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Screening of wild rice species against blast disease incidence

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ABSTRACT

Wild rice varieties are great store house of genetic diverseness and have survived in the nature for thousands of years and so they definitely therefore must carry substantial amount of genes that are responsible for resistance to different diseases and pests. This chapter reveals the screening of blast disease incidence of 27 accessions representing 19 wild rice species. The disease incidence was evaluated in wet season of 1994 against blast disease. Out of the wild rice species tested only six (Oryza eichingeri, O. grandiglumis 1085, O. latifolia 1007, O. longistaminata 1026, O. meridionalis and O. rufipogon 1011) showed resistance to blast isolated IC-1. The diseases intensity in the above cultivars ranged between 2.66 and 3.66 Mean Disease Index (MDI). Out of the wild rice species tested only six (O. eichingeri, O. grandiglumis 1085, O. latifolia 1007, O. longistaminata 1026, O. meridionalis and O. rufipogon 1011) showed resistance to blast isolate IC-1. The diseases intensity in the above cultivars ranged between 2.66 and 3.66 MDI. These resistant wild rice cultivars can also be used to develop blast resistant cultivated rice cultivars by incorporating the resistant genome from the resistant wild rice accessions.

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important staple food crops of the world especially for the people of Asia and provides both value and more than 50% global food energy. Wild rice varieties are great store house of genetic diverseness and can be used to enhance the quantity as well as quality of rice (Brar 2003; Sun et al. 2001). They have survived in the nature for thousands of years and so they definitely therefore must carry substantial amount of genes that are responsible for resistance to different diseases and pests. It is also well known that the wild rice is acclimatized to drastic habitats like flood, drought, saline and acidic conditions and so they must possess resistant genes conferring tolerance to the extreme environmental conditions. Genes from wild rice have found to have utmost influence for improvement of rice. Data revealed the introduction of genes of wild rice in to cultivated rice to get resistant cultivar of cultivated rice to bacterial leaf blight, grassy stunt virus and brown plant hopper (Kush 1997; Tanksley & McCouch 1997; Sanchez et al. 2013). *Oryza* genus consists of two cultivated rice species and 24 wild rice species (Sharma & Shastry 1965). Near about 1, 20, 000 different accessions are reported in rice and phenotypic variations also exists (Das et al. 2013). A number of rice germplasm belonging to different important cultivars are conserved in Global gene Banks (Brar 2003; Sun et al. 2001; Lu et al. 2005; Garris et al. 2005). A total of 4645 accessions of wild rice species are reported as per the data of International Rice Gene Bank Collection Information System (IRGCIS) in International Rice Research Institute (IRRI) gene bank, 838 accessions are of Indian origin. Indian gene bank at the National Bureau of Plant Genetic Resources (NBPGR) has 307 accessions of *O. rufipogon*, 726 accessions of *O. nivara*. Database was developed on 614 diverse wild rice accessions collected from different agro climatic zones of India along with the morphological description and total DNA fingerprint data. Among the diseases of rice, blast caused by *Pyricularia oryzae* cav. [the anamorph of *Magnoporthe grisea* (T.T. Herbert) Yaegashi and Udagawa] is considered as a serious disease and prevalent in almost all the rice growing tracts of the world. This disease was first reported from India in 1913 (Padmanabhan 1965). In India it is a major disease of both wet season (kharif) and summer crops. The blast fungus (*P. oryzae*) produces spores or lesions on leaves, nodes and different parts of the panicle including grains. Infection in the early state of the crop growth completely kills the plant while severe infection at a later stage drastically reduces the yield. In coastal belt of Odisha, where the relative humidity fluctuates between 82 and 97 per cent, there is frequent occurrence of blast disease in these areas. Wild *Oryza* species are adapted to varied habitats (Atwell et al. 2014). A survey of blast disease on 27 accessions representing 19 wild rice species viz. *O. afla*, *O. austaliansis*1061, *O. austaliansis*1062, *O. Brachyantha*, *O.brschyara*, *O. Barthii*, *O. eichingeri* ,*O. eichingeri* ,*O. Corolata*, *O. grandiglumis*1085, *O. latifolia* 1005, *O. latifolia* 1007, *O. longistaminata*1013, *O. longistaminata*1026, *O. minuta*1025, *O. minuta*1028, *O. malphu*, *O. marionalis*, *O. nivara*1054, *O. nivara*1055, *O. officinalis* 1040, *O. officinalis* 1041, *O. officinalis* 1042, *O. officinalis* 1065, *O. punctata*, *O. rufipogon*1011, *O. rufigo*1060 and *O. seneifoxthina* 1049 were investigated in wet season of 1994 to evaluate the blast disease incidence.

METHODOLOGY

Experimental site

The work was carried out at CRRI is located at 20^0 N latitude and 86' E longitude and 23 meters above the mean sea level. The climate of the place is warm, humid and the winter is mild. The temperature varies from 12-27^{0}C (minimum) and 27-42^0 C (maximum) and maximum relative humidity (RH) normally varies from 82-97%. The annual rainfall varies from 1225-1575 mm mostly received by South-West monsoon between June and October.

Source of experimental material

Seeds of 27 accessions representing 19 wild rice species of genus *Oryza* were obtained from the Genetic Resource Department of CRRI, Cuttack.

Raising up of seedlings

The seeds were grown separately for each accession in earthen pots measuring 30 cm diameter and 40 cm high during the wet season of 1994. The pots were filled with a mixture of field soil and compost (3: 1). Ten seedlings were raised for each cultivar. Nitrogen fertilizer in the form of ammonium sulphate was applied @ 80 kg/ha in two split doses, once at sowing and the other at 15 days after germination. Pot watering and other cultural operations were done as per the need. Twenty-one day-old-seedlings with dark green leaves were selected for artificial inoculation (Hashioka & Ikegami 1965) with the most virulent path type IC-1.

Preparation of media slants and culture plates

Oat meal agar (OMA) medium was prepared by taking 40 gm Oat meal, 20 gm agar-agar powder with 0.015 mg biotin and 0.025 mg thiamine per litre of water (Leaver et al. 1947; Tanaka & Katsuki 1951; Tanaka & Katsuki 1951). Then the culture media (5 ml) were dispensed into clean rimless Pyrex test tubes of size 6" x 5/8" and then plugged with non-absorbent cotton wool. Then the tubes were placed in the wire basket covered with grease proof paper and was tied with elastic rubber bands. Sterilization was done at 121°C by autoclaving the media. To secure this temperature, steam having 1.05 kg/cm' pressure was passed for 20 minutes in an autoclave. Then culture slants were prepared. For preparing the culture plates, the sterilized media was poured aseptically to the sterilized petri plates (dry heat in a hot air oven at 160^9C for three hours) to avoid contamination.

Isolation of the pathogens

Rice blast infected leaves, as well as panicle samples were collected from different places. Isolation of the pathogen was carried out in laminar flow which was first sterilized by 1: 1 alcohol (70%) and then formaldehyde (40%). Finally it was sterilized by exposing UV - light for 30 minutes. The infected plant samples were cut into small pieces by a sterile scissor. The surface sterilization of the infected pieces were done in 0.01% mercuric chloride ($HgCl_2$) solution for 1-2 minutes and properly washed for three times in sterile water. The sterilized blast infected leaf pieces were taken with the help of a sterilized transfer needle (dipped in alcohol and flamed) and pressed against the media inside the culture slants and culture plates. The slants and plates were incubated for 10-12 days for the growth of the pathogen. Growth of the fungus varied with the days of incubation. After subculture, the isolates were kept at 5 ± 1^0c inside a refrigerator.

Preparation of inoculums

Mycelia from 10-12 day-old-slants were transferred separately for each isolate and macerated aseptically in a tube with 10 ml of distilled water. The resulting suspension of mycelia and conidia were poured over sterilized agar plates at the rate of 2ml per plate. After a week of incubation at 28^0c the surface of the plates were scrapped with a sterile glass slide. The plate surface was exposed continuously to fluorescent light at room temperature for 3-4 days. This was done to stimulate sporulation. Conidia were scrapped from incubated plates into which 10-20 ml of distilled was added. Spore suspension was filtered through nylon gauze mesh and spore concentration was adjusted to 5 × 10^4 conidia per mm. The suspension had at least 8-10 spores per microscopic field under low power magnification.

Artificial inoculation

Plants were inoculated 21 days after seedling (3-4 leaf stage). Inoculation *was* carried out in the evening hours when the temperature came down to around 26^0c.For each isolate, a separate chamber was used. Seedlings were sprayed uniformly with the spore suspension of the appropriate isolate with a mist sprayer. To maintain high relative humidity, wet gunny bags were enclosed around the nursery trays and periodically water was sprinkled thoroughly. This condition was maintained overnight through plastic tubes with holes at 2.5 cm apart all through. The inoculations were repeated twice per each isolate tested. Precautions were taken to ensure that the inoculated seedlings were kept in humid atmosphere for a minimum period of 6-10 hours which was necessary for establishment of infection (Hemmi & Abe 1932; Andersen et al. 1947). After 24 hours, the test plants were transferred to a humid chamber with an automatic mister at 26-28°C temperature for 8-10 days.

Reactions of inoculated rice plants

Disease reaction was observed after 10 days of inoculation. Lesions of several reaction types were produced on the inoculated seedlings. The leaves of each of the inoculated seedlings were carefully examined and based on the type, as well as extent of expression of lesions, they were classified into three categories, namely resistant (R), moderately susceptible (MS) and moderately susceptible (MS) and observed at weekly intervals in every month and from each tray 100 seedlings were randomly taken and mean disease index (MDI) was calculated. Based upon the types of spots on the leaves, the MDI of 10 plants of each of the cultivars were calculated for each replications and the data was statistically analyzed as per standard procedure.

Disease scoring

The occurrence and development of disease was recorded by scoring its intensity at weekly intervals. The reaction of rice leaves to the invasion of the blast fungus was expressed, based on the type and number of lesions. The mean disease index (MDI) was calculated (Padmanabhan & Ganguly 1959).

RESULTS

The blast disease incidence in 27 accessions representing 19 wild rice species varied from 2.66 to 65.66 MDI (Table 1) under the same agro climatic conditions. Out of the wild rice species tested only six (*O. eichingeri*, *O. grandiglumis* 1085, *O. latifolia* 1007, *O. longistaminata* 1026, *O. meridionalis and O. rufipogon* 1011) showed resistance to blast isolate IC-1. The diseases intensity in the above cultivars ranged between 2.66 and 3.66 MDI. Two wild species i.e., *O. longistaminata* 1013 and *O. minuta* 1025 showed moderately susceptible (MS) reaction with 11.33 and

12.0 MDI, respectively and did not vary significantly from each other. In the suspeptible species, *O. alta*, *O. australiensis 1069*, *O. brachyantha*, *O. brachyara*, *O. malphu and O. corolata* the MDI dewindled between 25.08 to 27.72 which did not vary significantly. It was notices that *O.brachyantha*was to differ significantly from *O. officinalis* 1040, *O. minuta*1028, *O. nivara*1054, *O. sneifoxthina 1049* and *O. officinalis*1042. Eight species viz. *O. australiensis* 1061, *O. barthii*, *O. latifola* 1005 and *O. nivara*1055, *O. officinalis* with accession number 1041 and 1065, O. punctata and O. rufigo 1060 were found to be highly susceptible (HS) with a range between 42.66 and 65.66 MDI. Among the highly susceptible species *O. australiensis* 1061, *O. barthii*, *O. officinalis* 1041 and *O. punctata* that MDI varied from 60.0 to 65.66 and differed significantly from rest of the wild rice species included in the test (Figure 1).

Table1: Reaction of wild rice to leaf blast under artificial inoculation condition

Wild Rice Species	Mean of Three Replications	Reaction
O. afla	20.66 (27.00)	S
*O. austaliansis*1061	60.0 (50.79)	HS
*O. austaliansis*1062	21.66 (27.72)	S
O. brachyantha	18.0 (25.08)	S
O.brschyara	18.0 (25.09)	S
O. barthii	65.66 (54.18)	HS
O. corolata	19.33 (26.00)	S
O. eichingeri	3.66 (10.86)	R
*O. grandiglumis*1085	3.0 (9.73)	R
O. latifolia 1005	44.0 (41.54)	HS
O. latifolia 1007	2.66 (9.27)	R
*O. longistaminata*1013	11.33 (19.66)	MS
*O. longistaminata*1026	3.66 (10.86)	R
*O. minuta*1025	12.0 (20.09)	MS
*O. minuta*1028	24.66 (29.75)	S
O. malphu	19.33 (26.00)	S
O. marionalis	2.66 (9.27)	R
*O. nivara*1054	28.0 (31.91)	S
*O. nivara*1055	42.66 (40.76)	HS
O. officinalis 1040	24.33 (29.55)	S
O. officinalis 1041	65.66 (54.18)	HS
O. officinalis 1042	29.33 (32.76)	S
O. officinalis 1065	55.33 (48.08)	HS
O. punctata	62.0 (51.98)	HS
*O. rufipogon*1011	3.33(10.15)	R
*O. rufigo*1060	44.0 (41.54)	HS
*O. seneifoxthina*1049	28.66 (32.35)	S

(HS= Highly susceptible, MS= Moderately susceptible, R= Resistant, S=Susceptible)

Table 2: Reaction of wild rice species to leaf blast

Number of Wild Rice Species	Reaction to Blast Disease
08	HS
11	S
02	MS
06	R

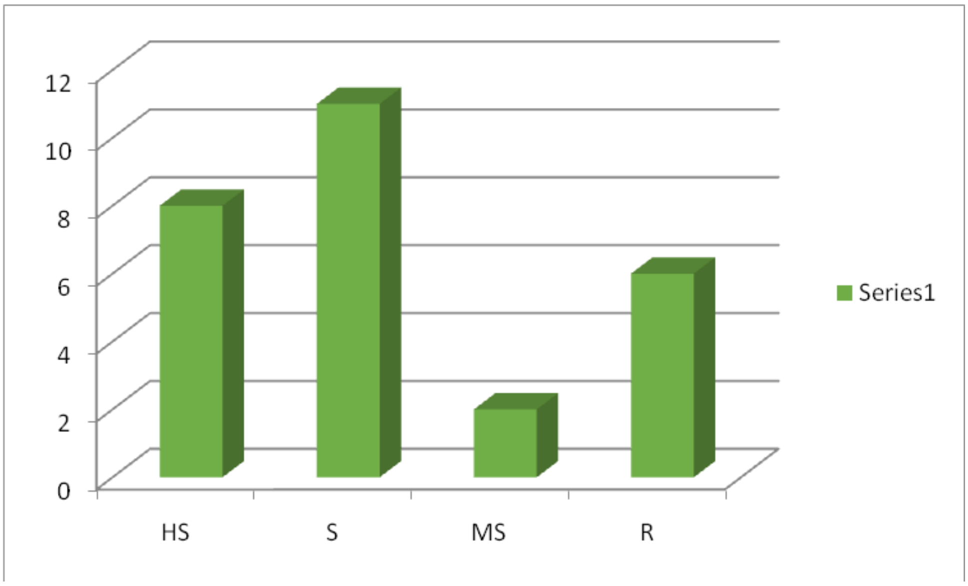

Figure 1: Number of wild rice cultivar to blast disease reaction

DISCUSSION

This study highlights the importance of wild rice species as alternative hosts harboring pathogens of cultivated rice and the likely disease threats to expansion of cultivated rice into the same region(s) where wild rice is endemic. Eight species viz. *O. australiensis* 1061, *O. barthii*, *O. latifola* 1005 and *O. nivara*1055, *O. officinalis* with accession no. 1041 and 1065, *O. punctata* and *O. rufigo* 1060 were found to be highly susceptible (HS), 11 species were susceptible namely *O. afla*, *O. austaliansis*1062, *O. brachyantha* ,*O.brschyara*, *O. corolata* , *O. minuta*1028 , *O. malphu*,*O. nivara*1054,*O. officinalis* 1040, *O.* officinalis 1042 and *O. seneifoxthina* 1049 while two wild species i.e., *O. longistaminata* 1013 and *O. minuta* 1025 showed moderately susceptible (MS). Out of the wild rice species tested only six (*O. eichingeri*, *O. grandiglumis* 1085, *O. latifolia* 1007, *O. longistaminata* 1026, *O. meridionalis and O. rufipogon* 1011) showed resistance to blast isolated IC-1. A survey of fungal diseases in North Queensland, Australia in May 2014 also reported diverse fungal genera on wild as well as cultivated rice but a single blast isolate of *M. grisea* was found from wild rice. Crop domestication process has left significant genetic diversity in their wild progenitor species which has the potential to use for developing varieties to various biotic and abiotic stresses in the crop development program. Literature data reported about the collection and morphological description of 614 wild rice accessions of *O. rufipogon* and *O. nivara* and their intermediate types from various agro-climatic regions (Pak et al. 2017).

CONCLUSION

This study highlights the importance of wild rice species as alternative hosts nurturing the pathogens of cultivated rice that can be a threat to the cultivated rice in the particular region, where wild rice is endemic. The resistant wild rice cultivars can also be used to develop blast resistant cultivated rice cultivars by incorporating the resistant genome from the resistant wild rice accessions.

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