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INHERITANCE OF BLAST DISEASE RESISTANCE IN THE CROSS HUR 3022 X TETEP OF RICE (*Oryza sativa* L.)

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KEYWORDS

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Disease severity per cent

Inheritance

Polymeric gene action

ABSTRACT

An experiment was carried out using six generations (P₁, P₂, F₁, F₂, B₁ and B₂) of cross HUR 3022 x Tetep in rice at BHU, Varanasi during year 2017-2018 to know the inheritance pattern of leaf blast disease under artificial inoculation with *LB-TN-2* isolate of *Magnaporthe oryzae* in the field condition. The blast disease resistant cultivar ‘Tetep’ showed 9.32% disease severity, while high yielding, early maturing susceptible cultivar HUR 3022 showed 43.65% disease severity against *M. oryzae*. The area under the disease progress curve (AUDPC) of resistance cultivar was observed 127.95 which are significantly less than the susceptible cultivar 605.62. The F₁ (HUR 3022 x Tetep) plants were observed to be resistant with an average disease severity and AUDPC are 17.54% and 224.7, respectively. The F₂ population was observed to show three distinct phenotypic classes resistant, moderately resistant and susceptible with a ratio of 9:6:1, respectively. Two backcross Populations, B₁ and B₂ showed different response from each other during evaluation which results in the phenotypic ratio of 1R:2MR:1S in B₁ and 1R:OS in B₂, respectively. The results showed that blast disease resistance occurs in the cross is due to duplicate cumulative effects or polymeric gene effect of two dominant resistant genes *i.e.*, *Pil* and *Pi54*.

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1 Introduction

Rice is a staple food crop provides food to more than half of the world's population. Further rice is grown all around the world from as far north Manchuria of China to far south Uruguay and at elevations >300 m of Bhutan to 3 m below sea level in Kerala state of India (Khush, 2005; Khush, 2013). With such diverse growing area, rice is also prone to 70 different types of diseases caused by several biotic agents like fungi, bacteria and viruses along with nematodes causing constitutively 5 % yield loss every year (Song & Goodman, 2001; Singh et al., 2013a; Arunakumari et al., 2016). Among these diseases, rice blast caused by the fungal pathogen *M. oryzae* reported as a devastating constraint to rice production occurring in more than 85 rice growing countries globally (Scardaci et al., 1997; Gilbert et al., 2004). In India 23 rice growing states possess endemic districts for rice blast so rice production time to time encounter different level of losses due to occurrence of blast (Prasad et al., 2011; Turaidar et al., 2018). During the period 2001-2014, rice leaf blast has emerged as a major constraint in areas of Chhattisgarh, Jharkhand, Tamil Nadu and Uttar Pradesh states (Laha et al., 2011). Although, chemicals can control blast but use of blast-resistant cultivars in rice production is considered an economical and environment friendly disease control strategy. Out of 100 blast resistant genes till identified, several resistance genes have been already introduced into elite rice varieties using Marker Assisted Breeding approaches (Abhilash Kumar et al., 2016; Ellur et al., 2016; Sabin et al., 2016; Usatov et al., 2016; Khan et al., 2018; Kumar et al., 2019; Swathi et al., 2019). Dynamic changes in the race composition of pathogen has often caused breakdown of resistance in most of the improved resistant varieties. Especially cultivars containing a single major resistance gene become susceptible within few years. Stacking of more than one major resistance gene has been proven one of the effective methods to deliver durable resistance against rice blast (Hittalmani et al., 2000; Joshi & Nayak, 2010; Koide et al., 2010). For breeding durable rice blast resistance and stacking a number of genes into a single cultivar, the knowledge of inheritance pattern of blast disease is prerequisite. Keeping all these fact in view an investigation was carried out using six generations (P₁, P₂, F₁, F₂, B₁ and B₂) in rice to know the inheritance of blast disease under artificial inoculation for blast pathogen in the field condition.

2 Materials and Methods

Two *indica* rice varieties HUR 3022 (Blast susceptible high yielding and early maturing variety) and Tetep (Blast resistant donor variety) were used for staggered sowing at agricultural research farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (Uttar Pradesh) during *Kharif* 2017 and crossed to produce F₁ seeds. These F₁ seeds were planted along with parents at ICAR-National Rice Research Institute, Cuttack

(Odisha) during *Rabi* 2017-18 to generate seeds of F₁, F₂, B₁ (F₁ x HUR 3022) and B₂ (F₁ x Tetep) generation, which constitutes the study material along with a blast disease check (Co 39) for present investigation. The seedlings of parents (HUR 3022 and Tetep) along with four generations (F₁, F₂, B₁ and B₂) and blast disease check (Co 39) were transplanted in the field in a complete family randomized block design with three replications maintaining a spacing of 15 x 20 cm plant to plant and row to row, respectively at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (Uttar Pradesh) during *Kharif* 2018.

The blast infected leaf samples due to *LB-TN-2* isolate of *M. oryzae* were obtained from the green house of Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Isolation of fungus was carried out under aseptic conditions by spore-drop method following the methodology described in Rajashekara et al. (2017). Isolate was cultured on Potato Dextrose Agar (PDA) and Oat Meal Agar (OMA) medium in petri plates incubated at 28°C. The morphological identification confirmed the characteristics of pathogen *M. oryzae* i.e. pyriform to oblong conidia which are hyaline in colour and bisepate measuring 19 - 27 X 8 - 10 µm in size (Figure 1b). Disease screening plots were sprayed with 15 days old culture obtained from Oat Meal Agar media (Figure 1a) at a concentration of 1 x 10⁵ conidia/ml and solution also contains Tween-20 (0.2 %). High humidity in the field was maintained during night by covering the plants with plastic bags to insure development of disease (Figure 1c). The inoculated plants were observed thrice i. e. 7, 14 and 21 days after inoculation (DAI). Disease scoring was performed using 0-9 scale of IRRI-SES scale as described in Table 1 (IRRI, 2013; Singh et al., 2013b).

Table 1 Scale for scoring of rice leaf blast disease (IRRI, 2013; Singh et al., 2013b)

Scale	Disease severity	Host response
0	Lesion are not present	Resistant (R)
1	Small brown specks of pin point size or larger brown specks without sporulating center	Resistant (R)
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesions are mostly found on the lower leaves	Resistant (R)
3	Lesions type is same as in scale 2, but a significant number of lesions on upper leaf area	Resistant (R)
4	Typical susceptible blast lesions, 3 mm or longer infecting less than 4 % of leaf area	Moderately Resistant (MR)
5	Typical susceptible blast lesions infecting 4-10% of the leaf area	Moderately Resistant (MR)
6	Typical susceptible blast lesions infecting 11 – 25% of the leaf area	Moderately Susceptible (S)
7	Typical susceptible blast lesions infecting 26 - 50% of the leaf area	Susceptible (S)
8	Typical susceptible blast lesions infecting 51- 75% of the leaf area and many leaves are dead	Susceptible (S)
9	More than 75% leaf area affected	Susceptible (S)

Figure 1 (a) 15 days old culture of *LB-TN-2* isolate of *Magnaportheoryzae* on Oat Meal Agar media used for inoculation; (b) Biseptate characteristic conidia of *Magnaportheoryzae* from 15 days old culture; (c) Maintenance of humidity using plastic bags in field; (d) Symptoms of blast obtained in field after artificial inoculation

The disease severity per cent (DSP) and area under disease progress curve (AUDPC) were calculated according to the formulae described by Sabin et al. (2016). Plants categorized as resistance and susceptible for rice leaf blast based on their disease scores. These observed frequencies were tested using χ^2 test for goodness-of-fit with expected frequencies of resistant and susceptible plants to study the pattern of inheritance of blast resistance in rice.

3 Results and Discussion

The donor variety 'Tetep' used in present investigation have shown resistant against *LB-TN-2* isolate of *M. oryzae* under

artificial inoculation in the field condition due to presence of two major dominant resistance genes *Pi1* and *Pi54*, which showed disease score 1 with 9.32 per cent disease severity. While recipient variety 'HUR 3022' displayed susceptible reaction with disease score 7 and 43.65 per cent disease severity due to absence of these two genes (Table 2). The inaugural symptoms of blast observed on the recipient variety 'HUR 3022' with variable intensities in the form of gray green and water-soaked lesions with a darker green border, which expanded rapidly to several centimeters in length, later resulted into typical diamond shaped lesions (Figure 1d). These results are in agreement with earlier findings for symptoms on susceptible cultivars (Singh et al., 2019). The area under the

Table 2 Comparison of Disease severity, Area Under Disease Progress Curve (AUDPC) and Lesion number in P₁, P₂, F₁, F₂, B₁ and B₂ generations of the cross HUR 3022 × Tetep against *M. oryzae* isolate LB-TN-2

Genotypes	Plant scored	Disease Severity (DS %)			AUDPC value	Disease score (21 DAI)	Host response (21 DAI)	Lesion number	Mean AUDPC	Mean DS %
		7 DAI ± SD	14 DAI ± SD	21 DAI ± SD						
Co 39 (C) (30 plants)	30	41.08 ± 3.87	54.12 ± 3.79	69.33 ± 3.06	765.28	8	S	67.38	765.29	55.26
HUR 3022 (30 plants)	30	28.44 ± 2.05	42.67 ± 3.38	59.26 ± 2.10	605.64	7	S	48.20	605.62	43.66
Tetep (30 plants)	30	7.22 ± 1.69	9.34 ± 2.08	10.66 ± 2.01	127.96	1	R	6.60	127.95	9.32
F ₁ 's - 30 plants	30	12.65 ± 1.90	17.37 ± 2.12	22.52 ± 2.16	244.69	2	R	4	224.7	17.54
B ₁ (F ₁ x HUR 3022) 60 plants	14	11.35 ± 0.61	18.51 ± 0.93	26.45 ± 1.01	323.37	3	R	17.38	464.75	32.86
	34	20.26 ± 2.35	28.19 ± 2.03	32.40 ± 2.62	470.84	5	MR			
	12	23.70 ± 3.12	43.63 ± 3.20	60.04 ± 3.16	99.53	7	S			
B ₂ (F ₁ x Tetep) 60 plants	60	12.69 ± 5.70	22.37 ± 4.89	33.48 ± 4.44	318.20	3	R	17.13	318.20	22.84
F ₂ 's - 210 plants	115	14.11 ± 0.47	16.20 ± 0.55	20.56 ± 1.03	299.85	3	R	15.95	477.51	34.22
	84	18.78 ± 5.32	29.86 ± 4.97	33.47 ± 5.04	475.9	5	MR			
	11	39.67 ± 4.03	41.34 ± 5.43	65.07 ± 7.28	655.97	7	S			

SD: Standard deviation, DAI: Days after inoculation, AUDPC: Area under disease progress curve

disease progress curve of resistant donor parent was found 127.95 which is significantly lower than the susceptible recipient parent 605.62. Above results are in accordance with earlier reports of wide difference between area under disease progress curve of resistant and susceptible cultivars (Mahapatra et al., 2008; Nguyen et al., 2015). All the plant of F₁ generation from the cross HUR 3022 x Tetep were observed as resistant with average disease severity 17.54% and AUDPC 224.7. These findings are in accordance with earlier reports on resistant response of F₁ generation in cross of susceptible and resistant cultivars (Gupta et al., 2012).

Two backcross generations, B₁ (F₁ x HUR 3022) and B₂ (F₁ x Tetep) of the cross showed different response from each other during evaluation for blast disease resistance. These findings also showed similarity with earlier reports (Persaud et al., 2007; Singh et al., 2014). The plants from B₁ generation showed three types of responses which included resistant, medium resistant and susceptible response. Average lesion number showed by B₁ generation was 17.38 with 464.75 mean AUDPC value and 32.86 per cent disease severity. Out of 60 plants observed in B₁ generation, 14 plants showed resistant response, 34 plant moderately resistant and 12 plants susceptible response with $\chi^2 = 1.20$, $P > 0.05$ indicating that observed data are in agreement with the expected ratio in backcross generation and confirmed modification of mendelian dihybrid ratio of 1:1:1:1 into 1:2:1 ratio. Like B₁, In B₂ generation also 60 plants observed and all the plants showed resistant response revealed the modification of mendelian dihybrid ratio of 1:1:1:1 into 1:0 ratio. Plants in B₂ generation was observed having average lesion number as 17.13, mean AUDPC

value as 318.20 and per cent disease severity as 22.84, respectively (Table 2; Table 3). These results showed that blast disease resistance in two backcross generations was governed by two dominant genes which showed polymeric gene action. Disease resistance governed by two dominant genes in interaction was earlier reported but they reported presence of two independent dominant genes or complementary gene interaction (Persaud et al., 2007; Zewdu et al., 2018).

Plants from F₂ generation individually scored and could be categorized into four genotypic classes in a ratio of 9:3:3:1 while three types of phenotypic responses- resistant, moderately resistant and susceptible response were observed during investigation in a ratio of 9:6:1 (Table 2; Table 3). Average lesion numbers in F₂ generation were recorded as 15.95 with 477.51 mean AUDPC value and 34.22 per cent disease severity, respectively. In F₂ generation 210 plants observed which resulted into 115 plants showing resistant response, 84 plants showing moderately resistant response and 11 plants showing susceptible response against blast in a ratio of 9:6:1 with $\chi^2 = 0.70$, $P > 0.05$ revealed that observed data are in accordance with expected ratio. These results confirmed the modification of mendelian dihybrid ratio 9:3:3:1 into 9:6:1 ratio which was due to presence of two dominant genes showing polymeric gene action or we can say duplicate genes with cumulative effect. These findings are in contradiction with reports of single dominant gene governing blast resistance in rice (Fuji & Saito, 2007; Sharma et al., 2007; Ashkani et al., 2011). These findings are in partial agreement with the earlier reports of two dominant genes showing interaction for governing blast resistance in rice (Phillip & Prabhu, 1996; Persaud et al., 2007; Zewdu et al., 2018).

Table 3 Inheritance of blast resistance in six generations P₁, P₂, F₁, F₂, B₁ and B₂ generations of the cross HUR 3022 × Tetep against *M. oryzae* isolate LB-TN-2

Parents and their populations	Expected gene combination	Genotypic frequency	Disease score at 21 DAI	Disease reaction at 21 DAI	Total no of plant observed	Observed frequencies	Expected frequencies	Phenotypic ratio	χ^2 value
HUR 3022	<i>pi1pi1pi54pi54</i>	-	7	S	30	30	30	-	-
Tetep	<i>Pi1Pi1Pi54Pi54</i>	-	1	R	30	30	30	-	-
F ₁	<i>Pi1pi1Pi54pi54</i>	-	2	R	30	30	30	-	-
B ₁	<i>Pi1pi1Pi54pi54</i>	1	3	R	60	14	15	1 R	1.20
	<i>Pi1pi1pi54pi54</i>	1	5	MR		34	30	2MR	
	<i>pi1pi1Pi54pi54</i>	1							
	<i>pi1pi1pi54pi54</i>	1	7	S		12	15	1S	
B ₂	<i>Pi1Pi1Pi54Pi54</i>	1	3	R	60	60	60	1R:0S	NA
	<i>Pi1Pi1pi54pi54</i>	1							
	<i>Pi1pi1Pi54Pi54</i>	1							
	<i>Pi1pi1pi54pi54</i>	1							
F ₂	<i>Pi1_Pi54_</i>	9	3	R	210	115	118	9R	0.70
	<i>Pi1_pi54pi54</i>	3	5	MR		84	79	6MR	
	<i>pi1pi1Pi54_</i>	3							
	<i>pi1pi1pi54pi54</i>	1	7	S		11	13	1S	

NA: Not applicable

Conclusion

In any breeding programme related to blast resistance achieving durable broad spectrum blast resistance is a major objective. Results of present investigation revealed that in case of *M. oryzae* isolate LB-TN-2 infection on populations of cross HUR 3022 × Tetep the resistance to blast is due to presence of two dominant genes showing polymeric gene action or we can say each gene provide resistance when present in dominant state but level of resistance increases when both dominant alleles of the genes present together. Hence it is concluded that to increase the level of blast resistance in a cultivar stacking of multiple resistance genes could be a suitable approach.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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