Rice Seedborne Infection in Southern Spain

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Abstract

Rice (*Oryza sativa*) seeds were analysed to determine their potential transmission of pathogenic and saprophytic microorganisms. In four and three hundred lots (stocks) of seeds, in 1998 and 1999 respectively, proceeding from mechanical drying sheds, none presence of *Pyricularia oryzae* was detected. It did not happen that way for *Drechslera* spp., whose presence was detected in 8.3% and 28% of the lots, in 1998 and 1999; within these ones the affected seed average was 1.3% in both years. *Fusarium* sp. appeared in 41% and 48% of the lots, in 1998 and 1999, respectively; the affected seed percentage, within these lots, oscillated between 1.7 and 2.4%. Regarding *Arthrobotris* sp. and *Curvularia* sp. the percentage of both, affected stocks and affected seed within these ones, were lower than 2%. About saprophytic microorganisms, *Alternaria* appeared in 51% of the stocks in 1998, and in 38% in 1999, while *Nigrospora* did it in 39% and 33%. The affected seeds average per stock was about 2%. These low seed infection percentage seems a consequence of the mechanical drying process, that disables parts of the inoculum seed. On the other hand, harvested grain samples from trials established to promote the infection of *Pyricularia oryzae* in 1998, 1999 and 2000, showed that this pathogen was not present in any of these three years, in line with the seed results mentioned above. However, *Pyricularia* was present in 55% of the harvested grain samples in 2001 trial, as well as in 40% of the seeds. Seed can be a way of transmission of fungi if proceed from campaigns with a high level of inoculum in field.

Keywords: *Pyricularia oryzae* rice; seed infection

INTRODUCTION

The rice culture (*Oryza sativa* L.) at the moment occupies in Andalucia 38 000 ha and from 1974 exists information of severe losses associated mainly with infections by *Pyricularia oryzae* (MARÍN SANCHEZ 1979; MARÍN SANCHEZ & JIMÉNEZ DIAZ 1981) and other pathogens like *Drechslera* spp. Both pathogens attack seedling, leaves, knots and panicles, including the seeds, in which the fungus can last several years (ITO 1932; MANANDHAR *et al*. 1998) getting to be a primary source of inoculum (ROBER & PAMALA S. GUNNELL 1992; LEE 1994) in tempered zones (OU 1985). On the other hand, it is known that the absence of foliar symptoms under field conditions do not necessarily means that the seeds are healthy (MANANDHALAR 1998; SUZUKI 1934) this affirmation is specially important at the time of seeds certification's programs which are based on lot of seeds selected on the basis of field inspection. By all the previous one, we focused the objectives of this investigation in showing the fytosanitary state of the sowing seeds, and harvested grains of rice, through the knowledge of the species associated to the infections of the seeds to know the influence as a possible via of inoculum.

MATERIAL AND METHODS

Different studies with seed were carried out from rice sowing to know their fytosanitary state as well as the possibility transmission of fungi organisms. From November to June of 1999–2000 and 2000–2001 four hundred and three hundred lots of non-certified rice seeds were analysed respectively, destined to sowing and coming from industrial dryers of the previous campaigns (1998 and 1999). In addition, 11 lots of certified sowing seeds from the campaign 2001 were analysed from each one of the following varieties Galatxo, Maso, Thainato, Marismas, Orellana and Fonsa. From each one of the 766 lots sampling a total of two hundred seeds that were used to prepare moist

chamber that incubated during seven days to 26 ± 1°C, and with photoperiod 12 h light/dark. After 2 days and until the eighth the seeds were examined under a stereoscopic binocular microscope with a magnification of 63 to determine the pathogenic organisms and present saprophytes in the same ones.

Simultaneously, and from the campaign of the 1998 to 2001 an experiment with 14 varieties with different tolerance to *P. oryzae* has settled down and under conditions of sowing, ad-sickle, favourable to originate infections by this pathogen, that means a delayed sowing (10 of June) and high dose of fertilizer approximately 250 U of nitrogen/ha. The experimental design was the one of blocks at random with 4 repetitions, with an elementary plot of two lines of 6 m (30 plants), and a separation between lines of 0.25 m, all it surrounded by a wide edge of lm variety Baixet (very susceptible to the pathogen under the conditions of the Salt marshes 'marismas' of the Guadalquivir) helping to maintain a greater humidity (OU 1987) and to assure an inoculate acceptable level. In this test 560 lots of harvested grains coming from 10 plants by repetition were analysed similarly that in the study with sowing seeds.

Finally, were calculated the percentage of affected lots (affected lots/analysed lots), as much of sowing seed as certified and grains harvested, affected and from them the mean frequency (MF) by lot, that means the average of affected seed (n° affected seeds/n° affected lots).

Pathogenicity test. In order to contrast the pathogenicity of the fungi species associated to the rice seeds, pathogenicity tests were made. Affected seeds were surface disinfected in a 1:9 (v/v) solution of a household bleach (5.25% sodium hipochloride) and placed in moist chambers. The last chambers were incubated at 26 ± 1°C and alternating 12 h periods of fluorescent and NUV light of about 2500–3000 lux. Monoconidial culture of each micro-organisms were made on Water Agar (WA) and later on Rice-Polish Agar (RPA), means that last one where previously abundant sporulation of different organisms had been observed to consider, when the were inoculated at previously described conditions. Once recollected the spores from the Petri dishes with sterile water the spore suspension was filtered with a gauze and its concentration was adjusted with hematocitometer, after which it is mixed to equal parts with a gelatine solution of 1% in sterile water. Inoculations were preformed (a) by spraying a suspension of conidia in sterile distillated water (10^5 conidia/ml) onto the seeds with an atomizer, applied at the rate of 4 ml/plate and (b) injecting inoculum in the seeds. Twenty-five seeds of each lot were disinfected with sodium hipocloride in relation 1:9 (v/v) and pre hydrated (12 h in sterile water), later were placed on a disc of paper of filter in each board of Petri and it repeated 4 times. After inoculation, the rice seeds were incubated under the conditions described previously and they were inspected under a stereoscopic binocular microscope to the 5–10–15 and 20 days from the inoculation according to species.

Finally, were determined the symptoms of the injuries, n° of seeds or affected grains, and affected surface.

RESULTS

Drechslera spp. and *Fusarium* sp. have been the organism observed most frequently in non-certified sowing seeds and coming from the campaign of year 1998 and 1999 (Figures 1 and 2). These pathogens

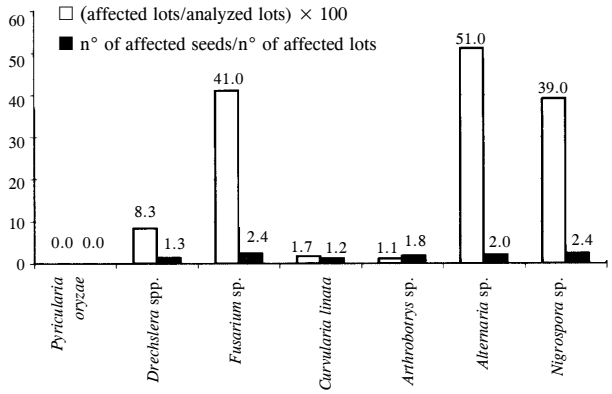

Figure 1. Fungus percentage found in the non-certified sowing seeds (campaign 1998)

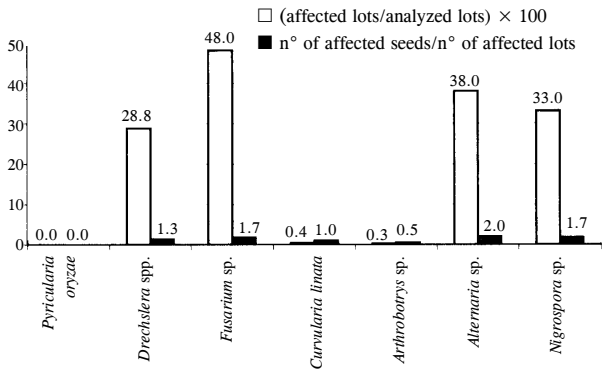

Figure 2. Fungus percentage found in non-certified sowing seeds (campaign 1999)

were present in the 8.3, 28.8 and 41–48 % of the studied lots, respectively, and appeared with a MF by lot between the 1.3 and 2.4%. Not thus it happened with *Curvularia lunata* and *Pyricularia oryzae* where the infection levels were despicable as in the case of *Curvularia* or total absence in the case of *P. oryzae*. In the year 2001 *P. oryzae* was isolated in the 45.5% of the lots of certified seeds from the Marismas cultivars, 36% of Orellana and the 45.5% of Fonsa (Table 1). The presence of *P. oryzae* in the first two years is was not strange since although one of the channels of transmission of this disease is the seed, the scientific bibliography makes reference to that the habitual measures that receives the grain of rice once collected as the drying to the sun or in mechanical dryer, disqualify inoculum of this present agent in the seed (MANANDHAR et al. 1998). As far as the saprophytic organisms is possible to emphasize *Alternaria* sp., 51% and 38% of the lots in 1998 and 1999 and *Nigrospora* sp. in 39% and 33%, respectively. In both cases the MF by lot was around 2%. *Arthrobotris* sp. always present very low levels of infestation.

In 1999 and 2000 *P. oryzae* did not infect the grain harvested nevertheless in 2001 the percentage of hatvested grain infected oscillated between 34% and 69% according to cultivating (Table 2) and with a mean frequency superior to the one found in that same year in certified sowing seeds.

Symptomatology of the injuries and pathogenicity of the agents

P. oryzae was isolated each other from sowing seeds as grain harvested where it rise to a dark brown tonalities in the surface of the seed which affected between 10% and 90% of the seed and in cases of severe infections got to produce empty grain with straw-colours. In any case the infection caused defined spots and characteristics. All the isolations were able to infect inoculated seeds artificially and to reproduce the same injuries again. Four species of *Drechslera* have been isolated from injuries that affect the rice seeds. Of them *D. cynodontis* attacked the grain severely affecting 50% of the surface, *D. oryzae* infected in smaller degree followed by *D. biseptata* and *D. hawaiiensis*.

Table 1. Percentage of certified sowing seeds lots affected by different organisms according to variety (campaign 2001)

Variety	*Pyricularia oryzae*	*Drechslera* spp.	*Alternaria* sp.
Galatxo	–	09 (02)	81 (03)
Maso	–	09 (01)	72 (04)
Thainato	–		72 (03)
Marismas	45.5 (08)		81 (03)
Orellana	36.4 (05)		72 (02)
Fonsa	45.5 (07)		90 (07)

Between brackets n° of affected seeds/n° of affected lots

Table 2. Percentage of infection by *Pyricularia oryzae* in harvested grains of in 2001

Variety	% of infected lots	Variety	% of infected lots
Senia	34 (04)	Alena	55 (11)
Galatxo	69 (12)	Thainato	49 (07)
Bahía	58 (09)	Jacinto	51 (09)
Maratelli	63 (18)	Thaibonnet_	69 (14)
Baixet	82 (21)	Puntal	41 (05)
Maso	64 (11)	Fonsa	45 (06)
Ullal	41 (06)	Puebla	47 (06)

Between brackets n° of affected seeds/n° of affected lots

The symptomatology which we have observed in our investigations was coincident with the one referred by MARÍN SANCHEZ and JIMÉNEZ DIAZ (1981), that is to say, generally made oval injuries, of brown colour and more clear centre partially occupying the surface of the seeds.

The symptoms caused by *Fusarium* sp. were the reddish coloration and black in some cases, and the ones caused by *Curvularia lunata* were those of discoloration of the grain. *Nigrospora* when inoculating artificially rise to brown necrotic spots of approximately 2.5 mm of diameter and in no case they surpassed 4% of the surface of the grain. The necrosis generalized in this device were associate with other fungi species of saprophytic organisms like *Arthrobotri* sp. and *Alternaria* sp. On the other hand, the high values of production of spores of *Nigrospora* sp. as opposed to the low values of severity of the grain infections could show their saprophytic capacity, question not only shown in our isolations in which repeatedly was isolated of seeds with symptoms induced by other pathogens but also described by MARÍN SANCHEZ and JIMÉNEZ DIAZ (1981).

In general, the results obtained until 1999 in non-certified sowing seeds show a good health of these since it was not observed the presence of the fungi *P. oryzae* and the frequency of appearance of other fungi causes of problematic diseases in the south Spain, like *Drechslera* spp. or *Fusarium* sp., were low. Despite the appearance of *P. oryzae* in 2001 in certified sowing seeds as much harvested grains makes you think that in previous years the cause of the absence of the pathogen in seeds was caused by the lack of inoculum or so that the environmental conditions were not the appropriate ones for the development of the disease. Also it is possible to emphasize that the smaller percentage of infection found in certified sowing seeds respecting to harvested grain can be consequence of the mechanical drying that disqualifies part of inoculo of this agent in the sowing seeds (MANANDHAR *et al.* 1998). Although the wind is the main via for conidia dissemination of *P. oryzae* the seeds also considers an important channel of transmission (LEE 1994; OU 1987) of fytopathogenes fungi when these come from campaigns with a considerable level of inoculum and appropriate climatic conditions for the development of the disease, therefore are advisable to know the fytosanitary state of the seeds before being used for sowing time.

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