Indian J. Genet., 74(4) Suppl., 670-673 (2014)
DOI: 10.5958/0975-6906.2014.00908.0

Evaluation of rice germplasm for multiple disease resistance under artificial inoculation conditions

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(Received : September 2014; Revised : November 2014; Accepted: November 2014)

Abstract

One thousand and eighteen rice germplasm lines were evaluated against the prevalent pathotypes viz., PbXo-7 and PbXo-10 of *Xanthomonas oryzae* pv. *oryzae* causing bacterial blight, brown spot and sheath blight diseases under artificial inoculation conditions. Virulence/avirulence of the two pathotypes to *Xa/xa* genes indicated that all the pathotypes were virulent on the most of bacterial blight resistant single *Xa/xa* gene (s). Pathotype PbXo-7 was virulent to known *Xa* genes namely *Xa 1, Xa 3, Xa 4, xa 5, Xa 7, xa 8, Xa 10* and *Xa 11* and cultivars PR 114, PR 116 and PR 118 growing in Northern regions of India. PbXo-10 was virulent on all these genes including newly identified gene *Xa38*. None of the single gene is effective against the pathotypes prevalent in Punjab. Two years data revealed that 46 lines (5.4%) showed resistant reaction to pathotype PbXo-7 and 30 lines (2.9%) showed resistance to newly emerged pathotype PbXo-10. Twenty two lines showed resistant reaction to both the pathotypes. Most of the entries which were resistant to PbXo-10 also showed resistant to the PbXo-7. Germplasm line IC No.346207 showed resistant reaction and IC Nos. 114315 and 320826 showed moderately resistant reaction to brown spot. None of the germplasm line showed resistance to sheath blight. These resistant entries can either be released as new varieties or further utilized as donors in multiple disease resistance breeding programmes.

Key words: *Oryza sativa*, bacterial blight, brown spot, pathotype, resistance, *Xanthomonas oryzae* pv. *oryzae*

Introduction

Rice (*Oryza sativa* L.) is one of the most important crop and primary source of food for more than half of the world population. It plays an important role in national economy of many countries. There is need to use rice varieties with higher yield potential, durable resistance to diseases and insects and tolerance to abiotic stresses [1]. As for India, rice is not only a food commodity but also a source of foreign exchange earning about 46000 crores annually. A critical aspect of enhancing production at any range of time-scale is to minimize losses to diseases [2].

Rice crop is affected by various diseases, out of which Bacterial Blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae*, sheath blight caused by *Rhizoctonia solani* and brown spot caused by *Drechslera oryzae* are of major concern. Bacterial blight can cause losses up to 50% depending on stage, weather, location and varieties [3]. Bacterial blight is widely distributed and endemic to most parts of Asia [4]. The disease appears every year in varying degrees throughout the Punjab state in India causing yield losses of 60 to 70% during epiphytotic years [5]. Chemical control of BB is impractical and no truly effective bactericide is commercially available for its control. Development and deployment of host plant resistance is the only effective means of BB management. Currently, more than 40 *Xa/xa* gene (s) in rice conferring resistance against *X. oryzae* pv. *oryzae* have been designated globally [4-6-7]. Since the effectiveness of these genes varies in time and space and relatively few are currently being used in rice improvement. The genetic base of resistance to BB in the working germplasm at the national level is quite narrow and transfer of BB resistance gene (s) from many of wild *Oryza* species. On the other hand it has been reported that sheath blight and brown spot account for highest yield loss across all the production situations in South and Southeast Asia [2]. Although, rice resistance breeding has made large achievements in managing BB disease, but, due to emergence of new

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Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110012
Online management by indianjournals.com

races of the pathogen, breeding of resistant cultivars always confronts difficulties in terms of durability of resistance. To date, no complete resistance against sheath blight and brown spot has been reported [8]. So in view of this, rice germplasm/landraces were evaluated for multiple disease resistance under artificial inoculation conditions.

Materials and methods

Plant materials

A total of 1018 rice germplasm lines received from Directorate of Rice Research, Hyderabad were evaluated against highly virulent pathotypes *viz*., PbXo-7 and PbXo-10 of the BB pathogen, sheath blight and brown spot under artificial inoculation conditions during the year 2012-13. Cultivars TN1 and Ajay (IET 8585) were used as susceptible and resistant check respectively for BB. Cultivar PR116 was used as susceptible check for sheath blight and brown spot diseases. The selected resistant entries were re-evaluated during the year 2013-14.

Artificial inoculation and disease assessment

Bacterial blight

For bacterial blight inoculation, pathotypes (PbXo-7 and PbXo-10) of *X. oryzae* pv. *oryzae* were isolated on Waki Moto medium. Seventy two hours old single colony virulent culture of *X. oryzae* pv. *oryzae* was used for artificial inoculations. The pathotypes were tested on near isogenic lines/differentials before screening the rice germplasm. All the test genotypes were inoculated at maximum tillering stage by clip-inoculation technique with bacterial suspension of approximately 10^{-9} cells/ml [9]. Ten plants of each test entry were inoculated with the aggressive races separately. Reaction of plants was recorded 14 days after inoculation according to Standard Evaluation System (0-9 scale) for rice [10]. Plants were characterized as resistant or susceptible based on scale 0-3 (resistant); 5 (Moderately resistant); 7-9 (Susceptible).

Sheath blight

The pathogen was isolated from infected rice plants on PDA medium and was mass multiplied in 500 ml flasks on *Typha* bits. Ten days old growth of *R. solani* was inoculated on plants by placing 3-4 *Typha* bits per hill in the whorl of each test genotype. The disease data were recorded 15 days after inoculation based on 0-9 scale of Standard Evaluation System (SES) for rice [10].

Brown spot

The pathogen was isolated from infected rice cultivar PR116 on PDA medium, mass multiplied in 250 ml conical flasks containing 100 ml potato dextrose broth by inoculating with 8 days old small bits of actively growing culture of the pathogen and incubated at 28 ± 2^{o}C. One week old culture was homogenized and the spore suspension was filtered through double-layered muslin cloth. Conidial concentration was adjusted to $1X10^{6}$ conidia per ml with the help of a haemocytometer and sprayed on the plants with hand sprayer at the booting stage to have uniform disease. Disease was scored as per Standard Evaluation System for rice [10].

Results and discussion

Testing of X. oryzae *pv.* oryzae *pathotypes*

Ten pathotypes of the *X. oryzae* pv. *oryzae* have been reported in Punjab state of India [11-12]. The pathotype PbXo-7 is predominant with frequency distribution of 40.1% in Punjab and virulent to known *Xa* genes namely *Xa 1, Xa 3, Xa 4, xa 5, Xa 7, xa 8, Xa 10 and Xa 11* and cultivars PR 114, PR 116 and PR 118 growing in Northern regions of India [11]. In the present study, virulence of the pathotypes PbXo-7 and PbXo-10 of *X. oryzae* pv. *oryzae* was tested on a set of near isogenic lines/ differentials before inoculations to the rice germplasm. Virulence/avirulence of the pathotypes to *Xa/xa* genes indicated that the pathotypes PbXo-7 was virulent on above said bacterial blight resistant genes and broken down the resistance to rice cultivars PR114, PR116 and PR118, in the Punjab State (Table 1). Another emerging pathotype, PbXo-10 has been found to be virulent on the most of single *Xa/xa* gene (s) including *Xa38*, a new BB resistant gene identified from

Table 1. Emerging and dominant pathotypes of *Xanthomonas oryzae* pv. *oryzae* showing virulence and avirulence to *Xa/xa* gene (s)/ cultivars in Punjab

Pathotype of *X. oryzae* pv. *oryzae*	Virulence	Avirulence
PbXo-7	*Xa4, xa5*, *Xa7, xa8,* PR 114, PR 115, PR 116, PR 118	*xa13, Xa21, Xa38, xa13+Xa21, Xa4+ xa5,* IR 64, PR 111, PR 113, PAU 201, PR 120, PR121, PR122, PR123
PbXo-10	*Xa4, xa5, Xa7, xa8, Xa38, Xa4+xa5,* IR 64, PR 111, PR 114, PR 116, PR 118, PR 120	*xa13, Xa21, xa13+ Xa21,* PR 113, PR 115, PAU 201, PR121, PR122, PR123

Oryza nivara [13].

Resistance to bacterial blight

Out of 1018 germplasm lines tested, 46 lines (5.4%) showed resistant reaction to pathotype PbXo-7 and 30 lines (2.9%) showed resistance to newly emerged pathotype PbXo-10 (Fig. 1). Twenty two lines viz. IC Nos. 114180, 145446, 252166, 320824, 330813, 381978, 449553, 449598, 449607, 449621, 449706, 449756, 449782, 449798, 449829, 449935, 450036, 450499, 450574, 461818, 544851 and 544893 showed resistant reaction to both the pathotypes (Table 2), however, 14 lines i.e. IC Nos. 334173, 347244, 400034, 413639, 449555, 449575, 449673, 449679, 449984, 450010, 450100, 450130, 460015 including IR 64 showed resistance to PbXo-7 but susceptible to PbXo-10. On the other hand four lines 17065, 450481, 450597, 450544 showed resistant reaction to PbXo-10 but susceptible to PbXo-7 (Table 2).

Comparatively higher number of germplasm lines (46 lines, 4.5%) showed resistant reaction to pathotype PbXo-7 in this study. Similarly, a number of advanced breeding lines with resistant to the pathotypes PbXo-7 have been reported in earlier study [14]. These lines might be susceptible to the emerging pathotype PbXo-10. In the present study, 14 lines including IR 64 showed susceptible reaction to this emerging pathotype but resistant to PbXo-7 (Table 2). It indicated that the diverse reaction of the pathotypes was observed and PbXo-10 is highly virulent. Those entries showed resistant reaction to both the pathotypes can be exploited in bacterial blight resistance breeding program.

Resistance to brown spot

Germplasm line IC No.346207 showed resistant reaction

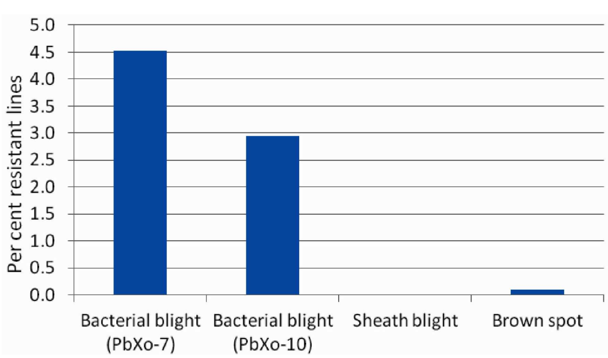

Fig. 1. Frequency of resistant rice germplasm lines against two pathotypes of *Xanthomonas oryzae* pv. *oryzae* (bacterial blight), sheath blight and brown spot diseases

Table 2. Disease reaction to pathotypes of *Xanthomonas oryzae* pv. *oryzae* (bacterial blight), sheath blight and brown spot

IC No.	Bacterial blight (Pathotypes)		Sheath blight	Brown spot
	PbXo-7	PbXo-10		
114180	3	3	7	7
145446	3	3	7	7
252166	3	3	7	7
320824	3	3	7	7
330813	3	3	7	7
381978	3	3	7	7
449553	3	3	7	7
449598	3	3	7	7
449607	3	3	7	7
449621	3	3	7	7
449706	3	3	7	7
449756	3	3	7	7
449782	3	3	7	7
449798	3	3	7	7
449829	3	3	7	7
449935	3	3	7	7
450036	3	3	7	7
450499	3	3	7	7
450574	3	3	7	7
461818	3	3	7	7
544851	3	3	7	7
544893	3	3	7	7
450123	7	7	7	5
449906	7	5	7	5
346207	7	7	7	3
334173	3	7	7	7
347244	3	7	7	7
400034	3	7	7	7
413639	3	7	7	7
449555	3	7	7	7
449575	3	7	7	7
449673	3	7	7	7
449679	3	7	7	7
449984	3	7	7	7
450010	3	7	7	7
450100	3	7	7	7
450130	3	7	7	7
460015	3	7	7	7
170065	7	3	7	7
450481	7	3	7	7
450544	7	3	7	7
450597	7	3	7	7
IR64 (Check)	3	7	7	7
TN-1	9	9	7	7
Ajay (IET 8585)	3	3	7	7
PR116	9	9	9	9

Score 0-3 (resistant); 5 (Moderately resistant); 7-9 (Susceptible) and two lines IC Nos. 114315 and 320826 showed MR reaction to brown spot after two years of evaluation under artificially inoculation in field conditions (Table 2). Very limited studies have been made for genotyping variability and rice cultivars for brown spot resistance [15]. In the present study, very few germplasm lines (0.29%) showed R/MR reaction to brown spot (Fig. 1). These resistant lines can be used for brown spot resistance breeding program

Resistance to Sheath blight

None of the germplasm line showed resistance to sheath blight. Though some of the lines showed R/MR reactions to sheath blight during the first year (2012-13) testing, but none of the entries showed R/MR reactions to sheath blight during second year (2013-14) (Fig.1). High level of resistance has not been observed among cultivated rice varieties, despite, testing more than 30,000 accessions of rice germplasm at different research centres, including International Rice Research Institute [16]. Similarly, large scale screening of the national and international rice elite material and germplasm has been done in the past against sheath blight and so far none of the material has been found to possess clear cut resistance [8].

It is concluded that the sources of resistance after determining their genetics can be exploited in disease resistance breeding programmes for the development of resistant commercial cultivars.

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