Identification of blast (*Magnaporthe grisea*) resistance genes in rice

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The blast, *Magnaporthe grisea* (Hebert) is one of the major diseases of rice worldwide, causing heavy yield losses ranging from 35 to 50%. The breakdown of resistance is the prime concern to search for new genes to develop durable resistance against this disease in rice. The present study was undertaken to know the mode of inheritance and allelic relationship of genes for blast resistance against the Directorate of Rice Research (DRR) isolate. The donors with unknown genes, i.e. Carreon and CNM4140 were crossed with the donors of known genes, i.e. Dular, Tetep, Zenith and Tadukan and with susceptible check, CO 39. Crosses were also made among the donors with known genes to confirm the allelic relationship. The inheritance pattern of resistance genes in donors when crossed with CO 39 indicated the presence of monogenic dominant gene. CNM 4140 when crossed with Dular, Tetep, Zenith and Tadukan segregated in 15 : 1 (resistant : susceptible) in F_2 generation, indicating the involvement of different genes governing resistance against the DRR isolate. The allelic test revealed that Carreon, Dular and Tetep possessed the same gene (*Pi-k*), while Zenith, CNM 4140 and Tadukan have different genes.

BLAST disease caused by *Magnaporthe grisea* is the most serious fungal disease of rice causing heavy yield losses worldwide particularly in temperate, flooded and tropical upland ecosystems[1]. Surveys[2,3] confirmed that blast remains among the most serious constraints to yield in South Asia. Host plant resistance is the most promising method of blast disease control[4].

Genetic analysis of blast resistance studied by several workers indicated either monogenic dominant[5–7], monogenic recessive[8], two dominant independent genes[9], two dominant complementary genes[10] and two recessive duplicate genes[11] or parental resistance controlled by minor genes[12–15]. The identification of blast resistance genes indicated 30 different loci in rice[16–18]. Among these, 20 are major genes and 10 are putative quantitative trait loci. Twelve major genes have been confirmed to be non-allelic and are officially registered with the rice genetics cooperative[16,19].

Host-specificity as well as genetic instability of rice blast fungus has made breeding for blast resistance difficult. Padmanabhan *et al.*[20] identified 31 races using international blast differentials, viz. Raminad Strain no 3, Zenith, NP 125, Usen, Dular, Kanto 51, CI 8970 and Calaro, whereas Veeraraghavan and Padmanabhan[7] emphasized the prevalence of a single race IC 17 in India.

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Dynamic changes in race composition of the pathogen have often resulted in short-lived efficiency of host resistance in the improved varieties. Thus breeding for durable resistance to blast needs to be directed towards the employment of multiple genes. Therefore, a need to identify new gene sources is imperative for gene pyramiding using various donors having divergent genes. Hence the present investigation was undertaken to study genetic relationship of different donors of known genes (Dular, Tetep, Zenith and Tadukan) and unknown genes (Carreon and CNM 4140) against a virulent Directorate of Rice Research (DRR) isolate.

The resistant donors, viz. Carreon, Zenith (*Pi-Z* + *Pi-a* + *Pi-i*), CNM 4140, Dular (*Pi-k*^{a}), Tetep (*Pi-k*^{h}), Tadukan (*Pi-ta*) and CO 39 (susceptible) were used to study the allelic relationship of resistance genes to blast against DRR isolate available at DRR, Hyderabad. All the donors were crossed with CO 39 and also among themselves in all possible combinations. The parents, F_1 and F_2s were screened under artificial inoculation.

Parents and F_1s were sown in a single row, 50 cm long and 10 cm apart, and F_2s were sown in 15 lines each. After each parent, F_1, F_2, HR 12 (susceptible) and IR 64 (resistant check) were grown. The entire nursery was surrounded on all sides by two rows of HR 12 to function as a spreader source for the pathogen. Fertilizer dose of 100 kg each of N and P and 30 kg K per hectare and 100 kg FYM/100 m^2 was applied in the nursery bed. Inoculation was carried out by placing pieces of infected leaves over the test material as well as by transplanting infected plants between test rows after 25 days of sowing. The humidity (95%) in the nursery bed was maintained with the use of sprinklers. The observation on disease reaction was recorded when the susceptible check was severely infected by blast. Individual plants in each parent, F_1 and F_2 populations were scored based on leaf blast severity following standard evaluation system (SES, IRRI, 1996) on 0–9 scale. Similarly, the scoring was repeated 10–15 days after the first observation to avoid the escapes.

Table 1. Seedling reaction to blast (DRR isolate) in parents and F_1s

Parent	Gene(s)	Total plants screened	Resistant	Susceptible
CO 39	Susceptible	30	0	30
Carreon	–	30	30	0
Zenith	*Pi-z* + *Pi-a* + *Pi-i*	30	30	0
CNM 4140	–	30	30	0
Dular	*Pi-k*^{a}	30	30	0
Tetep	*Pi-k*^{h}	30	30	0
Tadukan	*Pi-ta*	30	30	0
Cross (F_1)				
Co 39 × Carreon		10	10	0
Co 39 × Zenith		15	15	0
Co 39 × CNM 4140		12	12	0
Co 39 × Dular		18	18	0
Co 39 × Tetep		20	20	0
Co 39 × Tadukan		19	19	0

Table 2. F_2 segregation pattern for blast resistance in crosses between susceptible parent and donors

Cross	T	Observed		Expected ratio	Expected		χ^2
		R	S		R	S	
Co 39 × Carreon	131	101	30	3 : 1	98.2	32.8	0.32
Co 39 × Zenith	143	110	33	3 : 1	107.3	35.8	0.29
Co 39 × CNM 4140	17	13	4	3 : 1	12.8	4.3	0.21
Co 39 × Dular	145	111	34	3 : 1	108.8	36.3	0.19
Co 39 × Tetep	96	68	28	3 : 1	72.0	24.0	0.88
Co 39 × Tadukan	66	51	15	3 : 1	49.5	16.5	0.18

T, Total; R, Resistant; S, Susceptible.

Table 3. F_2 segregation for blast resistance in crosses among resistant donors

Cross	Total	Observed		Expected ratio	Expected		χ^2
		R	S		R	S	
Carreon × Zenith	490	461	29	15 : 1	459.37	30.62	0.09
Carreon × CNM 4140	450	422	28	15:1	421.87	28.12	0.00
Carreon × Dular	470	469	1	–	–	–	–
Carreon × Tetep	500	500	0	–	–	–	–
Carreon × Tadukan	505	473	32	15 : 1	473.4	31.56	0.01
Zenith × CNM 4140	502	471	31	15 : 1	470.62	31.37	0.01
Zenith × Tetep	498	467	31	15 : 1	466.87	31.12	0.00
Zenith × Tadukan	459	435	24	15 : 1	430.31	28.68	0.81
CNM 4140 × Tetep	496	460	36	15 : 1	465.0	31.0	0.85
CNM 4140 × Tadukan	466	435	31	15 : 1	436.87	29.12	0.13
Dular × Tetep	490	490	0	–	–	–	–
Dular × Tadukan	480	448	32	15 : 1	450	30	0.14
Tetep × Tadukan	460	430	30	15 : 1	431.25	28.75	0.06

R, Resistant; S, Susceptible.

The seedlings were scored as resistant (≤3 score) and susceptible (>3 score). The maximum scores of each plant from two observations were considered for categorizing resistance and susceptible reaction. χ^2 test was employed to test goodness-of-fit of observed and expected frequency in segregating generations.

All the six donors, viz. Carreon, Zenith, CNM 4140, Dular, Tetep and Tadukan showed resistant reaction and CO 39 was the susceptible check (Table 1). All F_1 plants derived from crosses of CO 39 and the donors were resistant (Table 1), indicating dominant gene conferring blast resistance. The F_2 population of all the crosses involving CO 39 and donors segregated in a ratio of 3 resistant : 1 susceptible (Table 2). These results showed that resistance in the donors against DRR isolate of *M. grisea* is governed by a single dominant gene, though the plant population in the F_2 generation of Co39 × CNM 4140 was less, but the pattern was similar to that in other crosses. The dominant gene controlling resistance to blast was also reported[5,7,21]. Among the six donors studied for allelic test, Zenith has *Pi-z* + *Pi-a* + P_{ii}, Dular *Pi-k*^{a}, Tetep, *Pi-k*^{h} and Tadukan *Pi-ta* genes controlling blast resistance[17,18], whereas the gene involved in Carreon and CNM 4140 was not known.

The reaction to DRR isolate in F_2 population derived from the crosses among donors, i.e. Carreon, Zenith, CNM 4140, Dular, Tetep and Tadukan was studied to determine allelic relationship of resistant genes (Table 3). F_2 populations from the crosses Carreon × Dular, Carreon × Tetep and Dular × Tetep did not segregate for susceptibility, indicating that the single dominant genes (*Pi-k*) present in Carreon, Dular and Tetep are allelic with each other, while Carreon, Tetep and Dular when crossed with Zenith, CNM 4140 and Tadukan segregated in 15R : 1S, ratio indicating that the gene present in Tetep, Dular and Carreon is different from that of Zenith, CNM 4140 and Tadukan.

Similarly, the segregation pattern of resistant and susceptible plants in F_2 generations of CNM 4140 × Carreon, CNM 4140 × Zenith, CNM 4140 × Tetep, CNM 4140 × Tadukan and Zenith × Tadukan was observed in 15 resistant and 1 susceptible (15 : 1) ratio, indicating two independent dominant genes are involved in expression of resistance and both are non-allelic (Table 3). Therefore, it is suggested that CNM 4140 has a different gene for blast resistance, which was also evident from the χ^2 test of goodness-of-fit, where the χ^2 values did not deviate significantly (Table 3). Crosses Zenith × Tetep, Zenith × Tadukan, Tetep × Tadukan, Dular × Tadukan and Carreon × Tadukan segregated in 15 : 1 ratio for resistance and susceptible plants, indicating two duplicate dominant genes. Similar results for the presence of two duplicate dominant genes were also reported by Padmanabhan *et al.*[22] in different sets of donors. Similarly, the monogenic dominant gene for blast resistance in several other donors was also reported[5,6,17,22–25]. The segregation ratio of the known genes in Zenith, Dular and Tadukan for resistance confirms the earlier gene nomenclature of these donors.

Table 4. Reaction of donors to blast disease at different test locations during 2001

Donor	ALM	BNK	CRRI	HZB	JDP	KHU	MGD	MLN	MND	NWG	PNP	Mean
Zenith	7	5	4	4	2	5	5	8	0	8	9	5.3
Dular	9	7	5	7	4	4	3	9	0	9	6	5.9
Tetep	0	9	5	–	–	4	8	8	6	0	9	4.5
Tadukan	2	9	–	2	2	5	1	9	3	5	5	3.9
IR64 (resistant check)	3	9	–	5	4	5	4	9	6	3	9	5.7
Co39 (susceptible check)	8	4	5	–	9	6	8	3	8	8	9	5.0
HR12 (susceptible check)	9	8	–	8	8	5	9	8	6	9	9	5.5
IR50 (susceptible check)	6	5	5	5	8	4	5	9	5	7	9	7.2

Adapted from DRR Annual Progress Report, 2001, vol. 2.
ALM, Almora; BNK, Bankura; CRRI, Central Rice Research Institute, Cuttack; HZB, Hazaribagh; JDP, Jagadalpur; KHU, Khudwani; MGD, Mugad; MLN, Malan; MND, Mandya; NWG, Nawagam; PNP, Ponnampet.

The donors (Dular, Tetep, Zenith and Tadukan) with known genes for blast resistance showed uniformly high level of resistance against the DRR isolate, but when screened at 11 hotspot locations[26] with divergent virulent races showed different reaction (Table 4). For example, Dular and Tetep have the same known gene (*Pi-k*) which showed similar level of resistance against the DRR isolate and the races present at CRRI (Cuttack) and Khudwani, and similar susceptible reaction against races of Malan, Bankura, and Ponnampet, while variable reaction at Almora, Mandya, Hazaribagh and Nawagam. Dular showed resistant reaction against Mandya and Mugad race, while Tetep was found to be susceptible at those locations. At Almora and Nawagam, Tetep was resistant but Dular was found susceptible. These observations suggested involvement of additional genes along with *Pi-k* in Dular and Tetep expressing divergent reactions at different locations. This was also true for other donors. Among all the donors, Tadukan showed more stable reaction against the races under test locations, except at Malan, Nawagam, Ponnampet and Bankura. In order to develop durable and broad spectrum resistance in high-yielding cultivars, information on the number of genes involved in the donors and their effectiveness against variable virulent races at different geographic areas is essential. Use of different donors exhibiting variable reaction against different virulent races is suggested for developing durable resistance.

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