ARTIGOS

Mycobiota evolution during storage of paddy, brown and milled rice in different genotypes

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ABSTRACT

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The rice grain is frequently infected by a series of pathogens (fungi) during its storage, producing damages to the economy and health of humans. The aim of this study was to identify the fungal genera present in different rice genotypes and to quantify their variation during storage. Paddy, brown and milled rice fractions of Nutriar, (N) H329-5(H329) and Don Ignacio genotypes were analyzed at 4, 8 and 12 months of storage. Fungi were identified based on their micromorphological characteristics and colonies. The observed genera according to their frequency were*: Alternaria, Nigrospora, Epicoccum, Bipolaris, Curvularia, Cladosporium* and *Fusarium* (field fungi) and *Penicillium* and *Aspergillus* (storage fungi). The mycobiota composition was different depending on the grain fraction and the period of storage: field fungi were located in the hulls and bran layers, while storage fungi were mainly in the endosperm. The different genotypes showed different susceptibility to contamination.

Additional keywords: *Oryza sativa*, seed pathology, seed health.

RESUMO

Pinciroli, M.; Gribaldo, A.; Vidal, A.; Bezus, R.; Sisterna, M. Evolução da micobiota durante o armazenamento do grão de arroz com casca, integral e polido em diferentes genótipos. ***Summa Phytopathologica,*** v.39, n.3, p.157-161, 2013.

O grão de arroz é, com frequência, atacado por uma série de patógenos (fungos) durante o armazenamento produzindo danos econômicos e à saúde humana. O objetivo do trabalho foi identificar os gêneros fúngicos presentes em diferentes genótipos de arroz e quantificar sua variação durante o armazenamento. Analisaram-se as frações casca, integral e polido dos genótipos Nutriar (N), H329-5 (H329) e Don Ignácio aos 4, 8 e 12 meses de armazenamento. Os fungos foram identificados por suas características micromorfológicas e suas colônias. Os gêneros observados segundo sua frequência foram: *Alternaria, Nigrospora, Epicoccum, Bipolaris, Curvularia, Cladosporium* e *Fusarium* (fungos de campo) e *Penicillium* e *Aspergillus* (fungos de armazenamento). A composição da micoflora foi diferente segundo fração de grão e tempo de armazenamento: os fungos de campo localizaram-se na casca e no farelo, enquanto os de armazenamento, no endosperma. Os diferentes genótipos apresentaram diferente suscetibilidade à contaminação.

Palavras-chave adicionais: *Oryza sativa*, sanidade de sementes, patologia de sementes.

Rice (*Oryza sativa* L.) is one of the major commercial cereal grains in the world, together with wheat and corn. Around 433.8 million tons of milled rice have been produced all over the world (30). Most of the cultivated rice is consumed as head rice, without processing. Therefore, its health is important for both seed use and human consumption. Rice processing involves the milling process. The milled fractions are: paddy rice (without elaboration), brown rice and milled rice.

Fungi grown on seeds can cause severe damage (seed abortion, necroses, discoloration, shrunken seeds) and reduce seed yield (17). The mycobiota associated to the grain can be modified by the cultivar (6) and the milling fraction (12) during storage. Neninger et al. (19) reported over 99 species and 59 fungal genera, the most complete list of organisms associated to rice seed. These fungi can be grouped into two categories: "field fungi" which are more or less parasitic and infect the grains before harvest, and "storage fungi" which usually are saprophytes and develop after harvest (3).

Alternaria is the most common contaminating fungi detected in cereal grains before harvest. This genus is frequently found on glumes but may cause black spots on the endosperm and may contribute to a decrease in grain quality (20). *Alternaria* presents several species parasitizing rice grains (21, 18). Gutierrez et al. (7) reported *A. padwickii* and *A. longuissima*, while *A. alternata* was observed by Pinciroli et al. (22).

Nigrospora has been reported worldwide although it has little economic importance in rice production. Four species are found in rice but *N. oryzae* and *N. sphaerica* are most common. They are all considered saprophytes. *Nigrospora* has been mentioned to affect glumes, culms, leaves or other parts of rice plants that are weakened because of nutritional or climate conditions or that suffer from diseases or insect attack (14). This organism is frequently recorded in rice grains in Argentina (7) and in other countries (19, 9).

The genus *Epicoccum* is responsible for the "red blotch" disease of harvested rice seeds. This disease occurs when the rice panicles fall on the ground before or after maturity. Infected seeds fail to germinate and damage can be severe at frequent times (21). These fungi have been reported by other authors and in other areas parasitizing seeds (18). Their presence is constant in the complex causing discoloration to grains in our country (27, 7).

Several *Bipolaris* species have been mentioned in rice seeds worldwide (16), especially in Argentina (27, 7, 22). *Bipolaris oryzae,* the causal agent of brown spot, produces important economic damages, especially in tropical regions (10).

The genus *Curvularia* has been found in rice grains causing discoloration (21). More than ten species have been reported in rice worldwide (14). The most common species are *C. lunata* and *C. geniculata* (14). In Argentina, *C. lunata* (7; 22; 27), *C. pallescens* (7) and *C. protuberata* (26) have been reported, while *C. oryza-sativae* was first recorded by Sisterna & Carranza (25).

The genus *Cladosporium* in seed has been recorded by several authors (12, 19, 14). In Argentina, it has only been mentioned by Mamone & Gaetán (13).

Fusarium is a relevant field genus detected in rice (19), although sometimes it behaves as an important toxicogenic storage fungus (9). In Argentina, *F. moniliforme, F. graminearum* and *F. semitectum* (27, 29, 2, 7, 22) were reported.

At harvest, seeds carry a combination of field and storage fungi. Storage eliminates the most field fungi that are originally present on freshly harvested rice seeds (11).

Aspergillus and *Penicillium* are mostly saprophytes in nature. The toxins produced by these fungi, particularly from *Aspergillus,* are harmful to humans and to animals (9). All over the world, the following *Aspergillus* spp. have been cited: *A. flavus, A. candidus, A. sydowii, A. versicolor, A. tamarii, A. alutaceus, A. chevalieri, A. amstelodami, A. ruber* (12), *A. niger, A. flavus-oryzae, A. clavatus* (14), *A. ochraceus, A. versicolor* (23). *Aspergillus amstelodami* and *A. ruber* cause grain deterioration during storage. In Argentina, *A. flavus* (29, 2) and *A. niger* (29; 13) were reported. *Penicillium* was mentioned in rice grains by Lima et al. (12), Karunakara & Manonmani (9). In Argentina, *P. citrinum* (29; 2); *P. islandicum, P. funiculosum, P. aurantiogriseum* (29) were observed.

The aim of this study was to identify the mycobiota present in the different genotypes and to quantify its variation during storage.

MATERIALS AND METHODS

Rice samples

Samples harvested from trials conducted at the Experimental Station Julio Hirschhorn of the "Facultad de Ciencias Agrarias y Forestales de la Universidad Nacional de La Plata", Buenos Aires Province, Argentina (34° 52' S and 57° 58'W), crop 2005/06, were analyzed. Genotypes Nutriar FCAyF (N), H329-5-2-1-1-2-1-1-1 (H329) and Don Ignacio (DI), belonging to "Programa Arroz" from the same University, were used. The cultivars N and DI correspond to the long-grain type, while H329 is a long-width grain type. The cultivar N has about 30% more protein content than the normal varieties. Samples were processed to obtain the milling fractions by using an experimental mill (universal type Guidetti and Artioli, USA). Usually, rice is harvested with intact hulls (lemma, palea and glumes). This type of rice is known as *rough* or *paddy rice.* The first step of the rice milling process involves removing the hulls. Rice in this form is called *brown rice*. The second step includes removing of the bran layers, producing the *milled white* or *polished rice*, the fraction for human consumption.

Methodology

Paddy rice samples, maintained in cotton bags under laboratory conditions, were milled to obtain the brown and milled fractions at 4, 8 and 12 months of storage (MOS). Samples were analysed according to the routine phytopathological techniques based on the standard methodology. The health of grains was analyzed by the agar method (ISTA rules: International Seed Testing Association) (17). The grains had their surface disinfected with sodium hypochlorite (5%) during five minutes and washed with sterile water. Fifty grains per sample were then plated on 90mm Petri dishes with potato dextrose agar (PDA) at 2%. Each sample was replicated three times. The plates were incubated in a growth chamber at 21±1°C, 80% RH, with light alternation (3500 lux dark cycles of 12 h plus UV light) for 6 days, and then examined under a stereoscope microscope. The isolation and identification of fungi were carried out by using standard taxonomic schemes based on cultural features and on the microscopic observation of their vegetative and reproductive structures, with the support of specific bibliography (28, 4, 5, 21).

Statistical analysis

The values of presence, previously transformed to square root function, were used to carry out a multifactorial analysis of variance (ANOVA) for each milling fraction and each group of fungi. Statgraphics Plus 4.0, a software package from Statgraphics Corp. Rockville, MD, was used. The mean separation was tested by Tukey's test (P d" 0.05).

RESULTS AND DISCUSSION

Qualitative analysis

The most frequently isolated fungal genera were: *Alternaria*, *Nigrospora, Epicoccum, Bipolaris, Curvularia, Cladosporium* and *Fusarium* (field fungi) and *Penicillium* and *Aspergillus* (storage fungi). Changes in the composition of the mycobiota according to MOS, genotypes and milling fractions are presented in Table 1.

Alternaria, and its preponderant species *Alternata*, was the most common genus, in accordance to the results of Broggi & Moltó (2) and Pinciroli et al. (22). This fungus, an organism that contaminates grains in the field before harvest, was found in all genotypes and months of storage (Table 1). It is more abundant in paddy rice and brown rice, remarkably decreasing in milled rice. In this sense, there are references (20) of the beneficial relationship, because of its nutritive quality, between its outer coats and *Alternaria*.

Table 1 - Mycobiota changes for different months of storage, genotypes and milling fractions (expressed as %).

NUTRIAR	**4 MOS[1]**			**8 MOS**			**12 MOS**		
	paddy rice	**brown rice**	**milled rice**	**paddy rice**	**brown rice**	**milled rice**	**paddy rice**	**brown rice**	**milled rice**
Alternaria	55,7	39.7	9.7	55.4	47.6	0	50.5	1.9	0
Nigrospora	0	0	0	34.9	19.0	30.0	19.1	70.4	0
Epicoccum	17.2	16.2	0	2.4	0	0	0	1.9	0
Bipolaris	2.9	19.8	25	2.4	0	0	1.5	0	0
Curvularia	1.1	7.3	15.3	3.6	14.3	0	1.5	0	0
Cladosporium	17.8	0.7	0	1.2	4.8	10.0	0	1.9	0
Fusarium	0	0	0	0	0	0	0	3.7	0
Penicillum	5.2	16.2	50	0	14.3	60.0	4.4	11.1	19.0
Aspergillus	0	0	0	0	0	0	23.5	9.3	81.0

H 329	**4 MOS**			**8 MOS**			**12 MOS**		
	paddy rice	**brown rice**	**milled rice**	**paddy rice**	**brown rice**	**milled rice**	**paddy rice**	**brown rice**	**milled rice**
Alternaria	64.9	72.7	0	77.1	72.2	0	55.6	9.6	0
Nigrospora	1.8	0	0	0	0	0	25.9	79.1	49.0
Epicoccum	29.8	15.6	0	12.9	0	0	1.9	0	0
Bipolaris	0	1.3	0	1.4	0	0	0	0	0
Curvularia	0	1.3	0	2.9	11.1	0	3.7	0	0
Cladosporium	3.0	1.3	15.4	4.3	0	25.0	3.7	0	0
Fusarium	0.6	5.2	0	0	0	0	0	7.0	17.0
Penicillum	0	2.6	84.6	1.4	16.7	75.0	3.7	1.7	20.8
Aspergillus	0	0	0	0	0	0	5.6	2.6	13.2

DON IGNACIO	**4 MOS**			**8 MOS**			**12 MOS**		
	paddy rice	**brown rice**	**milled rice**	**paddy rice**	**brown rice**	**milled rice**	**paddy rice**	**brown rice**	**milled rice**
Alternaria	62.0	76.1	0	51.6	40.0	13.3	54.8	1.3	4.8
Nigrospora	0	4.4	0	0	0	0	22.6	83.9	0
Epicoccum	38.0	11.5	2.9	48.4	0	0	0	0	0
Bipolaris	0	0	0	0	0	6.7	0	0	0
Curvularia	0	0.9	0	0	0	0	3.2	3.9	0
Cladosporium	0	2.7	0	0	0	13.3	0	0	0
Fusarium	0	0.9	0	0	0	0	0	0	0
Penicillum	0	2.7	97.1	0	60	66.7	9.7	3.9	19.0
Aspergillus	0	0.9	0	0	0	0	9.7	7.1	76.2

[1] MOS: months of storage.

The levels of *Alternaria* in paddy grains and brown grains were similar for all genotypes and in the first 8 MOS, while at 12 MOS it only remained in paddy grains. At 12 MOS, for all genotypes, *Nigrospora oryzae* was more abundant in brown grains (Table 1). The presence of *Nigrospora* coincided with the decrease in *Alternaria.* These results disagree with those found by Sempere & Santamaria (24), who studied the interaction between these fungal species under different experimental conditions and observed no dominance. *Epicoccum nigrum* was observed in the three genotypes, especially in paddy grains, where values had a tendency to decrease with MOS (Table 1). *Bipolaris* had the highest levels in Nutriar at 4 MOS (Table 1), increasing in the inner parts of the grain (milled). Several species (*B. cynodontis*, *B. sorokiniana, B. spicifera*), at different proportions, were recorded. The genus *Curvularia* was more abundant in Nutriar at 4 MOS (Table 1) and milled grains were highly infected. Two species were isolated: *C. lunata* and *C. oryza-sativa. Cladosporium* had an erratic behaviour, without a defined occurrence pattern, and *Cladosporioides* was the predominant fungal species. In these rice samples, *Fusarium* showed low values, not agreeing with those previously cited by Sisterna et al. (27) and

Pinciroli et al. (22). *Fusarium graminearum* was the prevalent species. Bateman (1), studying wheat and barley seeds, observed that some *Fusarium* species were generally inhibited by *Alternaria*. In this sense, the high frequency of *Alternaria* spp. in our data could be the cause of the scarce contamination by *Fusarium*.

Regarding storage fungi, *Penicillium* was observed at an increasing pattern when the outer coats were eliminated and was more abundant in milled grains, the main fraction for human consumption. This fungus showed a tendency to decrease with storage, while *Aspergillus* was present at a higher frequency.

Quantitative analysis

The quantitative general characterization of the mycobiota at different milling fractions is shown in Table 2. Field fungi were mainly located in outer coats, while storage fungi, which are mostly toxigenic, in the endosperm (milled rice).

Paddy rice. The analysis did not show significant interaction between genotype and MOS, either for field or for storage fungi. In general, the presence of field fungi decreased over the MOS, while for the storage fungi of the two homogeneous statistical groups, the highest value corresponded to the 12 MOS (Table 3). These results agree with those previously cited by several authors (11). Nutriar was the most contaminated genotype, probably due to its protein content, while DI was the healthiest genotype.

Table 2 - Grand mean of fungi grain contamination in the different milling fractions (expressed as %).

	paddy rice	brown rice	milled rice
field fungi	59.63	41.11	8.96
storage fungi	2.96	4.55	23.41

Table 3 - Mean values of contamination of field and storage fungi at MOS and genotypes in paddy grain (expressed as %).

	field fungi	storage fungi
Months of storage		
4 MOS[1]	110.89 a	2.0 b
8 MOS	40.67 b	0.22 b
12 MOS	27.33 b	6.67 a
Genotypes		
Nutriar	66.0 a	6.22 a
H 329	63.56 a	1.33 b
Don Ignacio	49.33 b	1.33 b
Interaction MOS x G	ns	ns

[1]MOS: months of storage. Different letters within the same column (for each factor or feature) mean significant differences at $P \leq 0.05$ according to Tukey's test.

Table 4 - Mean values of contamination of field fungi at MOS and genotypes in brown grain (expressed as %).

	4 MOS[1]	8 MOS	12 MOS
Nutriar	76.0 a A	12.0 a B	28.67 b B
H 329	50.0 a A	10.0 a B	73.33a A
Don Ignacio	72.67 a A	1.33 bB	46.0 ab A

[1]MOS: months of storage. Different lowercase letters within the same column and uppercase letters on the same row mean significant differences at $P \leq 0.05$ according to Tukey's test.

Brown rice. Field mycobiota evolution differed according to the genotype (Table 4). Nutriar showed highest contamination at 4 MOS. This initial contamination was characterized by great genus diversity (*Alternaria, Bipolaris, Epicoccum, Curvularia, Cladosporium*). Genotypes H329 and DI had important fungi presence at 4 MOS, with prevalence of *Alternaria*, and at 12 MOS, when *Nigrospora* prevailed. The contamination of storage fungi was scarce. There were no differences between MOS and genotypes. The mean values were 6.22, 2.0 and 5.44% for 4, 8 and 12 MOS, respectively, and 8.0, 2.22 and 3.44% for Nutriar, H329 and DI genotypes, respectively.

Milled rice. Field and storage mycobiota evolution differed according to the genotype (Table 5). Regarding field fungi, Nutriar presented high contamination at 4 MOS with prevalence of *Bipolaris, Curvularia* and *Alternaria*. On the other hand, H329 had the highest contamination at 12 MOS with *Nigrospora* and *Fusarium*. DI led to low values of field fungi which were constant over MOS. For storage fungi, Nutriar was the most contaminated genotype at 12 MOS (Table 5). DI contamination values did not change over the MOS.

The mycobiota composition differed according to the milling fraction and MOS: field fungi were found in the hulls and bran layers, while storage fungi were mainly in the endosperm. In the two groups, some genera substituted others: for field fungi in the first observations *Alternaria*, *Epicoccum* and then *Nigrospora* prevailed, while for storage fungi *Aspergillus* first appeared, followed by *Penicillium*. The latter organisms are important toxin producers that predominated in the grain fraction for human consumption. Genotypes had different behavioural responses towards contamination. In conclusion, the evolution of the grain

Table 5 - Mean values of contamination of field and storage fungi at MOS and genotypes in milled grain (expressed as %).

	field fungi			storage fungi		
	4 MOS[1]	8 MOS	12 MOS	4 MOS	8 MOS	12 MOS
Nutriar	48.0 a A	2.67 a B	0 b B	48.0 a B	4.0 a C	94.67 a A
H 329	1.33 bB	0.67 a B	23.33 a A	7.33 b AB	2.0 a B	12.0 bA
Don Ignacio	0.67 bA	3.33 a A	0.67 b A	22.67 ab A	6.67 a A	13.33 bA

[1]MOS: months of storage. Different lowercase letters within the same column and uppercase letters on the same row mean significant differences at P d” 0.05 according to Tukey's test, for field and storage fungi.

rice mycobiota changed according to the genotype and the milling fraction during storage, findings that could provide preventive measures for safe consumption.

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