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Short Communication

SALIENT RESEARCH FINDINGS ON VARIABILITY, FUNGICIDAL SENSITIVITY AND PROFILING OF *AVR* GENES AMONG ISOLATES OF RICE BLAST PATHOGEN (*MAGNAPORTHE ORYZAE*)

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Rice blast caused by *Magnaporthe oryzae* (Herbert) Barr is one of the most economically significant diseases of rice (Silva *et al*., 2009). The pathogen attacks the crop at all the growth stages and causes huge grain yield losses. Keeping in view the importance of the disease in rice production, the present investigation was carried out to identify the causal organism associated with rice blast in different regions of Telangana and Andhra Pradesh; to study morphological and genetic diversity among rice blast pathogens; profiling of avirulent genes in pathogenic isolates; and evaluating the *in vitro* sensitivity of *M. oryzae* isolates to different fungicides. The present research work was carried out at Department of Plant Pathology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar and Indian Institute of Rice Research (IIRR), Rajendranagar, Hyderabad. Khammam, Warangal, Nalgonda, Mahbubnagar, Medak, Adilabad, Rangareddy, Nizamabad districts of Telangana and Guntur, Kurnool, Nellore, West Godavari, Srikakulam districts of Andhra Pradesh were selected for collecting the foliar disease samples from rice blast affected fields. The *M. oryzae* cultures were isolated and purified on Oat Meal Agar medium (OMA) by adopting single spore isolation method. The single spore cultures were maintained on OMA medium, preserved on filter paper discs and stored at 4°C temperature.

Variations in morphological characters of *M. oryzae* isolates such as colony growth, colour, size of the conidia and sporulation index were observed. The growth of the mycelium was measured at 24 hrs interval and from the radial growth as measured after 15 days ranged from 71.67 mm to 87.67 mm. Maximum radial growth was recorded in isolate MG11 (87.67 mm), whereas minimum growth was observed in isolate MG18 (71.67 mm). The colony texture of *M. orzyae* isolates varied from rough surface to smooth surface. Further, the conidial size ranged from 8.6-11.8 µm in length and 3-3.8 µm in width in different isolates. The shape of conidia in all the isolates was pyriform, and were hyaline to pale olive, 2 septate and 3 celled. Further, the sporulation of isolates as measured at 15 days after incubation indicated that 11 isolates (MG1, MG2, MG3, MG5, MG6, MG7, MG12, MG13, MG14, MG18 and MG19) exhibited "Good" sporulation index (rating of 3). Only four *M. oryzae* isolate *viz*., MG4, MG8, MG11 and MG16 exhibited "Excellent" sporulation index (rating of 4). Further, the four isolates *viz*., MG9, MG 10, MG15 and MG17 have exhibited "Fair" sporulation with a scale of 2. Only one out of the 20 isolates, MG20 exhibited "Poor" sporulation index (rating of 1). Morphological and cultural variability in *M. oryzae* have earlier been reported among isolates within India (Srivastava *et al*., 2014) as well as in other countries (Silva *et al*., 2009).

Besides morphological variability, our studies also involved determining the genetic variability among the blast pathogenic isolates under study. Our results indicated that the genetic diversity of *M. oryzae* isolates from different locations using three MGM markers showed less polymorphism at DNA level. The cluster analysis of MGM data grouped the isolates on the basis of their origin with few exceptions. All the isolates were grouped into three major groups. Cluster-I consists of 11 isolates, which were further divided into two sub clusters (IA and IB). The sub cluster IA consists of four isolates, in which two isolates (MG1 and MG5) were collected from Khammam, one isolate (MG2) from Medak and one isolate (MG3) from Atmakur. All the isolates of this sub cluster shares 65% similarity. Whereas, sub cluster IB is comprised of seven isolates (MG7, MG11, MG14, MG15, MG16, MG18 and MG19) collected from Adilabad, IIRR, Nizambad, Palem, Madhira, Warangal and Piduguralla. All the isolates of this sub cluster shares 35% similarity.

Cluster-II consists of 7 isolates, which were further divided into two sub clusters (II A and II B). The sub cluster II A consists of five isolates in which, isolate MG4 was from Bapatla (Guntur), isolate MG9 was from Nandyal, isolate MG10 was from Mahanandi, isolate MG17 was from Aleru (Nalgonda) and isolate MG20 was from Ragolu (Srikakulam). All the isolates of this sub cluster share 52% similarity. Whereas, sub cluster II B is comprised of 2 isolates (MG6 and MG8) from Nalgonda and Mahbubnagar. The two isolates of this sub cluster shares 28% similarity. Cluster-III consists of 2 isolates, MG12 from Nellore and MG14 from Maruteru sharing 100% similarity.

Twenty isolates were screened for the presence of 5 *AVR* genes *viz*., *AVR-Pi7, AVR-Pia*, *AVR-Pit, AVR-MedNoi* and *AVR-Pi15*.*AVR-Pi7* was foundin following 11 isolates *viz*., MG4, MG9, MG12, MG13, MG14, MG15, MG16, MG17, MG18, MG19 and MG20. *AVR-Pit* gene was foundin three isolates *viz*., MG15, MG16 and MG17 and *AVR-Pia* in three isolates *viz*., MG5, MG9 and MG12. The isolates MG15, MG16 and MG17 contained two avirulent genes *AVR-Pi7* and *AVR-Pit*. These genetic studies among rice blast pathogenic isolates help in comprehensive understanding on the variability. Further, these studies will be useful in devising strategies for area-wise blast management in rice. Earlier studies from India (Mohan *et al*., 2012) and other countries (Suzuki *et al*., 2009) have also reported genetic variability among *M. oryzae* isolates. Especially, the *AVR* genes and their profiling shall help in reliable determination of variability.

The *in vitro* sensitivity of *M. oryzae* isolates to different fungicides such as carbendazim, tricyclazole, isoprothiolane and kasugamycin was tested at different concentrations using poisoned food technique. Carbendazim (125, 250 and 500 ppm) showed complete inhibition of the radial growth of all the *M. oryzae* isolates at all the tested concentrations. In case of tricyclazole, isoprothiolane and kasugamycin, the isolates showed differential reaction indicating variability among the isolates in terms of sensitivity to different concentrations of these fungicides under study. For tricyclazole, at 50 ppm, the per cent mycelial inhibition for the isolates ranged from 25.57 to 31.90. The minimum inhibition was recorded by the isolate MG1 and maximum inhibition was recorded by MG10. At 100 ppm, per cent mycelial inhibition ranged from 45.50 to 52.27. Minimum inhibition was recorded by isolate MG13 and maximum inhibition was recorded by MG16. At 200 ppm, per cent mycelial inhibition ranged from 56 to 60.38. The minimum inhibition was recorded by the isolate MG1 and maximum inhibition was recorded by MG15. For isoprothiolane, at 100 ppm, the per cent mycelial inhibitions of isolates ranged from 76.42 to 86.60. The minimum inhibition was recorded by the isolates MG1 and MG7 (76.42%) and maximum inhibition was recorded by MG2 (86.22%), MG8 (86.00%) and MG19 (86.60%). At 200ppm, all the isolates showed high mycelial inhibition ranging from 84.52 to 91.78%. The maximum inhibition was recorded with MG6 (91.78%). The isolates MG6, MG16, MG17 and MG19 showed complete inhibition at 500 ppm concentration. At this concentration the isolates MG2, MG3 and MG14 have recorded 95.67, 95.00 and 95.00 per cent inhibitions in mycelial growth respectively.

All the isolates showed lower mycelial inhibition when screened with kasugamycin. At 100 ppm, the isolate MG20 (Ragolu of Srikakulam) has recorded least inhibition of 1.48%, while isolates MG4 and MG7 showed highest per cent inhibition of 10.52. The per cent inhibition among all the isolates were significant. At 200 ppm concentration the isolate MG8 has recorded the least per cent inhibition of 10.52 while isolate MG9 showed highest percent inhibition of 26.34. Similarly, at 500 ppm concentration the percentmycelial inhibition ranged from 40.67 to 49.07. The minimum inhibition was recorded by MG20 (40.67) and maximum inhibition was recorded by MG13 (49.07). Overall, our results indicated that carbendazim is highly effective at all the concentrations tested followed by isoprothiolane. The fungicide, kasugamycin is least effective at 100 and 200 ppm concentration and tricyclazole is least effective at 50 ppm. Significant difference was observed with these fungicides on per cent mycelial growth inhibition among the isolates. Earlier studies by several researchers also reported the sensitivity of *M. oryzae* to various degrees to commonly used fungicides (Singh *et al*., 2014).

Overall, from our studies, it can be concluded that there was no correlation between the *Avr* genes in the pathogenic isolates and the PDI on rice cultivars. In order to conclude the role of *Avr* genes on acquiring fungicide resistance or virulence on different rice cultivars, studies should be carried out with large number of *Avr* genes present in the population of *M. oryzae*. As the present study was carried out with only five avirulence genes, it was not possible to judge the role of *Avr* genes either on acquisition of resistance against fungicides or gaining virulence.

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