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Introgression of broad-spectrum blast resistance gene(s) into cultivated rice (*Oryza sativa* ssp *indica*) from wild rice *O. rufipogon*

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Rice blast disease caused by *Magnaporthe grisea* (Hebert) Barr, is one of the major diseases of the crop. Host specificity as well as genetic instability of rice blast fungus is the major cause for the breakdown of resistance in many varieties over a period of time. Here we report the introgression of broad-spectrum blast resistance gene(s) from *Oryza rufipogon* into a cultivated rice variety. An accession (Coll-4) of *O. rufipogon*, highly resistant to blast, was crossed with a susceptible line B 32-Sel-4 and the F_1 was top-crossed with another susceptible line B 29-6. The F_2 population of the three-way cross was screened for blast and salinity tolerance. Twenty selected plants, each with blast resistance and salinity tolerance, were intermated. The process of intermating was repeated in the F_2 progenies derived from the first intermated populations. After two cycles of intermating, pedigree selections in the resulting segregating generations were followed under saline conditions. The 42 introgression lines (F_5 generation) were screened for blast reaction under artificial inoculation. Eight lines were observed to be immune, 20 resistant and six lines moderately resistant to an isolate of blast isolated in Andamans. The remaining eight lines, the two *indica* rice parents and check CO39 were susceptible. Two promising lines with higher yield potential were evaluated in multilocation trials (AICRIP) during 1999, 2000 and 2001 at 17 hotspot locations for blast infection. The culture B 90-15 (IET 15420) showed resistance reaction against 14 isolates and moderate resistance against another two isolates of blast indicating the introgression of a broad spectrum of resistance to blast from *O. rufipogon*.

Keywords: Blast resistance gene(s), introgression, *Oryza rufipogon*, *O. sativa*.

BLAST disease of rice caused by the fungus *Magnaporthe grisea* (Hebert) Barr, is one of the most destructive diseases of the crop causing severe yield loss. The fungus can attack the rice plant at any growth stage and can cause severe leaf necrosis and impede grain filling, resulting in decreased grain number and weight. When the last node is attacked, it causes partial to complete sterility. Host plant resistance is the most promising method to minimize yield loss due to blast disease[1]. Several major genes with complete resistance to a specific subset of isolate have been deployed in the development of varieties. The resistance in several varieties having major genes for blast often breaks down due to the evolution of virulent races of pathogen after few years of the release of resistant varieties[2].

Identification of blast resistance genes indicated 40 different loci in rice[3–5], including the *Pi9* gene introgressed from *Oryza minuta*. A number of these genes have been mapped on the molecular linkage map of rice[5–9]. In addition to genes that confer complete resistance through hypersensitive reaction, genes for partial resistance to blast have also been characterized[10]. This quantitative resistance is also referred to as field resistance[11], slow blasting[12] and dilatory resistance[13]. Partial resistance is characterized by lesions that are typically spindle-shaped but may be fewer in number, reduced in size, slower to develop, or shorter lived. The net effect is a reduced inoculum potential and a

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lower probability of blast epidemic. Compared to complete resistance, partial resistance is more difficult to use by breeders because it is quantitatively inherited and sensitive to environmental factors such as temperature, leaf wetness duration, high N-fertilization, soil type and water stress[14,15].

The development of high yielding varieties with durable/broad-spectrum resistance to major diseases under varying environmental conditions is one of the major objectives of plant breeders to ensure sustainable crop production. The wild relatives of *Oryza* are an important reservoir for several agronomically useful genes such as biotic, abiotic stress resistance and yield trait[16,17]. Some of the major genes showing broad-spectrum resistance to bacterial blight (*Xa21*, *Xa23*), blast (*Pi9*), rice tungro virus, brown planthopper (*Bph10*, *bph12*, *Bph13*, *Bph14*, *Bph15*), and grassy stunt virus have been introgressed from different wild species of *Oryza*[16]. The objective of this study was to introgress gene(s) for resistance to blast from wild rice (*O. rufipogon*) into elite breeding lines of *O. sativa* (*indica*).

One of the accessions of *O. rufipogon* (Coll-4) collected from the brackish-water submerged areas of Andaman and Nicobar group of islands, and which showed immune reaction to blast and tolerance to salinity, was crossed with B32-Sel-4, a medium-duration (130 days), semi-dwarf, high-yielding line, susceptible to blast and salinity. The F_1 of B32-Sel-4/*O. rufipogon* was top-crossed with another high-yielding, semi-dwarf susceptible line B29-6. Around 100 seeds each from a total of 107 F_1 plants of the cross B32-Sel 4/*O. rufipogon*//B29-6 were subjected to blast screening at seedling stage and the resistant plants were planted in field. The remaining seeds were sown in a nursery bed and evaluated under saline conditions (ECe 10.0 dS/m) along with parents and checks following recommended agronomic practices.

In the F_2 generation, 20 individual segregants resistant to blast and 20 segregants tolerant to salinity were crossed. The resulting 20 F_1s were grown in normal soil. Plants with weedy traits were rejected. Eighty-six F_1 plants were selfed to get F_2 seeds. Again, half of the F_2 seeds of each selected F_1 plant was subjected to blast screening in nursery stage and the remaining seeds were used for salinity screening. One hundred and sixty plants from each F_1 plant were planted along with two parents (B32-Sel-4 and B29-6) at a spacing of 20 × 15 cm (row to plant) in saline soils (ECe 8–10 dS/m) to screen for salt tolerance. Similarly, a second cycle of intermating was followed. Plants of 20 crosses were grown in normal soil conditions. One hundred and seventy-one plants with desirable agronomic traits were selected and the F_2 seeds of individual plants were harvested. Single plant pedigree method of selection was followed from the F_2 generation of the second cycle of intermating in saline conditions considering plant height, number of effective tillers/plant, panicle weight, fertility, grain type and salinity tolerance. In the F_5 generation, 42 homozygous lines were selected with superior agronomic traits.

Preliminary screening of introgression lines was done against blast isolates of Andamans. Two most promising genotypes (B90-15 and B90-15-4R) with superior yield, salinity tolerance and blast resistance were screened in the National Screening Nursery under All India Coordinated Rice Improvement Programme (AICRIP)[18] during 1999–2001 at hotspot locations covering all rice growing ecosystems such as rainfed upland (Ponnampet, Rewa, Jagadalpur, Wangbal, Hazaribagh), irrigated (Hyderabad, Nellore, Mandya, Pattambi, Ambasamudram, Coimbatore, Chiplima), rainfed lowland (Ghaghraghat, Gudalur, Lonavala) and irrigated hills (Malan).

Forty-two introgression lines in the F_5 generation along with their parents (B32-Sel-4, B29-6 and *O. rufipogon*) and checks (IR 64 and CO 39) were screened against a blast isolate of Andamans. In the National Screening Nursery, B90-15 was screened along with 359 and 356 test entries in 1999 and 2000 respectively. In 2001, 329 test entries in the National Screening Nursery and ten near isogenic lines (NILs) of blast resistance genes (*Pia*, *Pi1*, *Pi2*, *Pi3*, *Pi4a*, *Pi4b*, *Pi5*, *Pi7*, *Pi9*, *Pi12*), four pyramided lines with 2–3 gene combinations (*Pi1* + *Pi2*, *Pi1* + *Pi4*, *Pi2* + *Pi4*, *Pi1* + *Pi2* + *Pi4*), eight international differentials (Raminad Str – 3, Zenith, NP 125, Usen, Dular, Kanto 51, Shia – tia-tsao and Calaro), five resistant checks (Tadukan, IR 64, C102PKT, Tetep, Rasi) and two susceptible checks (IR 50, HR 12) in the trial monitoring of virulence of *M. grisea* were screened with B90-15.

At the Andamans all the test entries were sown in nursery bed, one line each of 50 cm length at the distance of 10 cm. The nursery bed was surrounded with two lines of CO 39 (susceptible check). The parents along with resistant and susceptible checks were sown after each set of ten test entries. A fertilizer dose of NPK at the rate of 120 : 60 : 30 kg/ha was applied in the nursery bed. The experiment was replicated twice in blast favourable weather conditions with 90–95% relative humidity. To ensure severe blast infection, additional inoculum was sprayed with suspension of 10^5 spores/ml. Also, the additional diseased leaves were collected, chopped into pieces of 3–6 cm length and scattered over the test entries after two weeks of sowing. The same screening method was adapted for the National Screening Nursery and the trial conducted for monitoring of virulence of *M. grisea*, except replacing the susceptible check HR 12.

Observations on disease reaction were recorded twice; first, when the susceptible check was severely infected with blast and later at 10–15 days after the first scoring to avoid the escapes. The disease reaction was scored in a 0–9 scale following Standard Evaluation System[19]. A score of 0–3 was considered as resistant, 4–5 as moderately resistant, 6 as moderately susceptible and 7–9 as susceptible.

Screening results of the parents B32-Sel-4, B29-6, *O. rufipogon* (Coll-4) and checks against the blast isolates present in the Andamans and Directorate of Rice Re-

Table 1. Reaction of B90-15 (IET 15420) and resistant check to blast at different hotspot locations in India

	Disease score in 0–9 scale and disease reaction									
	B90-15 (IET 15420)					IR 64 (resistant check)				
Location	1999	2000	2001	Mean	Overall reaction	1999	2000	2001	Mean	Overall reaction
South India										
DRR, Hyderabad	0	–	–	0	R	2	–	2	2.0	R
Nellore	0	1	–	0.5	R	3	1	–	2.0	R
Ambasamuddram	0	–	–	0	R	0	–	–	0	R
Mandya	–	3	2	2.5	R	–	0	4	2.0	R
Ponnampet	0	–	0	0	R	6	9	9	8.0	S
Coimbatore	–	–	2	2.0	R	–	–	2	2.0	R
Gudalur	–	3	–	3.0	R	–	4	–	4.0	MR
Pattambi	1	–	3	2.0	R	4	–	5	4.5	MR
East and Central India										
Chiplima	0	0	0	0	R	0	3	1	1.6	R
Rewa	6	6	6	6	MS	4	5	3	4.0	MR
Jagadalpur	3	2	2	2.3	R	3	4	6	4.3	MR
Wangbal	3	–	3	3.0	R	5	–	4	4.5	MR
Hazaribagh	0	1	1	0.6	R	2	4	–	3.0	R
Ghagharghat	4	4	3	3.6	MR	5	2	3	3.3	MR
North and West India										
Lonavala	4	5	5	4.7	MR	2	6	6	4.6	MR
Malan	–	2	2	2.0	R	–	4	4	4.0	MR

R, Resistant; MR, Moderately resistant, S, Susceptible; –, Not screened.
HR12 (susceptible check) showed susceptible reaction across the years and locations.

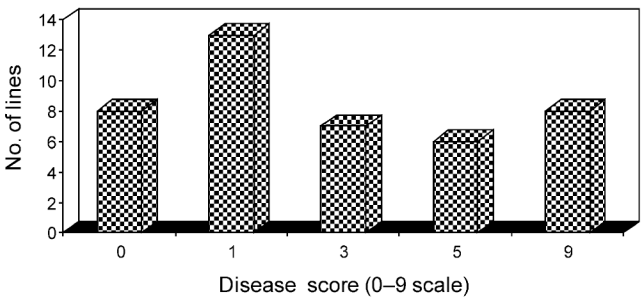

Figure 1. Reaction of B32-Sel-4/*O. rufipogon*//B29-6-derived lines (F_5 generation) against Andaman isolate of *Magnaporthe grisea*.

search (DRR), Hyderabad, indicated that *O. rufipogon* and IR 64 were immune (score 0) and resistant (score 3), respectively to blast while the *indica* parents (B32-Sel-4 and B29-6) and CO 39 were susceptible. Among the 42 introgression lines screened, 28 showed resistance (score 0–3), six moderate resistance (score 4–5) and eight lines showed susceptible (score 7 and 9) reaction. Among the 28 resistant introgression lines, eight lines were immune (score 0), 13 lines had score-1 and 7 lines along with IR 64 had score-3 (Figure 1). The variability for resistance reaction in the introgression lines suggests that the wild rice may have more than one gene conferring resistance and moderate resistance to blast.

The results of blast reaction on introgression line B90-15 and checks at 16 hotspot locations in upland, irrigated, rainfed lowland and hilly regions in the National Screening Nursery during 1999, 2000 and 2001 are presented in Table 1. In 1999 at Mandya and in 2000 at Ponnampet and Hyderabad disease pressure was low. Hence data were not considered for overall reaction. During three years of screening, the check HR 12 showed susceptible reaction at all the locations. The resistant check IR 64 showed susceptible reaction at Ponnampet and moderate resistance reaction at Pattambi, Rewa, Jagadalpur, Wangbal, Lonavala and Gudalur. The introgression line B 90-15 (IET 15420) showed resistance reaction consistently for 3 years against all the isolates of *M. grisea* in upland, rainfed lowland, hills, and irrigated ecosystems, except at Lonovala and Ghagharghat, where it recorded moderate resistance. Only at Rewa, B 90-15 showed moderately susceptible reaction. It showed immune reaction against the isolates of Andamans, Hyderabad, Ponnampet, Ambasamudram, Chiplima and highly resistant reaction to the isolates of Nellore, Hazaribagh and Malan (Table 1). Hence, the resistant gene(s) introgressed from *O. rufipogon* in B90-15 showed broad-spectrum resistance against 16 isolates of blast present in India. The other introgression line B90-15-4-R showed resistance reaction at six locations against blast during 1999 and was inferior in yield compared to the checks under saline conditions, hence it was discontinued from the trial.

Results from the National Screening Nursery and experiment on field monitoring of virulence of *M. grisea* conducted at Hazaribagh, Jagadalpur, Malan, Ponnampet and Mandya in 2001, indicated that culture B90-15 was the only entry with high level of resistance at all the loca-

Table 2. Reaction of rice genotypes to *M. grisea* at locations with high disease pressure during Kharif 2001

Genotype	Gene	Disease score in 0–9 scale at the location				
		Hazaribagh	Jagadalpur	Malan	Ponnampet	Mandya
B90-15 (IET15420)	–	1	2	2	0	2
CO 39	*Pia*	–	9	3	9	8
C 101LAC	*Pi1*	4	6	4	0	8
C 101 A51	*Pi2*	4	5	9	5	8
C 104 PKT	*Pi3*	8	7	6	9	8
C 101PKT	*Pi4a*	4	6	9	9	0
C 105 TTP	*Pi4b*	4	2	7	0	7
RIL-45	*Pi5*	4	5	6	9	7
RIL-29	*Pi7*	4	4	8	9	7
RIL-10	*Pi12*	8	8	6	9	7
O. minuta-derived line	*Pi9*	4	6	5	7	7
BL122	*Pi1* + *Pi2*	3	4	4	9	7
BL142	*Pi1* + *Pi4*	6	5	6	5	8
BL245	*Pi2* + *Pi4*	4	2	3	0	8
A57	*Pi1* + *Pi2* + *Pi4*	2	1	3	0	8
International differentials						
Raminad Str-3	–	4	2	7	9	0
Zenith	*Piz* + *Pia* + *Pii*	4	2	8	9	0
NP 125	–	8	3	5	9	7
Usen	Pia^{+}	8	3	9	9	6
Dular	Pik^{a+}	7	4	9	6	0
Kanto 51	*Pik*	8	5	7	9	0
Shia-tia-tsao	Pik^{s}	4	2	9	9	6
Calaro	Pik^{s}	6	4	2	9	5
Donors						
Tadukan	*Pita*	2	2	9	5	3
C 102 PKT	*Pi4a*	9	9	–	9	–
Tetep	Pik^{h+}	–	–	8	9	6
Resistant checks						
IR 64		5	6	4	9	4
Rasi		4	1	9	9	6
Susceptible checks						
IR 50		5	–	9	9	5
HR 12		7	9	9	9	9

–, Not tested.
Score 0–3 Resistance, 4–5 moderate resistance, 6 moderate susceptible and 7–9 susceptible.
Source: DRR Progress Report 2001, vol. 2.

tions (Table 2). The NILs, the *Pi9* gene derived from *O. minuta*, international differentials, donors and resistant cultivars showed susceptible reaction at 2–3 locations. The pyramided lines BL 245 (*Pi2* + *Pi4*) and A57 (*Pi1* + *Pi2* + *Pi4*) showed resistance/moderate resistance at four locations, but were susceptible at Mandya. BL 122 (*Pi1* + *Pi2*) was resistant at Hazaribagh and moderately resistant at Jagadalpur and Malan and BL 142 (*Pi1* + *Pi4*) showed only moderate resistance at four locations. At Ponnampet five entries (B 90-15, C 101 LAC, C 105 TTD, BL 245 and A 57) showed immune reaction, while at Mandya B 90-15, C 101PKT, Reminad Str-3, Zenith, Dular, Kanto 51 were found resistant and Calaro and IR 50 were moderately resistant.

Rice blast disease is the most serious fungal disease of the crop causing severe yield loss worldwide, particularly in temperate flooded and tropical upland ecosystems[20]. There have been significant achievements in the development of blast-resistant varieties, particularly using vertical resistance genes. Due to wide variability in virulence among the isolates of the blast pathogen and the limited exposure of breeding materials to the full range of virulent pathogen, most of the resistant rice varieties succumb to the disease within 2–3 years of their introduction in the disease-prone areas[1]. Variability in the blast pathogen population from different ecosystems and locations has been documented by several workers using differential host reaction and DNA fingerprinting[21,22]. In India, the physiological races of blast have already been identified[22–25].

Nevertheless, durable host resistance alone can adequately protect the rice crop in the tropics. Rice cultivars with durable resistance have been reported from several countries: cv Moroberekan and OS 6 in West Africa,

IR36, IR64 in Asia, CICA 7 in South America[26] and Lemont in the US[27]. Exploitation of durable resistance has been proposed for less blast-conducive environment[1,28].

Durable and broad-spectrum resistance to blast has been associated in some cases with multiple genes with additive effect and in some cases with major genes along with QTLs. Moroberekan, a West African *japonica* variety showed durable resistance across locations, due to the combined effect of more than two major genes along with several QTLs conferring resistance to blast[5]. Similarly, IR 36 and IR 64 have the *Pita* gene and IR64 has another closely linked gene *Pi20* in addition to several minor genes with complex lineage, which help them possess broad-spectrum resistance to blast[29,30]. The pyramided line A 57 carrying three major resistance genes (*Pi1* + *Pi2* + *Pi4*) was reported to show broad spectrum of resistance in multi-environment tests[31]. The introgression line B90-15 was effective against the blast isolates present at 16 locations across the diverse rice growing ecosystems in India. It is assumed that B 90-15 may have, besides major genes, additional QTLs introgressed from *O. rufipogon* resulting in broad-spectrum resistance. The gene(s) for biotic stress resistance introgressed from wild species for bacterial blight (*Xa*21 and *Xa23*), blast (*Pi9*), brown planthopper (*Bph10*, *bph12*, *Bph13*), grassy stunt virus and rice tungro disease are in general more effective across the environments[36]. These findings suggest that transferring genes for biotic stress resistance from wild species is important for developing varieties with durable resistance, since these genes are novel and less exposed to pathogen variability.

All the NILs, international differentials, donors and resistant varieties screened showed differential reaction at the test locations indicating wide variability in virulence among blast pathogen populations (Table 2). The line B 90-15 showed high level of resistance against isolates present at Hazaribagh, Jagadalpur, Malan, Ponnampet, and Mandya. At Ponnampet, B90-15, NILs with genes *Pi1*, *Pi4b* and the pyramided lines with 2 and 3 gene combinations *Pi2* + *Pi4* and *Pi1* + *Pi2* + *Pi4* were resistant. Whereas the gene *Pi1* in combination with genes *Pi2* and *Pi4* showed susceptible and moderate resistance reaction respectively. The susceptible reaction of pyramided line BL122 having *Pi1* + *Pi2* genes at Ponnampet may be due to the antagonistic effect[33]. At Mandya, all the NILs (except *Pi4a*) and pyramided lines with 2–3 gene combinations showed susceptible reaction. The blast resistance genes *Pi5*, *Pi9* and *Pi2* indicated earlier[31,32] with broad-spectrum resistance were not effective against some of the isolates. Similar observations on differential reaction of NILs and pyramided lines against different blast isolates, were also reported[21,33]. The backcross inbred lines or near isogenic lines with known gene or QTLs are useful to evaluate the effectiveness of each gene/QTL conferring resistance to blast races[34,35]. Information on race-specificity of a single gene/QTL is crucial for pyramiding the effective gene combinations for broad-spectrum and durable resistance in rice.

In the present study we introgressed broad-spectrum resistance gene(s) from wild rice (*O. rufipogon*) effective against most of the isolates present in India. We developed line B90-15 by introgressing gene(s) from *O. rufipogon* for broad-spectrum resistance to blast and moderate resistance to brown plant hopper and tolerance to salinity, which has been released as variety Jarava for cultivation in coastal saline areas of West Bengal, Pondicherry and Andamans. It is observed that effectiveness of individual resistance genes in NILs and gene combinations in pyramided lines vary between locations. Hence identification of effective gene combinations against several virulent races is important in gene pyramiding for durable and broad-spectrum resistance to blast. The present study also indicated that for introgression of multiple genes from closely related species of rice such as *O. rufipogon,* 1–2 back crosses or top-crosses followed by selective intermating in segregating generations is useful in bringing together the desirable traits and breaking undesirable linkages.

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Characterization of variability among isolates of *Fusarium graminearum* associated with head scab of wheat using DNA markers

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Head scab of wheat caused by *Fusarium* species is characterized by bleaching of the wheat spike, shrivelled kernels and accumulation of mycotoxins which may cause various ailments in humans and animals. Understanding the variability of the fungal population associated with head scab could improve disease control strategies. RAPD was used to study genetic variation in 15 isolates of *Fusarium graminearum*, collected from naturally infected wheat from Punjab, Tamil Nadu and high ranges of Himachal Pradesh during 2000–02. A screening of sixty-one 10-mer oligonucleotide primers (OPAA 1-20, OPAC 1-20, OPAD 1-20, OPV 14), revealed 19 RAPD primers which produced strong and reproducible DNA amplicons by PCR. The amplification products were in the range of 300 bp to 1.2 kb. Maximum number of bands (11) was obtained with primer OPAD 12 followed by ten bands with OPAA 12. Punjab isolates of *F. graminearum* from Gurdaspur (G 31) and Ludhiana (L23) were found genetically most similar (91.38%), whereas Wellington isolates of *F. graminearum* (W 5 and W 7) were found genetically most dissimilar (14.92%). Cluster analysis of band-sharing coefficients separated isolates of *F. graminearum* into four clusters. Lahaul valley isolates of *F. graminearum* (D 3, D 4 and D 5) grouped together (Group I), while *F. graminearum* isolates of Punjab

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