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Resistance of rice cultivars to *Pyricularia oryzae* in Southern Spain

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Abstract

The susceptibility of 14 commercial rice cultivars to *Pyricularia oryzae* was tested under field conditions agronomically appropriate for natural infection to occur. The first symptoms of infection were detected on the leafs when the crops were at the medium tillering stage. Generally speaking, the incidence of infection for each particular cultivar was similar in the 2002 and 2003 seasons and at the two trial locations (the Sartenejales and Casudis estates in the Province of Seville). The pathogen infected 80-100% of 'Maratelli', 'Baixet', 'Thainato' and 'Galatxo' plants. Differences were seen among the cultivars with respect to the parts of the plant affected. 'Baixet' and 'Maratelli' were the most susceptible cultivars, followed by 'Thainato'. 'Ullal' was the most tolerant, followed by 'Marisma', 'Puebla', 'Maso', 'L-202', 'Susan', 'Doongara', 'Fonsa' and 'Jacinto'; these last six cultivars showed medium disease severity according to the scale used in this work. Further studies on the interactions between different strains of *P. oryzae* and rice cultivars are underway.

Additional key words: blast, *Oryza sativa*, varietal susceptibility.

Resumen

Evaluación de la resistencia de cultivares de arroz a *Pyricularia oryzae* en el sur español

Se sembró en campo una colección de 14 cultivares comerciales de arroz bajo condiciones agronómicas apropiadas para que se produzca la infección natural por *Pyricularia oryzae,* a fin de estudiar su susceptibilidad al patógeno. Los primeros síntomas de la infección se detectaron en las hojas cuando el cultivo se encontraba en estado de ahijado medio. Generalmente, la incidencia de la enfermedad fue similar para cada cultivar en dos campañas (2002-2003) y localidades (Sartenejales y Casudis, Sevilla). El patógeno infectó el 80-100% de las plantas de 'Maratelli', 'Baixet', 'Thainato' y 'Galatxo'. Se observaron diferencias entre los cultivares estudiados relativas a las partes de las plantas afectadas. Considerando el porcentaje de infección ponderado, 'Baixet' y 'Maratelli' fueron los cultivares más susceptibles al patógeno, seguidos de 'Thainato'. Por otro lado, 'Ullal' fue el más resistente a *P. oryzae*, seguido de 'Marisma', 'Puebla', 'Maso', 'L202', 'Susan', 'Doongara', 'Fonsa' and 'Jacinto'. Estos últimos seis cultivares mostraron una severidad de enfermedad media. Se están llevando a cabo estudios adicionales para conocer el espectro de razas de *P. oryzae* y su respuesta patogénica frente a una colección de cultivares diferenciales.

Palabras clave adicionales: *Oryza sativa,* seca, susceptibilidad varietal.

Introduction

About 450,000 ha of land are devoted to rice (*Oryza sativa* Linn) cultivation in the European Union (EU); Spain alone has some 111,000 ha given over to this crop. The rice-growing region of Andalusia (southern Spain) mainly lies between Seville and the mouth of the River Guadalquivir – the *Marismas del Guadalquivir* area. This is characterised by its flat, clayey, saline soils of sedimentary origin. Some 38,000 ha of rice are cultivated in this area, the production of which represents about 40% of the total Spanish crop (Aguilar, 2001). Two cultivars, 'Doongara' (of Australian origin, known as 'Puntal' in Spain) and 'L202' (of Californian origin, known as 'Thaibonnet' in Spain), both of the Indica (long grain) subgroup, are currently the most widely grown in this area, the first making up 75% of the entire crop, the second making up some 15%. The remaining 10% is produced by rices of the Japonica (medium grain) subgroup. In total, the *Marismas* area represents half of all the land in the EU devoted to Indica rice.

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'Rice blast', caused by the fungus *Pyricularia oryzae* Cav. is the most important disease of rice worldwide, both in terms of its distribution (Anonymous, 1968; Pans, 1976) and the damage it causes (Ou, 1972, 1980). Its great destructive potential can cause serious yield losses; controlling this disease is therefore one of the main goals of rice growers.

The presence of the disease in Spain was first mentioned in 1968 (Anonymous, 1968; Benlloch, 1975), and in 1978 it was reported in the paddies of the *Marismas del Guadalquivir* (Marín-Sánchez and Jiménez-Díaz, 1981). The aetiology and importance of *P. oryzae* disease was studied by Marín-Sánchez (1979), who reported *Pyricularia* infections to occur all over the *Marismas* rice area. These infections affected all the plant organs and caused serious yield losses when the climatic conditions where favourable to the disease; over 90% of the plants were affected. In 1997, rice blast seriously affected all the cultivars in this area; yield losses reached 15% —in economic terms some € 10 million were lost.

Pyricularia oryzae can cause damage to any aerial part of a rice plant, although the leaves and panicles (necks) are the most commonly affected organs. Leaf infection reduces the photosynthetic area of the plant, which can lead to its death. Panicle infection is that which causes the most important economic losses via yield reduction (Roumen, 1992).

The severity of the damage inflicted differs depending on the part of the plant affected and the cultivar. The use of resistant cultivars has been the cheapest and most effective way of controlling this disease, however, the high epidemic-causing and evolutionary potentials of this pathogen have led to plant resistance being overcome via the emergence of new fungal strains. In addition, the different types and degrees of resistance of the different cultivars, and the influence of environmental factors (Xia *et al*., 1993), affect the expression of resistance (Ou, 1980).

Currently, nearly all commercially grown European rice cultivars are susceptible to the fungus, yet there is little scientific information about the resistance of commercial cultivars to blast under European field conditions.

The aims of this study were: a) to describe the disease symptoms caused by *P. oryzae* in rice growing in the *Marismas del Guadalquivir*, and b) to compare the resistance of 14 commercial cultivars of different origin to natural *P. oryzae* infection under field conditions.

Material and Methods

All assays were undertaken at the Sartenejales (La Puebla del Río, Sevilla; 850 ha, 37º 22' N, 6º 10' W) and Casudis (Los Palacios, Sevilla; 2,400 ha, 37º 20' N, 6º 13' W) estates, both of which have clayey soils, during the 2002 and 2003 rice growing seasons. The altitude of both estates is just 3 m above sea level. The tillage and agronomic practices selected were those that suited *P. oryzae* infection (late seeding date, high plant density and high N-fertilisation rate). This allowed the disease intensity (Teng and James, 2002) associated with the different cultivars to be determined.

Fourteen commercial cultivars (belonging to both the Japonica and Indica groups) of unknown resistance to the pathogen (Table 1) were seeded in independent seedbeds between June 4th and 10th of 2002 and 2003. All these cultivars have early cycles and are adapted to the conditions of the Mediterranean climate. The plantlets were transplanted to plots at the two sites 30-35 days later (between the 9th and the 13th of July). These plots consisted of three rows (10 m long) 20 cm apart; the plants were spaced 25 cm apart. The experiment had a random block design with three replicates. Four additional rows were sown with 'Baixet' (known to be very susceptible to the pathogen in the conditions of the *Marismas*) and two more with 'Maratelli', a cultivar internationally recognised as susceptible to *P. oryzae* (Roumen *et al.,* 1997). These, together with five additional terminal rows of 'Baixet' that surrounded the trial area, acted as an inoculum source.

Nitrogen fertiliser was applied on two occasions, on May 10th (100 kg ha^{-1} of 46% urea [46 U N_2] applied mechanically) and on August 1st (granulated 21% ammonium sulphate, to provide 250 units of nitrogen ha^{-1}).

Bearing in mind that in this area the first disease symptoms in previous years had appeared around mid July, samples (30 leaves, nodes, panicle bases and ligules per plot, depending on the phenological stage of the plants at the time of sampling) were taken and examined for infection every 10 days from July 15th onward. These were packed individually by cultivar and date. At the end of the crop cycle, 30 additional plants per plot were sampled to determine the degree of disease in each tiller. The pathometric variables studied for each cultivar were incidence (percentage of affected plants) and severity (level of disease caused by the pathogen). Severity was studied by measuring the total plant surface affected and the number of affected nodes, ligules and panicle bases. The percentage infection

Table 1. Incidence of *P. oryzae* infection (% damaged plants) by cultivar, site and season

Cultivar	Subgroup	Origin	No. tillers/ plant	Incidence (%)				Mean	Incidence level
				2002		2003			
				Casudis	Sartenejales	Casudis	Sartenejales		
Maratelli	Japonica	Italy	13.83	100.00	100.00	96.83	100.00	98.94	Very high (80-100%)
Baixet	Japonica	Valencia (Spain)	16.24	100.00	100.00	92.28	100.00	97.43	
Thainato	Japonica	California (USA)	15.90	92.03	91.42	86.43	91.21	89.96	
Galatxo	Japonica	Tarragona (Spain)	17.70	82.09	79.77	84.82	79.58	82.23	
Hispagrán	Japonica	California (USA)	17.53	80.90	70.28	71.80	70.52	74.33	High (60-80%)
Jacinto	Indica	California (USA)	17.57	53.40	49.70	55.53	49.84	52.88	Medium (40-60%)
Doongara	Indica	Australia	13.82	61.00	47.65	47.28	46.23	51.98	
Fonsa	Japonica	Tarragona (Spain)	17.56	50.71	47.56	54.84	46.18	51.04	
L-202	Indica	California (USA)	16.43	47.81	46.63	51.30	45.60	48.58	
Susan	Japonica	Tarragona (Spain)	19.63	49.13	46.02	42.38	46.42	45.84	
Maso	Japonica	Tarragona (Spain)	18.51	45.88	43.50	48.69	43.07	46.02	
Puebla	Japonica	California (USA)	15.11	45.87	39.09	44.65	39.81	43.20	
Marisma	Japonica	Tarragona (Spain)	15.59	33.42	34.20	31.13	33.42	32.92	Low (20-40%)
Ullal	Japonica	Valencia (Spain)	18.86	29.76	30.27	40.22	27.89	33.42	
LSD 95%				9.66	8.65	4.85	7.89		

of the whole plant, i.e., the weighted average of all the considered parts (adjusted percentage infection), was then calculated. This took into account the repercussion that each plant part might have on the grain yield if infected; for this weighting, the disease severity value for the ligule was multiplied by 1, that of the nodes and damaged leaf surface by 2, and that of the panicle base by 3.

A 0-100% scale was used to measure incidence: 0-20% (very low susceptibility), 20-40% (low susceptibility), 40-60% (medium susceptibility), 60-80% (high susceptibility), and 80-100% (very high susceptibility). A 0-50% scale was used for recording severity: 0-10% (very low severity), 10-20% (low severity), 20-30% (medium severity), 30-40% (high severity), and 40-50% (very high severity).

The results were analysed by ANOVA using Statistix v.7 software (Analytical Software, USA). Least significant differences (LSD) were calculated (significance was set at $P = 0.05$). When the range of percentages exceeded 40%, arcsine $x^{1/2}$ transformation was used (Little and Hills, 1999).

Results

Disease symptoms

The first symptoms of *P. oryzae* infection, whitish or green-greyish spots with darker greenish edges, appeared on the leaves in all cultivars when they were in the mid tillering stage. As the damage developed,

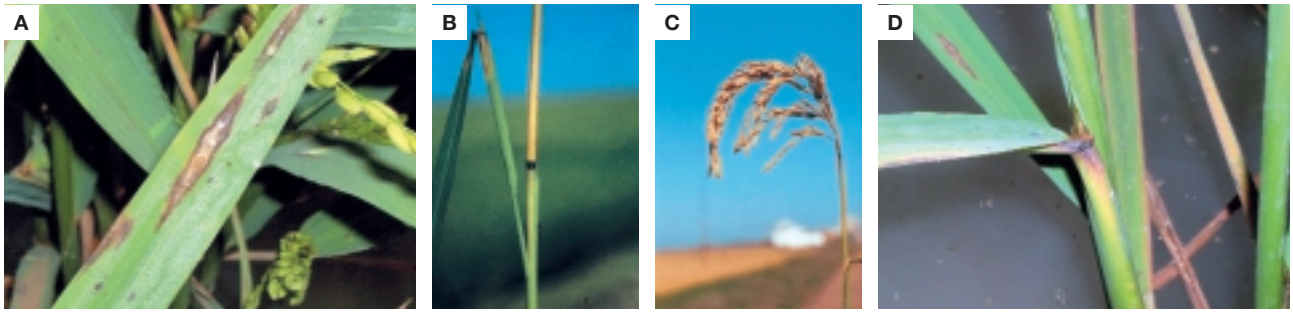

Figure 1. Symptoms caused by *Pyricularia oryzae* in rice: A, spots on leaves; B, node damage; C, ligule lesion; and D, panicle base damage.

the spots gradually became whitish-greenish in colour with brown-reddish necrotic edges (Fig. 1A). The spots were of different shapes although frequently oval-elliptic with the extremes more or less pointed and facing the same way as the veins.

When sporulation occurred, the centre of the spots turned ash grey due to the presence of conidia and hyphae. On average, the lesions were less than 1 cm long when the crop was in the plantlet stage, and reached 3 or 4 cm by the jointing stage.

In the culm (nodes and internodes) and panicle (base, axis, branches and glumes), necrotic dark brown lesions appeared that sometimes reduced or cut off the circulation of the sap. Node damage (Fig. 1B) was much more common and harmful than at the internodes; this sometimes broke the culm either partially or completely. The damage caused by *P. oryzae* was frequently occasioned by dewdrops carrying the fungus sliding down the flag leaf and collecting at the collar and affecting the ligule (Fig. 1C).

Infection of the panicle base (Fig. 1D) was usually the most destructive form of infection. As with node damage, this sometimes caused a reduction in the weight grain and even led to white, erect panicles with empty grains when the attack was early and severe. Infection of the panicle base and branches (axis, primary and secondary branches) and damage to the pedicels holding the spikelets were sometimes simultaneous, depending on the environmental conditions and cultivar. However, infections of the branches frequently appeared later, during the ripening phase.

Cultivar resistance

Table 1 shows the incidence of the damage caused by *P. oryzae* in the different cultivars, at the different estates, and in the different years of the study. Incidence figures varied among the different cultivars but were generally constant for each at the two sites and for both seasons. *Pyricularia oryzae* infected between 90% and 100% of the 'Maratelli' and 'Baixet' plants. 'Thainato', 'Galatxo' and 'Hispagrán' showed mean incidence levels of 89.96%, 82.23% and 74.33% respectively. A third group of cultivars including 'Jacinto', 'Doongara', 'Fonsa', 'L-202', 'Susan', 'Maso' and 'Puebla' suffered medium incidence (40-60%). 'Marisma' and 'Ullal', with 20-40% of plants infected, suffered low incidence of infection.

With respect to leaf disease severity (Table 2), 'Baixet' showed the highest percentage of spots, reaching values in 2002 and 2003 of 26.97% (i.e., leaf area affected) and 24.77% respectively at the Casudis site, and 28.26% and 28.35% respectively at the Sartenejales site. These values were significantly higher than those reached by the remaining cultivars in the same year and in the same locations. 'Baixet' was followed by 'Galatxo', 'Thainato', 'Hispagrán', 'Maratelli' and 'Jacinto', which showed only low disease severity (10-15%). The cultivars that showed the least affected leaf area were 'Marisma' and 'Ullal', with values of < 2%.

The cultivar that showed the worst nodal infection was 'Maratelli' (significantly different compared to the remaining cultivars; Table 3). This was followed by 'Baixet', 'Thainato', 'Galatxo' and 'Hispagrán', which showed values ranging between 20 and 40% (i.e., of nodes infected). 'Maso' and 'Ullal' were those that showed the least node susceptibility (or greatest node tolerance) to the pathogen.

'Maratelli' was the most susceptible to ligule infection, with a mean value of 39.38% (i.e., of ligules infected) (Table 4), a value significantly higher than that of the remaining cultivars. 'Baixet', 'Doongara', and 'Thainato' showed medium susceptibility to ligule

Table 2. Percentage leaf area infection caused by *P. oryzae* per cultivar, site and season

Cultivar	2002		2003		Mean
	Casudis	Sartenejales	Casudis	Sartenejales	
Baixet	26.97	28.26	24.77	28.35	27.09
Galatxo	13.36	11.45	12.49	15.32	13.16
Thainato	13.67	12.16	12.49	13.99	13.08
Maratelli	11.24	15.11	11.82	10.95	12.28
Hispagrán	14.57	10.89	13.90	10.31	12.42
Jacinto	12.00	10.15	10.00	10.11	10.57
L-202	7.07	6.16	6.30	6.04	6.39
Maso	5.08	6.29	4.84	6.06	5.57
Susan	5.71	6.18	5.20	6.59	5.92
Fonsa	3.41	6.96	3.37	7.52	5.32
Puebla	3.26	3.28	3.07	3.22	3.21
Doongara	6.05	5.80	5.40	5.57	5.71
Marisma	1.57	1.95	1.46	1.95	1.73
Ullal	1.16	1.37	1.25	1.41	1.30
LSD 95%	0.69	0.47	0.36	0.65	

Table 3. Percentage node infection caused by *P. oryzae* per cultivar, site and season

Cultivar	2002		2003		Mean
	Casudis	Sartenejales	Casudis	Sartenejales	
Maratelli	57.30	55.39	41.25	57.79	52.93
Baixet	39.69	38.91	33.56	38.56	37.68
Thainato	33.61	33.61	27.84	30.67	31.43
Galatxo	27.25	24.81	24.51	23.80	25.09
Hispagrán	22.06	21.94	17.08	19.85	20.23
Fonsa	22.03	19.71	13.37	16.10	17.80
Doongara	21.81	17.68	10.42	16.35	16.56
Susan	17.45	16.98	11.38	15.81	15.40
Puebla	16.70	16.59	11.74	14.12	14.78
Jacinto	16.69	16.32	11.72	13.21	14.48
L-202	12.41	12.61	8.76	10.14	10.98
Marisma	12.39	12.39	6.27	11.10	10.53
Maso	8.73	9.12	6.60	5.55	7.50
Ullal	7.77	8.18	5.70	5.70	6.83
LSD 95%	3.18	1.39	0.41	1.48	

infection (20-30%). 'Doongara', which is of great agricultural value and provides high yields, showed a percentage of affected ligules of almost 28% in 2002, a value similar —although significantly lower— to that for 'Baixet' in the same year. 'Galatxo', 'Hispagrán', 'Jacinto', 'Puebla', 'Fonsa' and 'L-202' showed average susceptibility to ligule infection (10 and 20%). 'L-202', which is agronomically similar to 'Doongara' also showed average susceptibility (10.05%). 'Ullal' was the only cultivar with very low ligule infection values.

The most susceptible cultivar to panicle base infection was 'Thainato', which showed a susceptibility level of over 75% (Table 5). 'Baixet' showed similar susceptibility (no significant differences were seen between 'Thainato' and 'Baixet' in Casudis in 2002 and Sartenejales in 2003. Compared to 'Thainato', 'L-202' and 'Doongara' showed significantly greater collar resistance to *P. oryzae*. 'L-202' was, however, more susceptible than 'Doongara'. 'Ullal', which usually performed as a very resistant cultivar, showed a remarkably high collar infection rate of 23.55% in 2003 at the Casudis site.

Table 4. Percentage ligule infection caused by *P. oryzae* per cultivar, site and season

Cultivar	2002		2003		Mean
	Casudis	Sartenejales	Casudis	Sartenejales	
Maratelli	39.99	37.89	40.54	39.13	39.38
Baixet	30.04	30.48	22.43	27.19	27.53
Doongara	27.80	27.95	20.71	27.50	25.99
Thainato	24.80	22.75	21.18	22.65	22.84
Galatxo	17.29	18.38	13.06	17.72	16.61
Hispagrán	19.15	16.48	12.09	15.38	15.77
Jacinto	16.06	15.79	10.49	14.77	14.27
Puebla	11.51	12.91	8.90	10.20	10.88
Fonsa	12.38	11.27	8.65	9.35	10.41
L-202	11.30	11.11	7.86	9.93	10.05
Maso	8.21	8.74	6.01	8.34	7.82
Susan	8.84	8.52	5.70	7.13	7.54
Marisma	6.54	5.91	4.23	7.89	6.14
Ullal	2.36	2.82	3.71	1.46	2.58
LSD 95%	1.82	1.17	0.95	1.38	

Table 5. Percentage panicle (neck) infection caused by *P. oryzae* per cultivar, site and season

Cultivar	2002		2003		Mean
	Casudis	Sartenejales	Casudis	Sartenejales	
Thainato	88.29	75.68	78.62	74.59	79.30
Galatxo	81.43	69.63	77.24	71.35	74.91
Baixet	78.71	60.78	65.32	71.42	69.05
Maratelli	74.80	56.81	63.41	53.91	62.23
Hispagrán	59.53	60.33	61.26	58.89	60.00
Jacinto	51.84	48.27	45.07	48.48	48.42
Fonsa	49.20	45.55	47.19	44.68	46.66
Maso	44.89	42.56	44.09	40.56	43.03
L-202	45.86	36.85	41.92	37.22	40.46
Susan	45.34	38.46	33.26	38.21	38.82
Doongara	33.21	31.95	35.78	30.14	32.77
Puebla	36.37	19.06	31.85	17.88	26.29
Marisma	29.10	17.54	25.52	16.82	22.25
Ullal	19.08	8.37	23.55	8.28	14.82
LSD 95%	13.06	5.42	5.44	5.68	

With respect to infection percentages of the plant as a whole (which takes into account the weighted data for each plant part) (Table 6), 'Ullal' was found to be the most resistant cultivar. 'Marisma' and 'Puebla' were classified in increasing order of susceptibility among the low severity cultivars (10-20%). 'Maso', 'L-202', 'Susan', 'Doongara' 'Fonsa' and 'Jacinto' showed medium disease severity (20-30%), while 'Hispagrán' and 'Galatxo' had high disease severity levels. 'Thainato', 'Maratelli' and 'Baixet' showed very high severity values (40-50%).

Discussion

Despite the low incidence of *P. oryzae* in recent years in the *Marismas del Guadalquivir*, the incidence and severity of infection under the trial conditions provided enough descriptive and comparative data to determine the susceptibility of the different cultivars.

'Baixet' and 'Maratelli' were the most susceptible. With respect to leaf and panicle base infection, 'L-202' was more susceptible than 'Doongara', and more susceptible than 'L203' and 'L204' which were tested

Table 6. Whole plant infection severity (taking into account the weighted data for each plant part) caused by *P. oryzae* per cultivar

Cultivar	2002		2003		Mean (%)	Severity
	Casudis (%)	Sartenejales (%)	Casudis (%)	Sartenejales (%)		
Baixet	49.93	43.39	41.88	46.9	45.52	Very high (40-50%)
Maratelli	50.18	43.66	42.11	42.29	44.56	
Thainato	44.27	42.66	42.21	41.96	42.77	
Galatxo	39.1	37.47	39.84	38.75	38.79	High (30-40%)
Hispagrán	33.87	32.89	32.22	31.54	32.63	
Jacinto	28.62	26.69	23.64	25.85	26.2	Medium (20-30%)
Fonsa	26.36	25.15	22.96	23.82	24.57	
Doongara	22.89	21.34	19.96	20.22	21.10	
Susan	23.89	21.27	17.33	20.82	20.82	
L-202	23.48	19.9	20.46	19.16	20.75	
Maso	21.31	20.9	20.14	19.15	20.37	
Puebla	20.06	13.72	16.75	12.31	15.71	Low (10-20%)
Marisma	15.22	10.9	12.03	9.44	11.89	
Ullal	9.68	5.87	11.03	5.06	7.91	Very low (0-10%)

by other authors in California (Greer and Webster, 2001). However, 'Doongara' was more susceptible than 'L-202' to node and ligule infection. Node infections did not usually completely stop the flow of sap to other parts of the plant, and were not as destructive as infections of the panicle base; the latter usually caused empty grains and yield losses. Both cultivars have a similar genetic origin, an early cycle, a high resistance to lodging, are long grain varieties, and both are very productive (Aguilar and Grau, 1996), although 'Doongara' is somewhat more productive and is widely used in the Guadalquivir Valley. 'L-202' has a slightly longer grain than 'Doongara' and is widely used in other countries.

The most resistant cultivars to the pathogen were 'Ullal' and 'Marisma'. 'Ullal' has a medium grain and a short growth cycle under the Mediterranean climate; its grain yield is high and the plants are quite short. However, due to its high vitrosity, its market demand is reduced. 'Marisma' stands out for its large grain size and culinary quality, it has a good agronomic performance, provides high yields, and is very interesting from an industry standpoint (Aguilar and Espinosa-Ruiz, 2003).

The results obtained in the present study may be useful when assessing the suitability of commercial cultivars for planting in areas where there is a risk of *P. oryzae* infection. In addition, the cultivars found to have a low susceptibility to *P. oryzae* could be selected as parentals in breeding programs.

Studies on the different strains of *P. oryzae* and their interactions with different cultivars are currently underway.

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