J Phytopathol **158**:523–526 (2010)
doi: 10.1111/j.1439-0434.2009.01651.x


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# Methods for Detection of *Alternaria padwickii* in Rice Seeds

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Received June 4, 2009; accepted October 19, 2009

**Keywords:** *Oryza sativa*, pathogen, seed health testing, seedborne pathogens

## Abstract

The fungus *Alternaria padwickii* has been frequently detected in seed tests of rice collected from commercial crops in Corrientes Province, Argentina. This pathogen causes germination inhibition, seedling death or spotted grains and is the causal agent of *Alternaria* leaf spot. The pathogen survives as mycelia and sclerotia on seeds, plant debris and soil. Four detection methods were compared in laboratory tests, to select the best for a quick identification of the fungus in seeds. The methods were (i) Blotter Test (ii) Potato glucose agar, (iii) Bean agar (BA) and (iv) Malt extract agar. Twenty seed samples of different varieties of rice collected from Empedrado, Goya, Itá Ibaté, La Cruz, Mercedes, Paso de los Libres and Perugorría localities (Corrientes, Argentina), were analyzed in the assays. The ANOVA test and the Tukey multiple range test were applied on the data to compare the *A. padwickii* incidence among the varieties and detection methods. BA method was found more sensitive than other methods for *A. padwickii*. The incidence values ranged from 3.6 to 76%. The statistical analysis demonstrated that the BA method was the most efficient for the detection of seed pathogens, and it could be useful in studies of transmission and chemical control.

## Introduction

Rice (*Oryza sativa* L.) is one of the most important crops in the North-east region of Argentina. It is produced in Corrientes, Entre Ríos, Chaco, Formosa and north of Santa Fe Provinces. Corrientes Province is the national first producer with 82 102 hectares (Asociación Correntina de Plantadores de Arroz, ACPA 2009 http://www.acpaarrozcorrientes.org.ar).

The rice seeds carry pathogenic and saprophytic micro-organisms according to the ecological regions of crop cultivation. Seeds are considered the main inoculum source and are responsible for dissemination of the pathogens (Ou 1985; Mew and Misra 1994; Mew and Gonzales 2002). Richardson (1990) mentioned that rice seeds can transmit 75 different micro-organisms, whose vegetative and reproductive structures have been found adhering to the grain external surface, inside the glumes, in coleoptiles or in the embryo.

In Argentina, Gutiérrez et al. (2002) identified fungal species associated with rice seeds from the north-east of the country. One of the most important seed pathogens was *Alternaria padwickii* (Ganguly) M. B. Ellis (Syn *Trichoconis padwickii* Ganguly), because of its incidence and prevalence in seed samples. *Alternaria padwickii* was observed causing rice grain spot and leaf spot (Mazzanti de Castañón and Gutiérrez de Arriola 2001). The pathogen was identified for the first time in Argentina by Winter et al. (1974), who analyzed rice seeds from Corrientes Province and observed up to 64% seed incidence. *Alternaria padwickii* has been detected in rice seeds in Africa, Asia and Latin America, either in irrigation or upland crops, where it is responsible for yield losses by causing seed, root and coleoptile rot as well as seedling decay (Gutiérrez et al. 2002; Ellis 1971; Farias et al. 2007; Kimati et al., 2005; Mew and Gonzales 2002; Mew and Misra 1994; Morato de Amaral 1987; Ou 1985; Rodríguez et al., 1988; Webster and Gunnell 1992). The damage is significant when the seeds are highly infected. According to Islam et al. (2000), the incidence of *A. padwickii* in rice seeds may range from 1.33 to 44% depending on the variety used. Incidence levels of up to 76% have been reported. The symptoms were observed on rice seedlings, adult plant leaves and grains (Mathur et al. 1972; Ou 1985).

The pathogen survives in the soil and on old rice straw and causes infection in the next season. Infected seeds are the source of primary inoculum. The patho-

gen infects wild grass species in rice fields (Padwick 1950; Ou 1985).

Blotter and agar methods have been considered the most sensitive to detect *A. padwickii*. Incidence values ranged from 1 to 52% (Mathur and Neergaard 1967; Guerrero et al. 1972; Kulik 1975; Shetty and Shetty 1985; Jayaweera et al. 1988; Shetty and Shetty 1988, Mathur and Kongsdal 2003).

The objective of this study was to compare different methods to detect *A. padwickii* in rice seeds collected from rice crops of Corrientes Province, Argentina. Some preliminary work in this respect was previously reported (Gutiérrez et al. 2008).

## Materials and Methods

Twenty seed samples of different varieties of rice (CT 6919, El Paso 144, Fortuna, Irga 417, Linea 363, Puitá, RP2, Supremo 1, Supremo 13 and Taim) naturally infected with *A. padwickii* were analyzed. The experimental design was a completely randomized design with two factors (methods and varieties) and their interaction.

The following assay methods were used for the analysis: (i) Blotter test (BT), (ii) Potato glucose agar (PGA), 1.5%, pH6, (iii) Bean agar (BA), 3%, pH6 and (iv) Malt extract agar (MEA), 2%, pH6 (Mew and Misra 1994; Mathur and Kongsdal 2003). Streptomycin sulphate (200 ppm) was added to the agar methods. For each method, two hundred seeds were surface sterilized for 10 min by soaking in 2% sodium hypochlorite solution. Seeds were then rinsed three times in sterile distilled water for 5 min each (modified by Mathur and Kongsdal 2003). The seeds were then plated on 9-cm Petri dishes; for the BT, 25 seeds were assayed per Petri dish and 10 seeds for the agar methods.

Plates were incubated at 24 ± 2°C in a growth chamber for up to 14 days with a 12-h photoperiod provided by two Philips lamps (model TL40W/52, near-ultraviolet light; Philips, Eindhoven, the Netherlands).

The incidence of *A. padwickii* on rice seeds was determined using stereobinocular microscope, and it was expressed as the percentage of seeds colonized by the fungus.

Data were analyzed by ANOVA and Tukey test 5%. To perform the analysis, data were transformed into $\sqrt{x} + 0.5$.

## Results

The efficiency of the compared methods for detecting *A. padwickii* on rice seeds is presented in Table 1. Results of the four types of assays used to detect *A. padwickii* differed significantly ($P < 0.05$). The average incidences were 1% in the MEA method (0–5%), 4.38% in the BT (0–10%), 13.5% in the PGA (2–45%) and 29.82% in the BA method (3.70–76%) assays, respectively.

In infected seeds, the pathogen caused root and coleoptile decay or inhibited germination.

Cotton-like and greyish aerial mycelial growth, conidiophores and conidia, and/or sclerotia were visible on the infected seeds of all detection methods when using a dissecting microscope. The fungus developed two types of growth on the infected seeds, one with more conidia and less mycelium and the other with more mycelium and sclerotia and less conidia. Conidiophores of *A. padwickii* are flat and straight, with straight to slightly curved, fusiform and flat conidia, with a long, light brown, rostrate beak and 3–4 transverse septa of 95–160 × 11–18 μm. Sclerotia were

Table 1
Incidence (%) of *Alternaria padwickii* in rice seeds in different health-testing methods

| Varieties | Location | MEA | BT | PGA | BA | Means (%) |
|---|---|---|---|---|---|---|
| CT 6919 | Itá Ibaté | 2.5 | 5.0 | 26 | 76.0 | 27.37 a |
| RP2 | Mercedes | 0.0 | 8.0 | 20 | 61.6 | 22.40 ab |
| Taim | Santo Tomé | 1,5 | 8.0 | 45 | 61.5 | 29.00 ab |
| Taim | Mercedes | 2.0 | 9.5 | 25 | 46.0 | 20.60 ab |
| Taim | La Cruz | 1.5 | 8.5 | 20 | 45.0 | 18.75 ab |
| Fortuna | Perugorría | 0.0 | 6.5 | 25 | 42.5 | 18.50 ab |
| Supremo 1 | Mercedes | 2.0 | 8.5 | 20 | 40.0 | 17.60 ab |
| Taim | Perugorría | 0.0 | 6.0 | 20 | 32.5 | 14.60 ab |
| Supremo 13 | Empedrado | 5.0 | 10 | 22 | 29.5 | 16.60 b |
| Linea 363 | Goya | 2.0 | 5.0 | 15.4 | 28.0 | 12.90 b |
| Puitá | Itá Ibaté | 0.0 | 0.0 | 10 | 28.0 | 9.50 b |
| Taim | Mercedes | 2.0 | 5.0 | 10 | 25.0 | 10.50 b |
| Supremo 13 | Mercedes | 0.0 | 2.0 | 10 | 24.4 | 9.10 b |
| Supremo 13 | Paso de los Libres | 0.0 | 0.0 | 10 | 19.0 | 7.25 b |
| Supremo 13 | Goya | 0.0 | 0.0 | 6.4 | 14.4 | 5.20 b |
| IRGA 417 | Goya | 0.0 | 0.0 | 2.5 | 10.0 | 3.12 b |
| CT 6919 | Goya | 0.0 | 0.0 | 2.0 | 7.4 | 2.35 b |
| Supremo 13 | Perugorría | 0.0 | 0.0 | 2.0 | 5.0 | 1.75 b |
| IRGA 417 | Mercedes | 0.0 | 0.0 | 2.0 | 3.8 | 1.45 b |
| Taim | Goya | 0.0 | 0.0 | 2.0 | 3.7 | 1.42 b |
| Means | – | 1.0 C | 4.38 BC | 13.5 B | 29.82 A | – |
| CV | | | 15.55% | | | |

MEA, Malt extract agar; BT, Blotter test; PGA, Potato glucose agar; BA, Bean agar.
Means followed by the same letter are not significantly different at $P = 0.05$ (Tukey test).

black, irregular to spherical with reticular walls and 55–180 $\mu$m of diameter.

In the BT method, the infected seeds were externally covered with a whitish, cotton-like mycelium, turning pink in some cases on the paper around the infected seeds. Conidia were also observed on the seeds with this method. The above-described characteristics help to identify the fungus, as reported by to Mathur et al. (1972) and Shetty and Shetty (1988).

When the seeds were applied to agar (PGA, BA and MEA), mycelium with abundant sclerotia formation was observed on the ungerminated seeds and/or on the affected tissues (coleoptiles and roots). Sclerotia immersed in the culture media were observed from the fourth day of incubation. Here, sporulation of *A. padwickii* was only detected when the incubation period was prolonged to 12–14 days.

## Discussion

The BA method was significantly ($P < 0.05$) more efficient than PGA, BT and MEA for pathogen detection on seeds. However, the interaction between the factors methods and varieties was not significant. Similar results were reported by Carmona et al. (1999, 2006) for other fungi belonging to the Dematiaceae family, such as *Drechslera teres* (Sacc). Shoem. and *Drechslera tritici-repentis* (Died.) Drechs. The agar methods, lima-BA and selective medium for *Cochliobolus sativus* (Reis 1983) were more sensitive to detect both pathogens when compared to the BT.

Our results are consistent with results presented by Mathur et al. (1972), Mathur and Neergaard (1967), Mew and Misra (1994) and Shetty and Shetty (1988). They detected *A. padwickii* using different analysis methods.

Mathur and Neergaard (1967) analyzed seed samples from Philippines, India, Portugal and Egypt, using the blotter and agar methods (dextrose potato agar, DPA). They considered the agar method as a macroscopic method for the detection of the fungus on seeds and recommended it as a routine method because the colonies can be easily differentiated from those developed by saprophyte fungi. They also considered that the seed analysts should be trained to identify this pathogen by the cultural characteristics of the colonies developed. Kulik (1975) found that the blotter method modified by freezing was more sensitive than the use of guaiacol agar for *A. padwickii* detection. Jayaweera et al. (1988) used the agar method also (DPA and MEA); the fungus presented a frequency of 8% in DPA but it did not grow in MEA.

Shetty and Shetty (1985) used six analysis methods (BT, 2.4-D, deep freeze, DPA, guaiacol agar and rice extract agar). All the methods, except the guaiacol agar, resulted to be efficient for the detection of the fungus on seeds.

According to Mew and Misra (1994), the BT method is considered as a standard test to detect seedborne fungi that respond to sporulation, while that the agar method detects and identifies seedborne fungi through colony characteristics which they exhibit when grown.

The International Seed Testing Association (2004) proposed the blotter method to work with seeds without previous treatment, as the valid one. However, the BA method assay in this work revealed a higher incidence of seed infection than other methods and allowed *A. padwickii* to be observed easy and rapidly.

The infection levels variation depends on the rice variety, the environmental conditions and crop management (Islam et al. 2000 and Farias et al. 2007). The decrease in seed germination caused by the pathogen might call for crop reseeding and although the seedlings derived from infected seeds die, the pathogen stays within the crop as inoculum source.

The BA method is suitable for routine detection of *A. padwickii* in infected seeds, and can be used for transmission and chemical control assays. The BA method could be useful to avoid the introduction of this pathogen into new areas due to accurate detection of infected seed lots.

According to our results, this study showed that this fungus is widely spread over all the rice-growing areas in Corrientes Province, Argentina, with 100% of prevalence in the sampled rice seeds and with variable infection levels in rice varieties analyzed.

Considering the importance of this pathogen on rice seeds as demonstrated through this work, it is advisable to implement integrated and efficient control strategies to achieve its complete eradication.

### References

Asociación Correntina de Plantadores de Arroz, ACPA. (2009) http://www.acpaarrozcorrientes.org.ar (verified February 26, 2009).

Carmona MA, Barreto DE, Reis EM. (1999) Detection, transmission and control of *Drechslera teres* in barley seed. *Seed Sci Technol* **27**:761–769.

Carmona M, Ferrazini M, Barreto D. (2006) Tan spot of wheat caused by *Drechslera tritici-repentis*: detection, transmission and control in wheat seed. *Cereal Res Commun* **34**:1043–1049.

Ellis MB. (1971) *Dematiaceous Hyphomycetes*. Kew, Surrey, Great Britain, Commonwealth Mycological Institute.

Farias CRJ, Afonso APS, Brancão MF, Pierobom CR. (2007) Ocorrência de *Alternaria padwickii* (Ganguly) em sementes de arroz (*Oryza sativa* L.) (Poaceae) produzidas em quatro regiões orizicolas do Rio Grande do Sul e seu efeito sobre plântulas. *Arq Inst Biol* **74**:245–249.

Guerrero FC, Mathur SB, Neergaard P. (1972) Seed health testing of rice V. Seed-borne fungi associated with abnormal seedlings of rice. *Proc Int Seed Test Ass* **37**:985–997.

Gutiérrez SA, Cúndom MA, Mazzanti de Castañón M. (2002) Hongos en semillas de arroz del noreste de Argentina. *Fitopatología* **37**:156–163.

Gutiérrez SA, Carmona MA, Reis EM. (2008) Detección y transmisión de *Alternaria padwickii* en semillas de arroz. In: *Primer Congreso Argentino de Fitopatología, 28–30 May 2008*. Córdoba, Argentina, pp. 168.

International Seed Testing Association (2004) *International Rules for Seed Testing. Anexe to Chapter 7 Seed Health Testing Methods*. Zürich, Switzerland, ISTA.

Islam MS, Jahan QSA, Bunnarith K, Viangkum S, Merca SD. (2000) Evaluation of seed health of some rice varieties under different conditions. *Bot Bull Acad Sinica* **41**:293–297.

Jayaweera KP, Wijesunera RLC, Medis SA. (1988) Seed-borne fungi of *Oryza sativa*. *Indian Phytopath* **41**:355–358.

Kimati H, Amorin A, Bergamin Filho A, Camargo LEA, Rezende JAM. (2005) *Manual de Fitopatologia* Vol. 2. *Doenças das Plantas Cultivadas*, 4ª ed. São Paulo SP, Agronômica Ceres, 663 p.

Kulik M. (1975) Comparison of blotter and guayacol agar for detection of *Helminthosporium oryzae* and *Trichoconis padwickii* in rice seeds. *Phytopathology* **65**:1325–1326.

Mathur SB, Kongsdal O. (2003) *Common Laboratory Seed Health Testing Methods for Detecting Fungi*, 3rd edn. Copenhagen, Denmark, Danish Government Institute of Seed Pathology for Developing Countries.

Mathur SB, Neergaard P. (1967) Seed health testing of rice II. Seed-borne fungi of rice in Philippines, India, Portugal and Egypt. Investigations on *Trichoconis padwickii*. In: *First International Sympossium on Plant Pathology, 26 December 1966–1 January 1967*. New Delhi, India, pp 69–81.

Mathur SB, Mallya JL, Neergaard P. (1972) Seed-borne infection of *Trichoconis padwickii* in rice, distribution, and damage to seeds and seedlings. *Proc Int Seed Test Ass* **37**:803–810.

Mazzanti de Castañón MA, Gutiérrez de Arriola SA. (2001) Enfermedades del cultivo del arroz en Argentina. *Fitopatol Bras* **26**:471.

Mew TW, Gonzales P. (2002) *A Handbook of Rice Seedborne Fungi*. Los Baños, Laguna, Philippines, IRRI.

Mew TW, Misra JK. (1994) *A Manual of Rice Seed Health Testing*. Manila, Philippines, IRRI.

Morato de Amaral H. (1987) Testes de sanidade de sementes de arroz. In: Soave J, Veloso Da Silva Wetzel MM. (eds) *Patología de sementes*. Campinas, SP, Brasil, Fundaçao Cargill, Capítulo XV, pp 358–370.

Ou H. (1985) *Rice Diseases*, 2nd edn. Kew, Surrey, Great Britain, Commonwealth Mycological Institute.

Padwick GW. (1950) *Manual of Rice Diseases*. Kew, Surrey, Great Britain, Commonwealth Mycological Institute.

Reis EM. (1983) Selective medium for isolating *Cochliobolus sativus* from soil. *Plant Dis* **67**:68–70.

Richardson MJ. (1990) *An Annotated List of Seed-Borne Diseases*, 4th edn. Zürich, Switzerland, The International Seed Testing Association.

Rodríguez HA, Nass H, Aleman L. (1988) Incidencia y control del manchado del grano de arroz. *Fitopatol Venez* **1**:5–7.

Shetty SA, Shetty HS. (1985) New methods to detect seedborne *Trichoconiella padwickii*. *Int Rice Res Notes* **10**:9.

Shetty SA, Shetty HS. (1988) Development and evaluation of methods for the detection of seed-borne fungi in rice. *Seed Sci Technol* **16**:693–698.

Webster RK, Gunnell PS. (1992) *Compendiun of Rice Diseases*. St Paul, MN, USA, APS Press.

Winter WE, Mathur SB, Neergaard P. (1974) Seedborne organism of Argentina: a survey. *Plant Dis Rep* **58**:507–511.