New sources identified for resistance to brown spot disease in rice

wn spot, caused by *Bipolaris oryzae* (Shoem), is a widespread disease in all rice-growing areas of the world. However, only limited information is available on resistance sources and their genetics. fungus can infect rice plant in all stages of growth — from germination to maturity. It causes seedling blight, reduces photosynthetic rate at the vegetative phase, poor grain filling, and grain coloration at the reproductive phase, and ultimately brings about considerable yield loss both in quality and quantity. This disease is more prevalent in rainfed lowlands and uplands or in rice areas with r soil condition. Rice plants get infected by externally and internally seedborne infection, spore inoculum on crop/weed residues and soils, as well as airborne spore inoculum. In this study, we eloped a screening protocol for brown spot development and assessment at the seedling stage, identified sources of resistance, and produced mapping populations for detecting linkage with lecular markers.

Pathogen

re than 40 isolates of the fungus from leaves re collected from farmers' field in Laguna, akan, and Cagayan in the Philippines. enty-two monoconidial pathogenic isolates re used to study the colony and conidial aracteristics and some differences were served. Eighteen isolates from the 22 isolates re used to identify the sources of resistance.

Bipolar germination of conidia at 40X

Host

A diverse germplasm of rice—comprising 22 modern high-yielding varieties, 6 traditional, wild species representing 9 genomes, 11 introgression lines, 10 hybrids, 5 CMS lines, and 10 restorers—was evaluated with a newly developed screening protocol at seedling stage. Significant differences were obtained between cultivars/lines for individual isolates based on lesion type and lesion density after seedling inoculation.

Inoculation of rice seedlings

Host-pathogen interaction

The 22 isolates were tested on six cultivars identified as resistant, susceptible, and intermediate. They showed a significant cultivar-isolate interaction, and susceptibility of isolates differed significantly on highly susceptible cultivar IR36 and UPLRi 7. Traditional cultivar Dinorado was found to be highly resistant (lesion type ratio =<0.25) to all 22 isolates. Besides Dinorado, wild species (*Oryza minuta*) was also found to be resistant to all 22 isolates tested.

Protocol for screening

- Multiplication of monoconidial isolates on freshly prepared PDA with pH around 6 in culture growth room under NUV and fluorescent light with temperature 28 ± 2 ºC in the daytime and 12 h photoperiod.
- Inoculation of 14 to16-d-old seedlings (from sowing) with spore concentration around 10^4 at 0.1 ml seedling^{-1} prepared from 10-d-old culture plate. Spores were washed two times by centrifugation with sterile water before estimation of final concentration. Inoculation of control was with sterile water.
- Incubation of inoculated seedlings in humid chamber in darkness for 40 h at room temperature 18±2 ºC.
- Incubation of seedlings for disease development in seedling growth room for 7 d at temperature 28± ºC, humidity above 80%, and 12-h photoperiod.
- Disease assessment on 2nd leaf from the top as number and size of lesion cm^{-2} (small = pin head, medium = up to 1 mm linear length, and large = above 1 mm linear length), which were converted into lesion type ratio (LTR) as large lesion cm^{-2} / (small lesion cm^{-2} + medium lesion cm^{-2}). Resistance = LTR <0.25. Intermediate = LTR 0.25-0.50. Susceptible = LTR >0.50.

Infected leaf for isolation of single spore

Comparison of different media for sporulation

Comparison of spore concentration 10^3, 10^4, and 10^5 ml^{-1} for inoculation

Comparison of 10, 14, and 21-d-old seedlings for symptom expression

Genetic analysis

F_3 progenies were produced from crosses between Dinorado (resistant) and IR36 (susceptible). Two hundred F_3 lines were screened using aggressive isolate SM2 to determine the inheritance of resistance. Of the 150 SSR markers tested, 10 revealed polymorphism between resistant and susceptible bulk comparable with that of the parents. Further analysis is in progress to tag the gene governing resistance to brown spot.

Confirmation of hybrids of IR36 (P1) and Dinorado (P2) by SSR marker RM 348

Disease expressions on $F_{3:4}$ resistant parent (P1) and susceptible parent (P2) with isolate SM2

SSR (RM 277) analysis of resistant bulk (1), susceptible bulk (2), resistant parent (3), and susceptible parent (4)

Screening of F_3 populations

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