgenic approach has problems associated with it such as the imposition of a fitness cost to the plant [121]. The ectopic expression of R-genes may also sometimes activate defence response pathway in the absence of pathogen, which is likely to reduce crop yield [88]. This implies that the use of a pathogen-responsive promoter instead of a constitutive promoter would be a smarter option for deployment of R-genes. The identification of pathogen-responsive promoters would also be crucial in testing the *R-Avr* two-component system for engineering resistance against rice blast disease.

Cloning and Use of Pathogen-Responsive Promoters

A number of pathogen-responsive promoters have been identified in different plant species during the past few years but only few have been well characterized. Ideally, a promoter that is desirable for use as a tool to enhance plant resistance should be exclusively induced by the pathogen in question and also in those tissues and developmental stages which are most amenable to infection by the pathogen. However, it would be difficult to identify such a promoter since plant promoters are comprised of modular elements each of which may have specific or overlapping functions. Indeed, a large number of genes whose promoters have been characterized and shown to be responsive to pathogens are also induced by other stresses [21, 45, 46, 59, 97]. However, advances in the understanding of natural pathogen-responsive promoters and their specific *cis*-elements would help us to explore the possibilities of allowing their modification to suit our interests and also in the development of synthetic promoters which may be a promising tool to achieve plant resistance [106]. Most of the earlier work on the identification of pathogen-responsive *cis*-acting elements in plants has already been reviewed by Rushton and Somssich [105].

It has been a daunting task to identify promoters that are specifically induced by pathogens alone and not by extraneous signals possibly due to the crosstalk between biotic and abiotic stress pathways. The identification of exclusively pathogen responsive promoter would indeed be a boon to researchers involved in engineering disease resistance. In rice, the *OsPR10a* promoter was found to be induced by the pathogen *Xanthomonas oryzae* pv. *oryzae* and also by salicylic acid, jasmonic acid, ethephon, abscisic acid and NaCl [49]. A W-box like element WLE1 present between –687 and –637 bp was found to be crucial for salicylic acid response. Another promoter that is responsive to the *X. oryzae* pv. *oryzae* is of the *OsWRKY13* gene which harbours two *cis*-elements PRE2 and PRE4 which are believed to negatively regulate gene expression in healthy plants. Under pathogen-challenged conditions, these two

elements positively influence gene expression most probably by binding of specific proteins [8]. So far, pathogen-responsive promoters identified in response to the blast pathogen *M. oryzae* have been few and not that well characterized. The rice *LTP1* gene was found to be upregulated 1 or 2 days following inoculation with *M. oryzae* in two rice cultivars exhibiting compatible and incompatible host-pathogen interactions. It was found that the induction was mainly restricted to the site of infection and also induced by wounding. However, although the promoter contained the TCA element that had been previously found in many genes induced by wounding or pathogen attack [29], the role of this element in the *LTP1* promoter has not been functionally validated. The rice phenylalanine ammonia lyase transcript *rPAL-5* was induced as early as 1 h post treatment with *M. oryzae* and its derived elicitor. Previously the same gene has also found to be induced at different developmental stages as well as by wounding, TMV infection and treatment with fungal elicitors [140]. The rice thaumatin-like protein (*Rtlp1*) has also been induced rapidly by *M. oryzae* apart from treatment with salicylic acid, methyl jasmonate and wounding. A 120 bp region of the promoter of rice thaumatin-like protein gene consisting of six W-boxes plays a major role in activating expression in elicitor-treated cells of rice. However, in all the blast pathogen-responsive genes discussed above, the role of individual cis-elements in gene expression has not been investigated. These promoters, however, have a broader application and could be useful in engineering resistance to chewing insects and larvae as well that also cause a lot of yield loss in rice. A promoter responsive exclusively to the rice blast pathogen would require that the motifs responsive to the pathogen and wounding be separate, which may complicate the process of promoter identification itself as the blast pathogen itself causes wounding during appressorium penetration of host tissues. The identification of early induced transcription factors such as *OsWRKY13*, antifungal proteins, phytoalexins and potent R-genes etc. have given hope for development of novel strategies to achieve immunity against the rice blast pathogen. Simultaneous identification of strong and selective promoters that lead to expression in the infected tissues instead of constitutive expression is the next step for the effective utilization of previous efforts. The discovery of such novel pathogen-responsive promoters that are activated early in the signal transduction pathway in rice would be greatly helpful in reducing yield losses.

Application of Avirulence Genes in Rice Blast Resistance

Avirulence (*Avr*) genes are the genes that encode molecules which function during normal growth and pathogenicity of

the pathogen. Most likely this activity is quite distinct from the role of the *Avr*-gene product in triggering *R*-gene mediated resistance in the host plant. *Avr* genes most probably have distinct and specific function to aid the pathogen in the process of infection but the resistant host plants have learned the way to detect them as foreign elements by employing specific *R*-genes which they have derived during the process of co-evolution and use them as a weapon against its enemy [57]. In plants, resistance to a particular pathogen is governed by incompatible interaction which follow gene-for-gene hypothesis [24]. So, the *Avr* genes specify the host range of the pathogens, by determining whether a pathogen carrying a set of *Avr* genes would be capable of producing disease on a particular host which contains complementary *R*-genes or not. Host range shift of the pathogen can also be achieved by the modification or shedding of avirulence genes and this phenomenon is quite common in the natural populations of *M. oryzae*.

Till date, 11 avirulence genes have been cloned and characterized in *M. oryzae*. Of these eight have been cloned by map based cloning approach and rest three by genome wide avirulence gene search followed by association mapping. The first cloned *Avr* gene of *M. oryzae* was *PWL* gene family consisting of four genes, viz., *PWL1*, *PWL3*, *PWL4* [61] and *PWL2* [117]. These are present as a rapidly evolving gene family of small, glycine rich hydrophilic secreted proteins. Another *Avr* gene, *Avr1-Co39* [22], has also been found to confer resistance against rice blast disease but its protein product is not well characterized. *Avr-Pi-ta* [89], the most well-studied *Avr* gene of *M. oryzae* which is avirulent on resistance gene *Pi-ta*, has been cloned by using map based cloning method and encodes a secreted metalloprotease expressed during infection and colonization of rice. The *ACE-1* avirulence gene [6] is unusual in that it encodes a polyketide synthase/non-ribosomal peptide synthetase (PKS/NRPS) fusion protein which is considered to be an enzyme, named, avirulence conferring enzyme-1 (ACE-1). It is a large protein of 4,035 amino acids and unlike the other *Avr*-proteins found in *M. oryzae* but not secreted out of the cell. Rather it produces toxic metabolite which aid in the aggressiveness of the fungus. Resistance to *M. oryzae* isolates containing active *ACE-1* gene is governed by resistance gene *Pi33*. *AvrPiz-t*, the latest one to be cloned by map based cloning approach is predicted to produce a secreted protein that triggers immunity in rice mediated by the blast resistance gene *Piz-t* [72]. With the availability of the whole genome sequence of *M. oryzae* (strain 70-15) in the public domain [18] and the advent of high throughput sequencing techniques, genome wide avirulence gene searching followed by association genetic studies have come up as an alternate approach to clone avirulence gene from *M. oryzae*.

Table 3 Important features of the *Avr* genes cloned from *M. oryzae*

Name	Protein length (aa)	Function	NCBI accession no.	Reference
<i>PWL1</i>	147	Small glycine rich hydrophillic secreted protein	U36923	[61]
<i>PWL2</i>	145		U26313	[117]
<i>PWL3</i>	137		U36995	[61]
<i>PWL4</i>	138		U36996	[61]
<i>Avr1 Co39</i>	45	Hypothetical protein	AF463528	[22]
<i>Avr Pi-ta</i>	223	Secreted metelloprotease	AF207841	[89]
<i>ACE1</i>	4034	Polyketide synthase/non-ribosomal peptide synthase fusion protein	AJ704622	[6]
<i>AvrPiz-t</i>	108	Predicted secreted protein capable to suppress programmed cell death	EU837058	[72]
<i>Avr-Pia</i>	85	Hypothetical protein	AB498873	[131]
<i>Avr-Pii</i>	70		AB498874	[131]
<i>Avr-Pik/km/kp</i>	113		AB498875	[131]

Following such strategy, three new avirulence genes, *Avr-Pia*, *Avr-Pii* and *Avr-Pik/km/kp*, have recently been discovered and cloned simultaneously [131] but their functions have not been characterized as yet. Few of the general information about the eleven avirulence genes cloned till date from *M. oryzae* or *M. grisea* is given in Table 3.

In our laboratory, an avirulence gene, *AvrPi54*, has been identified and cloned from an isolate of *M. oryzae* which is avirulent on rice genotypes containing resistance gene *Pi54*. We have used the whole genome sequence of *M. oryzae* strain 70-15 available in the public domain. A total of 474 candidate *Avr*-genes has been identified in the 37.878 Mb genome of *M. oryzae* strain 70-15. Of these 25 candidates were used for in silico protein modeling and performing protein–protein interaction with blast resistance gene *Pi54*. The study has revealed two candidate genes, *AvrPi54-1* and *AvrPi54-2* which have the potential of being the true candidate as the protein model of these two docked on the protein model of *Pi54* protein in silico annotation studies also showed that these two *Avr* genes hold the potential of being the perfect counterparts of blast resistance gene *Pi54*.

R–*Avr* Two Component System: Designing Broad Spectrum Blast Resistance

Evidently, a specific *R* gene gives protection against one pathogenic strain containing its cognate *Avr* gene. It seems to be a good ploy to stack number of *R* genes in a plant genome which will provide protection against a number of pathogen. This is, in fact, the strategy in practice which is commonly known as gene pyramiding. In a gene pyramided plant every *R* gene product will recognize its

counterpart *Avr* gene product individually and lead to a common signaling pathway of hypersensitive response (HR) that will ultimately lead to a broad spectrum disease response.

However, as discussed earlier, simply pyramiding of several *R* genes has some problems associated with it. As, introducing many *R* genes in a single plant may sometime have some negative effects on the plants, it may be better to deal with a single *R*-gene rather than stacking number of *R*-genes within a single plant genome. Standing up to this challenge De Wit gave the concept of *R*–*Avr* two component systems [17]. According to this concept *R*-gene and its counterpart *Avr* gene can be cloned in one gene construct which is introduced in the plant through transformation. The *R*-gene is driven by a constitutive promoter, so, *R*-gene product is always available inside the cell; whereas *Avr* gene is driven by a pathogen inducible promoter, so that it is expressed only when the pathogen causes infection in the host. Hence, in the absence of pathogen there is no *Avr* protein available inside the plant cell and consequently there will not be any HR. But when the pathogen attacks, *Avr* proteins begin to express and starts to interact with their counterpart *R*-protein already present in the cell and ultimately lead to HR mediated resistance response.

Some success has been achieved by using this system in tomato against the fungal pathogen *Cladosporium fulvum*. Resistant transgenic tomato has been produced by using resistance gene *Cf9* and avirulence gene *Avr9* in combination following the two component scheme discussed above [44]. The transgenic tomato plants are resistant to several bacterial pathogens besides *Cladosporium fulvum*. Such a two component system against rice blast disease can be designed where both blast resistance gene and its complementary *Avr* gene may have been cloned. For instance,

Fig. 5 Response of transformed rice cell containing *R-Avr* two component system in the absence and presence of *M. oryzae* infection. **a** The *Pi54* gene is driven by a constitutive promoter CaMV35S (P_{Con}), so its protein product, Pi54, is always present in the cell. But in the absence of the pathogen infection, the pathogen inducible promoter (P_{PI}) of *Avr* gene is not activated and hence *AvrPi54* gene product is not produced. As a consequence, there is no

hypersensitive response (HR) and cell remains alive (green cell with intact cell membrane). **b** In contrast, during pathogen infection the pathogen inducible promoter (P_{PI}) of *AvrPi54* gene gets activated and its protein product is produced inside the cell. This leads to interaction between Pi54 and AvrPi54 proteins followed by HR and as a consequence the infected cell dies (black cell with degenerated cell membrane) stopping the further spread of the pathogen

we have cloned blast resistance gene *Pi54* from rice cultivar 'Tetep'. It shows broad spectrum resistance against several isolates prevalent in India [100]. We are also in the process of cloning of its counterpart avirulence gene, *AvrPi54*, from *M. oryzae* isolate RML-29 which is avirulent on *Pi54* containing genotypes and the pathogen inducible promoter from rice plant itself which is responsive to a broad range of *M. oryzae* isolates and carry out early induction of downstream genes (unpublished findings). Once the *AvrPi54* gene and pathogen responsive promoter is cloned and characterized, we will be using them along with *Pi54* gene itself and constitutive promoter CaMV35S to constitute *R-Avr* two component system capable of imparting broad spectrum resistance against *M. oryzae*. The construct will be designed by hooking the *Pi54* gene downstream to CaMV35S promoter while the *AvrPi54* gene downstream to pathogen responsive promoter and will be used to transform susceptible rice line (Fig. 5). So, in absence of the pathogen *Pi54* gene will be expressed but not the *AvrPi54* and hence, there will be no cell death (Fig. 5a). But during the infection of *M. oryzae* the pathogen responsive promoter will get activated and it will drive the expression of *AvrPi54* gene. Under this circumstance, where both Pi54 and AvrPi54 proteins are present inside the cell, their interaction will lead to hypersensitive cell death, stopping further spread of the pathogen (Fig. 5b). This strategy would provide broad-spectrum resistance to different isolates of *M. oryzae*.

Acknowledgments T R Sharma is thankful to the NAIP-ICAR for financial assistance for cloning and characterization of avirulence gene and pathogen responsive promoters. He is also thankful to the

DBT for providing funding for the cloning of *Pi54* (*Pi-kh*) blast resistance gene from rice.

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