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INCIDENCE OF BACTERIAL STRIPE OF RICE IN IRAN[1]

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ABSTRACT

Bacterial stripe was observed on rice seedlings in eastern Mazandaran in 1983. The cultivars affected were Amol-2, Amol-3 and 6005. The disease was rarely found also on Tarom cultivar. On the basis of physiological and biochemical tests the causal bacterium was identified as *Pseudomonas avenae*. This is the first report of the disease in Iran.

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وقوع بیماری نواری باکتریائی برنج در ایران

حشمت اله رحیمیان

استادیار بیماریهای گیاهی آموزشکده کشاورزی دانشگاه مازندران - ساری

خلاصه

بیماری نواری باکتریائی درسال ۱۳۶۲ روی گیاهچه‌های برنج درخزانه‌های شرق مازندران مشاهده گردید. ارقام آلوده شامل آمل ۲، آمل ۳ و ۶۰۰۵ بودند ولی ندرتا بیماری در رقم طارم نیز وجود داشت. براساس نتایج آزمایشات فیزیولوژیکی وبیوشیمیائی باکتری عامل بیماری *Pseudomonas avenae* تشخیص داده شد. این اولین گزارش از وجود بیماری در ایران است.

INTRODUCTION

A disease resembling bacterial stripe was observed in May 1983 in some rice seedbeds of Sari and Amol. Symptoms included brown stripes of one to several centimeters long on leaves and the leaf sheaths. Frequently the lesions expanded, encompassed the full length of the sheath and often extended into the leaf blade. The pathogen affected primarily

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the sheaths and the lower leaves, but in severe cases the entire seedlings were blighted. These symptoms were similar to those of the bacterial stripe of rice caused by *Pseudomonas avenae* Manns (8, 11, 14) (Synonym *P. alboprecipitans* Rosen) (9, 13, 15). The causal agent has been described in the past under *P. setariae* (Okabe) Savulescu, *P. panici* (Elliott) Stapp, *P. panicimilliacei* (Ikata and Yamauchi) Savulescu, and *Xanthomonas panici* (Elliott) Savulescu (1, 2, 4). The disease has been observed in Sari and Amol on Amol-2 and Amol-3 rice cultivars. Generally less than 5% of the seedlings in an area have shown signs of infection. The purpose of the present study was to isolate and identify the causative agent of the disease.

MATERIALS AND METHODS

Isolation of the Pathogen

Infected rice seedlings of cultivar Amol-2, collected from Sari and Amol areas were used in isolation attempts. Infected leaves were washed thoroughly in tap water and finely chopped with a sterile scalpel in a few drops of sterile distilled water (SDW). A drop of the suspension was diluted 10- to 100- fold in SDW and a loopful was streaked on nutrient agar (NA). The plates were incubated at 28°C for 72 hr. Single colonies were picked and restreaked onto NA plates. Cultures were maintained on NA slopes at 4°C.

Pathogenicity Test

Inoculum was prepared from cultures grown on NA for 48 hr at 28°C. Bacterial suspensions were prepared in SDW and adjusted turbidometrically to contain 10^8 colony forming units per milliliter (cfu/ml). Rice cultivar Amol-2 was grown in 15-cm plastic pots containing sterilized field soil. Seedlings at 4-leaf stage were inoculated either by puncturing the leaves with fine needles and spraying with the bacterial suspension or by injecting a dilute bacterial

suspension containing 10^6 cfu/ml into the leaf until a 1-cm length of the leaf became watersoaked. All inoculated seedlings were enclosed in polyethylene bags for 72 hr at 18-28°C, then uncovered and placed on the greenhouse bench. Four plants were inoculated with each of the four strains or treated with SDW as the control.

Characterization of the Pathogen

The causal bacterium was identified on the basis of nutritional and biochemical tests performed on four strains of the bacterium. Tests for oxidase, oxygen requirement, arginine dihydrolase, urease, potato soft rot, sodium chloride tolerance, esculin hydrolysis, gelatin liquefaction, production of hydrogen sulfide from cystein, levan production, nitrate reduction, protein digestion, and Gram reaction performed according to Schaad (12). Hydrolysis of Tween 80 was tested according to Misaghi and Grogan (7). The bacterial isolates were checked for the presence and the arrangement of flagella (10). Tests for the production of catalase, indole and acetoin, methyl red reaction, and further tests for reduction of nitrate and liquefaction of gelatin were carried out by the procedure of Dye *et al.* (3). Fluorescin production was tested on medium B of King *et al.* (5). Tobacco hypersensitivity was determined by the procedure of Klement *et al.* (6). Carbon source utilization was tested on the medium of Ayer's *et al.* (12). All carbon sources were filter sterilized through 0.2 µm filters and added to the basal medium at a final concentration of 0.4% (w/v). All tests were carried out in triplicates.

RESULTS AND DISCUSSION

Isolation of the Pathogen

A gram- negative nonfluorescent bacterium was consistently isolated from infected rice leaves of cultivar Amol-2. Almost pure cultures were obtained when isolations were attempted from small young streaks. Four strains, two each

from Sari and Amol, were used in pathogenicity and characterization tests.

Pathogenicity Tests

All four strains tested were pathogenic to Amol-2 cultivar. Both inoculation methods were successful in eliciting disease reaction in rice, but injection infiltration produced a clearer and faster response. In needle puncture inoculation method streaks of 0.5 to 1 mm wide and 5 to 20 mm long (Fig. 1) appeared four to seven days after inoculation. The streaks were initially watersoaked but gradually turned yellow and finally became brown and necrotic. These lesions expanded rapidly, forming long stripes especially along the midvein. The same bacterium was reisolated from the lesions.

Characterization of the Pathogen

All four isolates of the stripe bacterium produced whitish, circular, raised colonies on NA. They were butryous when young but became viscid as the cultures aged. There was often a white precipitate at the periphery of the colonies. All isolates had one polar flagellum, were oxidase positive and nonfluorescent. Nutritional and biochemical tests (Table 1) helped identify these strains as *P. avenae* (2, 9, 13, 14, 15). The species have been reported, in one case, to be oxidase negative (9, 13) but the reverse has been the case in other reports (2, 14, 15). Results of the present work are in full agreement with those reported by Tsuchiya *et al.* (15). The strains used in this sutdy were also identical in all characters with some of the strains studied by Tominaga *et al.* (14); however, their other strains were capable of producing acid from maltose and lactose too.

In 1985 bacterial stripe was observed on rice lines 6001, 6005, 6008 and on Amol-2, Amol-3 and Sang-e-Tarom cultivars in Sari and Amol. The disease was mainly found in seedbeds but in rare occasions some infected plants could also be traced in the field after transplanting. The level of infection has been low in both locations during 1983- 1985, but

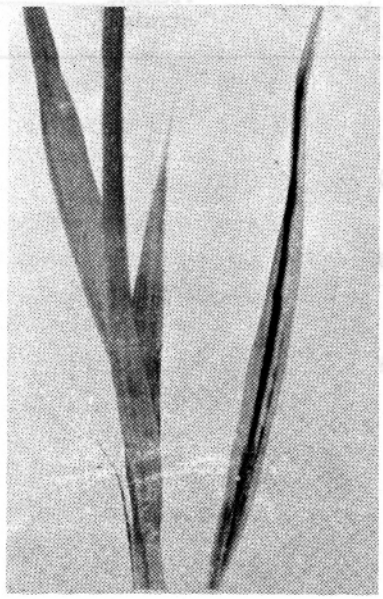

Fig. 1. Symptoms of bacterial stripe of rice on cultivar Amol-2 from artificial inoculation.

Table 1. Comparison of the present strains of rice stripe bacterium with the previously reported strains of *Pseudomonas avenae*.

Tests	Present strains†	*P. avenae* Tsuchiya *et al.*‡
Gram reaction	-§	-
Oxidase	+	+
Oxygen requirement (O/F)	+	+
Catalase	+	+
Arginine dihydrolase	-	-
Urease	+	+
Potato rot	-	-
Tobacco hypersensitivity	+	+
Levan	-	-
Methyl red	-	-
Aceton production	-	-
Production of H_2S	+	+
Production of indole	-	-
Starch hydrolysis	+	ND
Tween 80 hydrolysis	+	+
Gelatin liquefaction	+	+
Esculin hydrolysis	-	-
Casein hydrolysis	+	+
Nitrate reduction	+	+
Litmus milk	alkaline	alkaline
Maximum temperature for growth	42°C	41 - 42°C
Growth on 3% Nacl	+	+
Growth on 4% Nacl	-	-
Acid from:		
Xylose	+	+
Arabinose	+	+
Rhamnose	-	-
Mannose	+	ND
Galactose	+	ND
Glucose	+	+

Table 1. (Continued).

Tests	Present strains	*P. avenae* Tsuchiya *et al.*
Fructose	+	ND
Sucrose	-	-
Lactose	-	-
Maltose	-	-
Melibiose	-	-
Celubiose	-	-
Trehalose	-	-
Raffinose	-	-
Inulin	-	ND
Dextrin	-	-
Glycerol	+	+
Mannitol	+	+
Dulcitol	-	-
Inositol	-	ND
Sorbitol	+	+
Adonitol	-	-
Salicin	-	-
Utilization of:		
Acetate	+	+
Benzoate	-	ND
Citrate	+	+
Lactate	+	+
Malonate	+	+
Tartrate	-	-
Oxalate	-	ND

†Four strains.

‡Tsuchiya *et al.* (1983).

§+ = Positive reaction or growth, - = negative reaction or no growth, ND = not determined.

in 1986 a 10% seedling infection was noted in a Sang-e-Tarom seedbed in Sari. The disease does not seem to be economically destructive at the present, and most of the moderately infected seedlings usually survive.

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