Med. Fac. Landbouww. Rijksuniv. Gent, 55(3a), 1990

SEED DETECTION OF Pseudomonas fuscovaginae IN WHEAT

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Abstract:

Seed from seven bread wheat genotypes showing bacterial sheath rot symptoms after being inoculated at the booting stage with P. fuscovaginae, was harvested and tested after three months storage for seed transmission of the bacterium. Ten-gram seed samples were washed in sterile saline and the washing mixture was plated on KBC agar after dilution. In five genotypes, fluorescent pseudomonads obtained from single colonies proved to be P. fuscovaginae after determination using biochemical, serological and pathogenicity tests. Using a growing-on technique in sterile agar tubes for 100 grains of three genotypes, sheath rot lesions were obtained after 21 days incubation at 15°C. Fluorescent pseudomonads isolated from six different seedlings reacted with antiserum anti-P. fuscovaginae by slide agglutination.

Introduction

Pseudomonas fuscovaginae Miyajima, Tanii and Akita nom. rev. 1983, was first identified as the causal agent of bacterial sheath brown rot of rice (Oryza sativa L.) in northern Japan (Tanii et al. 1976; Miyajima, 1983; Miyajima et al.,1983). In 1982, the disease was, for the first time, found outside Japan in highland swamp rice of Burundi (Autrique and Maraite, 1983). The bacterium was then isolated from rice samples from IRRI (The Philippines), Rwanda and Madagascar (Duveiller et al., 1988; Rott et al., 1989; Duveiller et al., 1990), suggesting a widespread occurrence of the disease, particularly in highland environments. The isolation of P. fuscovaginae from maize and sorghum at locations distant from rice cultivation, confirmed this hypothesis and indicated that the disease was not limited only to rice (Duveiller et al., 1989).

In Mexico, the occurrence of the bacterium was suggested in rice (Zeigler and Alvarez, 1987; Diaz Balderas et al., 1989). In this country, bread wheat is grown in the wet season, in humid tropical highland environment with cool night temperatures, at elevations above 2300 meters. Bacterial sheath rot symptoms had been observed in bread wheat previously (Gilchrist, personal communication), but definitive identification of the causal agent, P. fuscovaginae was only made in 1987 (Duveiller, unpublished data). The disease incidence is usually below 0.1%.

In rice, the evidence for seed transmission of P. fuscovaginae has been shown (Miyajima, 1983; Goto et al., 1988). The purpose of the present study was to analyze whether the pathogen may also be seedborne in wheat.

Materials and methods

A preliminary screening trial to analyze the resistance of bread wheat genotypes to bacterial sheath brown rot was conducted in Toluca, Mexico during 1989. The inoculation was done at booting stage, using a concentrated bacterial suspension of P. fuscovaginae (CFBP3078 = CIMMYT Reference strain CB52) adjusted to 10^9 CFU/ml (Colony Forming Unit) with the help of a spectrophoto meter (Spectronic Mini 20, Milton Roy). Bacterial sheath rot symptoms with different severity levels were observed on inoculated tillers. The seed of nine genotypes from the trial (30 spikes per line) was harvested during the first week of November, 10 weeks after the inoculation, and was stored for three months at room temperature in the laboratory. Since the general disease incidence in Mexico is very low, these grains were used to analyze seed transmission of the bacterium.

Experiment 1. Washing seed and plating assay

Ten-gram seed samples of genotypes Chuan Mai #8, Anza, Seri M 82, Alondra, Thornbird, Anahuac F 75 and Shangai 8 were washed for 30 min. in 100 ml of sterile saline solution (Schaad and Donaldson, 1980). The 10^{-1} and 10^{-2} dilutions in sterile phosphate-buffered saline (PBS) 0.01 M, pH 7.2 (Lelliott and Stead, 1987) were assayed (0.1 ml) onto KBC agar (Mohan and Schaad, 1987), proved to be effective for the isolation of P. fuscovaginae (Duveiller, unpublished data). This medium was chosen because it may significantly reduce the number of fluorescent saprophytic bacteria frequently associated with P. fuscovaginae in old sheath rot lesions. Single colonies of fluorescent pseudomonads were noted after 5 days incubation at 30°C and were subcultivated on KB agar (King et al., 1954).

Basic biochemical identification tests allowing differentiation of P. fuscovaginae from non-pathogenic fluorescent pseudomonads (Duveiller et al., 1988) were done: Kovac's oxidase (Bradbury, 1970), production of 2- ketogluconate (Haynes, 1951), acid production from trehalose and from inositol (Dye, 1962). Results were compared with those of P. fuscovaginae (CFBP3078) and Pseudomonas syringae pv. syringae (CB21) CIMMYT reference strains.

The slide agglutination test was performed using antiserum anti-P. fuscovaginae -HMB266 provided by Dr. Maraite and following the same method as for rice strains (Duveiller et al., 1988).

Pathogenicity was tested by inoculating wheat plants of genotype Alondra, grown in the greenhouse up to the 4 leaf stage, by injecting the leaf sheath at 5 cm above soil level, with a bacterial suspension adjusted to 10^9 CFU/ml. The symptoms were assessed after a week incubation in a dew chamber (Percival E-54U-DL, Boone, Iowa), calibrated to 22/13°C day/night temperatures and a 12 hr photoperiod.

Experiment 2. Growing-on method

Individual tubes (160 mm) filled with 5 ml agar (3.5 g/l) and autoclaved were sown with one hundred grains of genotypes Kauz"s", Shanghai 7 and Shanghai 8. Tubes were incubated with a 12 hr photoperiod at 15°C. After one week, 5 ml sterile water was added to every tube. Symptoms were assessed after 21 days and a total of 20 isolations was performed on KBC agar medium. Fluorescent pseudomonads obtained in pure culture were only tested in slide agglutination with anti-P. fuscovaginae HMB266 antiserum, as in experiment 1.

Results

In experiment 1, fluorescent bacteria were observed on KBC, after 5 days incubation, in isolations from all seven genotypes (Table 1). Single colonies

were selected at random and a total of 26 pure cultures was obtained from the different plates. Fifteen strains had the same characteristics as CFBP3078 P. fuscovaginae reference strain in the basic biochemical tests (Table 2). They were positive for Kovac's oxidase and showed the simultaneous occurrence of no 2-ketogluconate production and of acid production from trehalose but not from inositol. These strains were all pathogenic on wheat. A typical brown rot lesion of more than 10 cm was noted extending on the plant sheaths after 8 days, inducing the collapse of the stem and later, a new tillering from the base of the plant as already observed for inoculations of wheat with P. fuscovaginae (Duveiller, unpublished data). Almost pure cultures of fluorescent Pseudomonas with the same characteristics as P. fuscovaginae were easily re-isolated from the borders of the lesions. All strains with the biochemical basic characters of P. fuscovaginae and which were pathogenic on Alondra wheat, reacted positively with P. fuscovaginae antiserum in the slide agglutination tests, as the CFBP3078 reference strain. The other fluorescent pseudomonads isolated from wheat seed did not have these characteristics. They did not react with the antiserum and were not pathogenic on wheat. They showed the production of 2-ketogluconate and acid from trehalose, and had a variable reaction for acid production from inositol.

In experiment 2, germination was better than 85% in the three seed lots and the agar concentration used, proved to be appropriate to allow the root to penetrate the medium. Seedlings with sheath browning were observed after 21 days in the three genotypes. Pure cultures from single fluorescent colonies on KBC were difficult to obtain due to the occurrence of numerous, presumably saprophytic, bacteria. Cultures from six different seedlings, showing a yellow brown fluorescence on KB typical of P. fuscovaginae, reacted positively with antiserum anti-P. fuscovaginae HMB266 in the slide agglutination test. Under the limitation of serological specificity of the antiserum used, this result suggests that P. fuscovaginae is associated with brown lesions observed on seedlings' coleoptiles and leaf sheaths.

Table 1. Number of fluorescent pseudomonads and Pseudomonas fuscovaginae from single colonies, after washing seed of bread wheat genotypes showing sheath rot symptoms and dilution plating on KBC agar

Lot	Genotype	Fluorescent pseudomonad strains selected from single colonies on KBC agar	P. fuscovaginae
1	Chuan Mai #18	4	1
2	Anza	1	0
3	Seri M 82	3	3
5	Alondra	7	5
6	Thornbird	4	3
7	Anahuac F 75	3	3
9	Shanghai 8	4	0

Discussion

P. fuscovaginae was detected on seed collected from wheat spikes showing bacterial sheath rot and was also found to be associated with sheath browning on young seedlings grown in sterile tubes incubated at moderate temperature and in wet conditions. The results and observations presented here support the finding that, as in rice, P. fuscovaginae is seedborne and seed-transmitted in wheat. This conclusion is not surprising and potentially any bacterial pathogen may probably be seed-transmitted (Neergaard, 1977).

Table 2. Characteristics of fluorescent pseudomonads strains isolated from wheat seed, compared to Pseudomonas fuscovaginae and Pseudomonas syringae pv. syringae reference strains from Mexico

	P. fuscovaginae		Saprophytic fluorescent pseudomonads	P.s. pv. syringae
	Reference strain	Strains isolated from wheat seed	Strains isolated from wheat seed	Reference strain
	CFBP3078 (CB52)	1.2, 3.1-3.3, 5.1-5.4, 5.7, 6.1-6.3, 7.1-7.3	1.1, 1.3, 1.4, 2.1, 5.5, 5.6, 6.4, 9.1-9.4	CB21
Fluorescence on KB	+[a]	+	+	+
Kovac's oxidase	+	+	+	-
Acid production from:				
- trehalose	+	+	+	-
- inositol	-	-	V	-
Production of 2-ketogluconate	-	-	+	-
Slide agglutination with P. fuscovaginae-HMB266 antiserum	+	+	-	-
Pathogenicity on wheat	+	+	-	+

a) + = positive reaction, - = negative, V = variable.

Table 3. Number of positive slide agglutination tests using anti-P. fuscovaginae HMB266 antiserum, for fluorescent pseudomonad strains isolated on KBC medium from different seedlings showing sheath browing after 21 days growth in sterile agar tubes at 15°C

Lot	Genotype	Seedlings with sheath browning	Isolations on KBC	Positive agglutination
4	Kauz"s"	16	5	2
9	Shanghai 8	19	5	1
10	Shanghai 7	25	10	3

The methods used in this study must be considered qualitative. It is difficult to recognize with the naked eye the slight difference between the yellow-brown colonies of P. fuscovaginae (Duveiller et al., 1989) and the more diffuse green fluorescence of saprophytic fluorescent pseudomonads commonly associated with plant samples. This is particularly true when using KBC where fluorescence is weaker than on KB. Therefore, single fluorescent colonies were selected at random on KBC, to be tested later with biochemical and serological tests.

The growing-on method used here to assess the possibility of P. fuscovaginae transmission to seedlings should not be considered quantitative. It may overestimate the rate of transmission of the bacterium if only the sheath discoloration percentage is taken into account. Rot lesions may also be caused by fungi frequently observed in association with untreated seed and Pseudomonas reacting with the P. fuscovaginae antiserum was only recovered in 6 of 20 isolations. In rice, seed desinfectation with prochloraz was used to assist isolation of P. fuscovaginae from young infected seedlings tested with a similar technique (Duveiller, unpublished data). In the present study no fungicide was used. Former preliminary detection trials showed that the addition of 0.5 ml per tube, of a 0.01 g a.i./l prochloraz solution, the day that grains were placed into the agar tubes, was deleterious to wheat.

In the second experiment the identification was based only on serology. Identification of strains isolated from sheath brown lesions using this method is valid under the limit of specificity of the antiserum. All the P. fuscovaginae strains from Mexico, however, react with the anti-HMB266 antiserum. Since bacterial sheath brown rot has a broad distribution and is found in various cereal crops, the improvement of routine serological seed detection techniques is justified.

Given the very low incidence of bacterial brown sheath rot of wheat in the field, the effective seed transmission rate in Mexican conditions is probably low. Since P. fuscovaginae is a widespread opportunistic bacterium, initial inoculum may not only come from seed but also from bacteria that survive in soil or on other wild graminaceous plants. However, little is known of the epidemiology of bacterial sheath brown rot, particularly in wheat, so that other studies are required.

Acknowledgements

This study is part of the project on Wheat Bacterial Diseases, funded by the Belgian Administration for Development Cooperation. The authors are grateful to Mr. J. Robinson and Mr. G. Hettel for reviewing the manuscript and to Dr. Maraite from UCL, Louvain-la-Neuve, Belgium, for providing the antiserum.

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Résumé

Détection de Pseudomonas fuscovaginae sur semences de blé

De la semence de sept genotypes de blé tendre présentant des symptômes de pourriture brune de la gaine foliaire, suite à l'inoculation au stade gonflement avec P. fuscovaginae, a été récoltée et testée pour la transmission de la bactérie par la semence, apres trois mois d'entreposage. Des échantillons de dix grammes de semences ont été lavés dans de la solution physiologique stérile et le liquide de lavage a été ensemencé sur KBC agar après dilution. Après détermination avec des tests biochimiques, sérologiques et de pathogénicité, des pseudomonas fluorescents obtenus à partir de colonies individuelles provenant de cinq génotypes s'avérèrent être des P. fuscovaginae. En utilisant une technique de detection sur jeunes plantules poussant dans des tubes steriles, pour 100 grains appartenant à trois génotypes, des lésions de pourriture de gaine ont été obtenues après 21 jours d'incubation à 15 degrés. Des pseudomonas fluorescents isolés de six plantules différentes ont réagi avec l'antisérum anti-P. fuscovaginae par agglutination sur lame.