CSIRO PUBLISHING
www.publish.csiro.au/journals/apdn *Australasian Plant Disease Notes*, 2008, **3**, 75–77

Detection and transmission of *Microdochium oryzae* from rice seed in Argentina

S. A. Gutiérrez[A,D], *E. M. Reis*[B] *and M. A. Carmona*[C]

[A]Cátedra de Fitopatología, Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, Sargento Cabral 2131 (3400), Corrientes, Argentina.
[B]Fitopatología, Facultade de Agronomia e Veterinaria, UPF, Cx. Postal 611, 99001-970, Passo Fundo, Brasil.
[C]Fitopatología, Facultad de Agronomia, Universidad de Buenos Aires, Av. San Martín 4453 (1417), Ciudad de Buenos Aires, Argentina.
[D]Corresponding author. Email: suarroz@yahoo.com.ar

Abstract. *Microdochium oryzae* is the causal agent of leaf scald of rice and is present in all stages of the crop. The seeds are considered the major source of inoculum. The fungus was isolated from diseased seeds obtained from commercial crops in the Province of Corrientes, Argentina. The pathogen was transmitted from seed to coleoptile tips in symptomless seedlings at a rate of 2.18%. Pathogenicity of the fungus was confirmed by spraying a spore suspension on healthy 15-day-old rice seedlings.

During the years 2005–07, routine screenings for seedborne fungi in samples of rice crops from the Corrientes Province, north-east Argentina, revealed a high incidence of *Microdochium oryzae* (Hashioka and Yokogi) Samuels & I.C. Hallett (obligate synonyms: *Gerlachia oryzae* and *Rhynchosporium oryzae*), the causal agent of leaf scald of rice.

Rice (*Oryza sativa*) is an important commercial crop in Corrientes Prov., and leaf scald has been a disease of increasing importance through this Province. The symptoms, characterised by zonated lesions, alternating light tan to dark brown bands, that dry out giving the leaves and foliar sheaths a scalded appearance, can be observed between maximum tillering stage and mature plants (Mazzanti de Castañón and Gutiérrez 2001). This disease has been reported worldwide in rice-growing regions (Farr *et al*. 2008) and seeds are considered the most important inoculum source (Ou 1985; Webster and Gunnell 1992). The teleomorph stage, *Monographella albescens* (Thüm) V.O. Parkinson, Sivan. & C. Booth (synonym: *Metasphaeria albescens* Thüm), was recently reported in green and senescent leaves of rice crops close to ripening in Argentina (Gutiérrez *et al*. 2007).

The objectives of the present work were: (1) isolate *M. oryzae* from rice seed; (2) to evaluate its possible transmission from diseased seeds to coleoptiles; and (3) to confirm its pathogenicity on rice seedlings.

Rice seeds of the cultivar Taim, obtained from commercial crops in the Mercedes locality (Corrientes Prov., Argentina), were incubated in agar medium following standard procedures (Mew and Misra 1994; Mathur and Kongsdal 2003). Four hundred seeds were surface-disinfested by soaking in 2.5% sodium hypochlorite solution for 10 min, followed by three rinses of 5 min each in sterile distilled water. Seeds were plated, 10 per plate, in Petri dishes containing 2% bean agar (BA) with streptomycin sulfate, pH adjusted to 6.0. Plates were maintained for 10–12 days in a climatic chamber at 25 ± 2°C, with photoperiods of 12 h provided by two Philips lamps (model TL40W/52, near-ultraviolet light). To determine the incidence of *M. oryzae*, the Petri dishes containing the seeds were observed under a stereoscopic microscope.

Studies of seed transmission were carried out using the same rice seed lot. Six hundred seeds, one seed per pot, were sown in plastic pots containing a sterilised soil mixture (soil : sand : peat, 1 : 1 : 1) and maintained in a greenhouse at 25–34°C. Symptomless coleoptiles (250) were randomly selected and cut off 1.0 cm above the soil, at 15 and 30 days after sowing. The coleoptiles were disinfested with 2.5% sodium hypochlorite following the same procedure as described above and then were plated on BA medium, at 10 per plate and incubated for 6–8 days. Transmission efficiency (TE) of the fungus from seeds to coleoptiles was estimated by the formula: $TE = C/S \times 100$, where C is the incidence of colonised coleoptiles and S the incidence of infected seeds (Reis *et al*. 1999).

To confirm pathogenicity, the fungus was previously isolated on 1.5% potato glucose agar (PGA), pH adjusted to 6.0, and was then inoculated with a spore suspension (1×10^6 spores/mL) onto healthy 15-day-old rice seedlings grown in plastic pots containing the same sterilised soil mix described above. The inoculum was obtained from 10-day-old sporulating colonies of the fungus grown in PGA. The controls used were rice seedlings sprayed only with sterile distilled water. The seedlings were then enclosed in plastic bags for 72 h after inoculation to maintain high humidity and the pots were maintained in a greenhouse at 24 ± 2°C.

Disease incidence was 55%. Symptoms and signals observed were: ungerminated seeds, decayed coleoptiles or root rot with

 10.1071/DN08030 1833-928X/08/010075

white mycelium and typical salmon-cream conidial masses (Fig. 1). Two kinds of symptoms were observed in the spray-inoculated seedlings 6 days after inoculation: typical scald lesions with zonation and leaf tip blight without zonation (Fig. 2). The fungus was reisolated on PGA. The cultural characteristics, whitish cotton-like colonies with creamy or salmon masses, reverse salmon, and microscopic characteristics, conidia hyaline, one-septate, cylindrical, slightly curved, $9–13 \times 3–4\,\mu m$, confirmed its pathogenicity and Koch's postulates.

Microdochium oryzae was not detected in coleoptiles after sowing 15 days later; nevertheless the fungus was detected at a TE of 2.2% 30 days later. The transmission efficiency of *M. oryzae* (2.2%) was low compared with the high incidence (55%) of infected seeds observed in the agar method. The transmission process of seedborne fungi could be affected by several factors. However, the fungus location is considered the most important in the transmission process of seedborne fungi (Neergaard 1979; Maude 1996; Agarwal and Sinclair 1997). In order to determine the location of *M. oryzae* on seeds, Singh and Sen Gupta (1981) used surface-sterilised and unsterilised seed and found the fungus only in the unsterilised seed. They therefore determined that the fungus is located externally. Nevertheless, Mia and Safeeulla (1984) and Mia *et al*. (1986), as well as Thomas (1984) reported that *M. oryzae* occurred frequently in seeds both externally and internally and that the fungus could be present in all parts of the seed (husk, endosperm and embryo) (Maude 1996). Also, according to Thomas (1984) symptoms of leaf scald are rarely apparent before the maximum tillering stage. In rice crops of Corrientes Prov., the symptoms caused by *M. oryzae* are often seen in leaves close to the soil towards the end of the tillering stage and the symptoms continue to develop until the panicle emerges, which is when the fungus infect the rice grains (Mazzanti de Castañón and Gutiérrez 2001; Gutiérrez 2002).

Fig. 1. Pionnotes of *Microdochium oryzae* on rice seed.

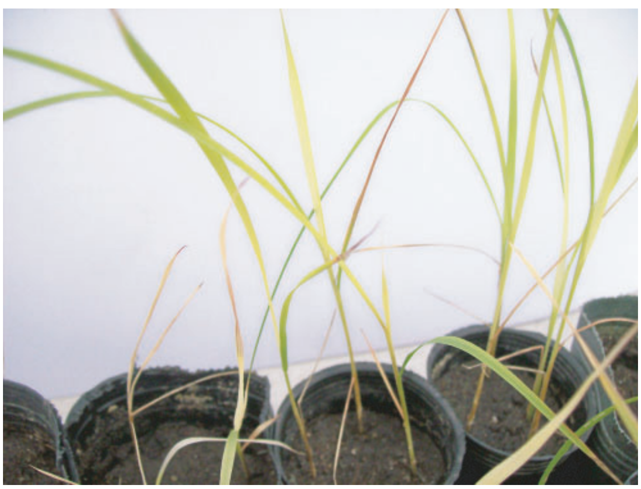

Fig. 2. Symptoms of leaf scald in inoculated seedlings.

Microdochium oryzae is considered to be a weak pathogen because some kind of injury is required for successful pathogenesis (Rao *et al*. 1977; Boratynski 1979). However, in our survey we found that injury is not necessary for disease development; the symptoms were observed in all inoculated seedlings (incidence 100%). These results indicate that spraying a spore suspension on rice seedlings could be used as an inoculation method for rapid screening of rice varieties for resistance to leaf scald in a greenhouse.

Microdochium oryzae was frequently detected in rice seed samples from Corrientes Prov. and it was able to be transmitted from seeds to coleoptiles. However, there is little published information about the epidemiological role of the seeds as a primary inoculum source for field epidemics of leaf scald in Argentina, and as the seed could introduce the pathogen in new fields, more studies are needed to elucidate this relationship as well as other factors that could affect field epidemics of *M. oryzae* in the north-east region of Argentina.

All isolates of *M. oryzae* obtained in this work were deposited in the Fungal Collection of the Laboratory of Plant Pathology, Faculty of Agronomy, University of North-east Corrientes Prov., Argentina.

References

Agarwal VK, Sinclair JB (1997) 'Principles of seed pathology.' 3rd edn. (CRC Press: Boca Raton, FL) 555 pp.

Boratynski TN (1979) La escaldadura de la hoja de arroz *Rhynchosporium oryzae* Hashioka e Ikegami, en Costa Rica. *Agronomia Costarricense* **3**, 21–27.

Farr DF, Rossman AY, Palm ME, McCray EB (2008) Fungal databases: Systematic mycology and microbiology. Available at http://nt.ars-grin.gov/fungaldatabases/ [Verified 27 May 2008]

Gutiérrez SA (2002) Micoflora asociada al manchado del grano de arroz. In 'I Jornadas Fitosanitarias Argentinas'. p. 29. (Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto: Río Cuarto, Córdoba)

Gutiérrez SA, Reis EM, Carmona MA (2007) First record of *Monographella albescens* in Corrientes Province, Argentina. *Australasian Plant Disease Notes* **2**, 19–20. doi: 10.1071/DN07008

Mathur SB, Kongsdal O (2003) 'Common laboratory seed health testing methods for detecting fungi.' 3rd edn. (Danish Government Institute of Seed Pathology for Developing Countries: Copenhagen) 427 pp.

Maude RB (1996) 'Seedborne diseases and their control: principle and practice.' (CAB International: Wallingford, UK) 293 pp.

Mazzanti de Castañón MA, Gutiérrez SA (2001) Enfermedades del cultivo del arroz en Argentina. *Fitopatologia Brasileira* **26**, 471. [Abstracts]

Mew TW, Misra JK (1994) 'A manual of rice seed health testing.' (International Rice Research Institute: Manila) 113 pp.
Mia MAT, Safeeulla KM (1984) Leaf scald of rice in Karnataka, India. *International Rice Research Notes* **9**, 19–20.
Mia MAT, Safeeulla KM, Shetty HS (1986) Seed-borne nature of *Gerlachia oryzae*, the incitant of leaf scald of rice in Karnataka. *Indian Phytopathology* **39**, 92–93.
Neergaard P (1979) 'Seed pathology. Vol. 1.' (The MacMillan Press Ltd: London) 861 pp.
Ou SH (1985) 'Rice diseases.' (Commonwealth Mycological Institute: Kew) 380 pp.
Rao PS, Hasanuddin A, Shagir S (1977) Leaf scald of rice, a new disease in Indonesia. *Plant Disease Reporter* **61**, 294–295.
Reis EM, Barreto D, Carmona MA (1999) 'Patologia de semillas en cereales de invierno.' (Gráfica Condal SRL: Buenos Aires, Argentina) 94 pp.
Singh AS, Sen Gupta PK (1981) Transmission of *Rhynchosporium oryzae* Hashioka and Yokogi by seed. *International Rice Research Notes* **6**, 11.
Thomas MD (1984) Dry-season survival of *Rhynchosporium oryzae* in rice leaves and stored seeds. *Mycologia* **76**, 1111–1113. doi: 10.2307/3793027
Webster RK, Gunnell PS (1992) 'Compendium of rice diseases.' (The American Phytopathological Society: St. Paul, MN) 92 pp.

Manuscript received 27 March 2008, accepted 23 May 2008