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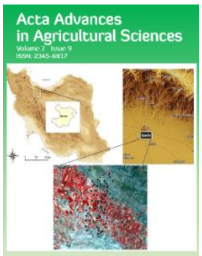

Research Article

Localization of *Trichoconiella padwickii* in the Different Components of Rice Grain

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ABSTRACT

The results of a phytosanitary survey of rice seeds in the province of Corrientes (Argentina) confirmed that *Trichoconiella padwickii* (Ganguly) Jain was present in 100% of the seeds samples analyzed, with values that reached 75% of the incidence. The localization of a pathogen in the different components of the seed provides valuable information for studies on transmission from seeds to seedlings and on the type of treatment to control the pathogen. The aim of the present work was to find out the localization of *T. padwickii* in the different components of rice seeds of the varieties BR-IRGA 424, Puitá INTA CL, Supremo 13 and Taim. Whole seeds were dissected in: palea, lemma, sterile lemmas and grain, and the incidence of the pathogen determined both in the whole seed and in each of the components. Then were incubated in Petri dishes with bean agar for 8-10 days. The results obtained show that the pathogen was present in all the components of the varieties analyzed, although mainly in the most internal components (endosperm and pericarp).

Introduction

In Argentina, the production of rice (*Oryza sativa* L.) comes from the northeastern region, where the rice cultivation area is recently expanding. The total area of rice harvested during the 2013-2014 was of 227.890 ha. The province of Corrientes, with 102.000 ha, represents 45 % of the total sowing area of the country, thus constituting the country's main province for rice production. The provinces of Entre Ríos, Santa Fe, Formosa, and Chaco follow represented 30%, 19%, 4% and 3%, respectively, of the total of rice cultivation area, respectively (ACPA, 2014). Limitations of the cultivation in the region include fungal diseases, mainly those transmitted by seeds (Echeverria *et al.*, 2013; Gutiérrez *et al.*, 2010).

Rice seeds are carriers of many disease-causing pathogens and constitute an efficient means of transport and survival of pathogens in nature, whose epidemic potential varies among species, strains and ecosystems (Archana & Prakash 2013; Huangh *et al.*, 2012; Hungh & Ashok 2004; Islam *et al.*, 2012). According to Mew & Gonzales (2002), more than 80 species of fungi have been detected in rice seeds from different cultivation regions in the world. Among them, *T. padwickii* is widely distributed worldwide, causes death of seedlings, roots and leaf spots and integrates the causal complex of grain spot of rice (Farias *et al.*, 2007). In the Corrientes province, it is considered the main pathogen associated with rice seeds being detected in rice leaves and grain (Gutierrez *et al*., 2010).

According to Singh & Mathur (2004) reported that, it is very important to find out both the exact location of the pathogen in the seed and whether more than one type of inoculum is associated with it. Falzi & Schroeder (1966), showed that the hyphae of *Helminthosporium oryzae* are able to invade the endosperm of the rice grain, causing deterioration in the quality of the seed. Ojeda & Subero (2004) quantified the incidence of *B. oryzae* in the pericarp, endosperm, and embryo of rice seeds and showed that 41-43% of the inoculum was localized in the endosperm. Vu & Somsiri (2006) found *B. oryzae* in all the components of rice seeds (lemma, palea, sterile lemmas, embryo, and endosperm), but recorded the highest values in sterile lemmas. Regarding the localization of *T. padwickii*, Padwick (1950) and Costa (1991) observed sclerotia of fungus in the endosperm of rice grains, whereas Morato de Amaral *et al.*,(1985) concluded that the pericarp of rice seeds contained a greater amount of inoculum than the endosperm, nevertheless the authors not mention the influence of the variety in the location of the fungus. The aim of the present work was to find out the localization of *T. padwickii* in rice seeds of different varieties BR-IRGA 424, Puitá INTA CL, Supremo 13 and Taim from Mercedes Department (Corrientes province, Argentina). The incidence of the pathogen in whole seeds and the different seed components was quantified.

Materials and Methods

Rice seeds of the varieties BR-IRGA 424, Puitá INTA CL, Supremo 13 and Taim were collected from Mercedes (Corrientes) during 2012-2013. Four hundred seeds per variety were dissected into their components: palea, lemmas, sterile lemmas and grain. Each component was sterilized for 10 min by soaking in 2% sodium hypochlorite solution and then grown in Petri dishes with bean agar (3%, pH 6) (40 dishes per component per variety). The dishes were then incubated for 12 days, at 24 ±2 ºC and a photoperiod of 12 hours light and 12 hours darkness (Phillips lamps TLD 36W/54). In addition, 400 seeds not dissecting (whole seed) of each variety were plated (10 seeds per dish with bean agar) and incubated under the same conditions of temperature and photoperiod as described for growing seed components. The identification of the *T. padwickii* was determined using a stereoscopic microscope (40 x) and compound microscope (400 x). The data obtained were expressed as: a) incidence (% In) of each component of seed colonized by the fungus and b) percentage of distribution of total inoculum present in the seed (% Di = % In of each component/ Incidence of fungus in the whole seed). The values in the endosperm and pericarp, which make up the rice grain, were obtained as proposed by Costa (1991), who showed that only 23% of the total inoculum present in the rice grain is located in the endosperm and that the remaining inoculum (77%) is located in the pericarp.

Results

The *in vitro* growth on bean agar of each of the components of rice seeds of the four varieties analyzed and their subsequent incubation allowed the development of colonies of *T. padwickii*. The incidence (%) of the fungus in each component was variable, depending on the component colonized (Table 1): grain: 12.5 to 53%, lemma: 12.2 to 38.5%, palea: 6.2 to 42.2%, sterile lemmas: 6.2 to 36.5%, pericarp: 9.6 to 40.8%, endosperm: 2.8 to 12.1%, and whole seed: 21.2 to 74.2%. The percentage of distribution total inoculum (Table 1) ranged from 45.0 to 99.3%

***Table 1**. Percentage of incidence (% In) and of distribution if the inoculum (% Di) of Trichoconiella padwickii.*

	BR-IRGA 424		Puitá INTA CL		Supremo 13		Taim	
Components	(%) In	(%) Di	(%) In	(%) Di	(%) In	(%) Di	(%) In	(%) Di
Lemma	38.5	54.4	12.25	44.1	25.5	34.3	20.5	96.5
Palea	42.2	59.7	6.25	22.5	22	29.6	10.1	51.4
Sterile lemmas	36.5	51.5	6.25	22.5	21.2	28.6	9.9	46.5
Pericarp	36.8	51.9	9.6	34.7	40.8	54.9	16.2	76.5
Endosperm	10.1	15.5	2.9	10.3	12.2	16.4	48.5	22.9
Whole seed	70.7	100.00	27.7	100.00	74.2	100.00	21.2	100.00

Fig. 1. Conidia (a) and sclerotia (b) of the *Ttrichoconiella padwickii*, (c) rice grains with symptoms of rot and death of embryos.

(grain), 34.3 to 96.4% (lemma), 22.5 to 59.7% (palea), 22.5 to 51.5% (sterile lemmas), 34.6 to 76.4% (pericarp) and 10.3 to 22.8% (endosperm). Infection by *T. padwickii* in each component was recognized by the formation of ash-gray aerial mycelium and hyaline conidia (83x13µm), usually 3-4 septate (Fig. 1a), on simple hyaline conidiophores 123µm long. Black and reticulate sclerotia, 123µm in diameter (Fig. 1b), were found in the culture medium. The rice grain of all the varieties analyzed showed development of sclerotia in the endosperm and pericarp, symptoms of rot, death of embryos and disintegration of tissue (Fig. 1c).

Discussion

Regardless of the variety of rice analyzed, *T. padwickii* was present in all the components of the seed, with values similar to those recorded by Mew & Gonzales (2002). According to Maude (1996), necrotrophic fungi are usually localized in the tissues of the seed cover (pericarp) and rarely deeper into the tissues of storage, and generally not present in the generative tissues of embryos of seeds. However, the observations made in this study indicate that *T. padwickii* was able to remain in the endosperm of the rice grain and cause rot and embryo death (Fig. 1c). These results are in agreement with those by Pineda *et al.*, (2007), who noted that the infection caused by *T. padwickii* in rice grains generates the rot and death of seeds. Our findings are also supported by Pham *et al.*, (2001), who concluded that the decrease in the physico-chemical properties of the rice grain was due to the presence of *T. padwickii* and *Curvularia lunata* in the endosperm of the grain. The results expressed as a distribution of total inoculum (% Di) showed that the variety Taim (99.3%) was the one with greatest ability to host the pathogen in internal tissues (pericarp and endosperm), followed by the varieties Supremo 13 (71.4 %), BR-IRGA 424 (67.5 %) and Puitá INTA CL (47.7%). These values were not coincident with those of the incidence levels detected in the whole seeds, being the variety Supremo 13 (74.3 %) the one with the highest value of presence of the pathogen, followed by BR-IRGA 424 (70.8%), Puitá INTA CL (27,8%) and Taim (21.2 %). In this regard, Singh & Mathur (2004) proposed that the distribution of inoculum in seeds affected by species of *Alternaria* is related to the level of incidence in the whole seed: the higher the incidence, the higher the proportion of components that will be colonized by the pathogen, but this would not explain the results obtained in the present study. Although the variety Taim concentrated the greatest amount of inoculum in the most internal components (pericarp and endosperm), it showed the lowest level of incidence in the whole seed. In contrast, the variety Puitá INTA CL presented a similar incidence value in the whole seed and the lowest values for the most internal components (pericarp and endosperm). This could be attributed to two main causes: a) the moment at which the infection occurs, as demonstrated for *Helmitosporium oryzae* by Falzi & Schroeder (1966), who concluded that in early infection, the pathogen has more time to colonize the grain tissues; and (b) to the nature of the internal tissues of seeds of each of the different varieties of rice, which would affect the colonization of *T. padwickii* within the grain. Another aspect to highlight regarding the localization of the pathogen in the seed is that the more internally the pathogen is localized, the higher its ability to survive because of the protection provided by the external tissues of the seed against the presence of adverse factors. In this regard, Maude (1996) mentioned that, according to their intrinsic morphology, fungi have different abilities to survive in the stage of seed. Strongly pigmented fungi with thick-walled conidia, such as Dematiaceous Hyphomycetes (which include the genus *Bipolaris*), persist in the seed for one to ten years. Although these morphological differences influence the persistence of fungi in the seed, they also influence the localization of the pathogen in the seed (Singh & Mathur 2004). The results obtained in the present study suggest that the pathogen is localized in all the components of the rice seed, with different values depending on the component colonized and variety analyzed. Resistance structures such as sclerotia, which contribute to the survival of the pathogen in the seed, were also observed. The precise knowledge of the localization of the pathogen in the different components of the rice genotypes provides valuable information to further study the transmission of rice pathogens and to assess the efficiency of chemical control with fungicides.

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