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Etiology of Chinaberry Gall Disease in Iran

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ABSTRACT-Chinaberry (*Melia azedarach* L.) is a beautiful tree indigenous to the Himalayas that grows as a native plant in Iran. Symptoms similar to bacterial gall have been observed on the crown, shoot and twig of chinaberry, a recent landscape tree since 2003-2004 in Shiraz, Fars province, south of Iran. A gram negative bacterium was isolated from the galls. The isolates did not produce fluorescent pigment on King's B medium, were negative in oxidase, levan production, potato soft rot, casein, gelatin hydrolysis, nitrate reduction, growth at 5°C, uease production and indol production, but were positive in arginine dihydrolase, catalase, growth at 35°C, and also produced hypersensitive reaction on tobacco. Wound inoculated bacterial suspension into chinaberry seedlings produced galls from which the bacterium was reisolated. Systemic movement of the isolates into the vascular system of chinaberry was shown by the formation of gall above the inoculation site on the stem. On the basis of biochemical, physiological and pathogenicity characteristics, the isolated bacteria were identified as *Pseudomonas meliae*. Electrophoretic pattern of cell proteins showed that chinaberry isolates were different from *P. syringae, P. viridiflava, P. fluorescens, P. savastanoi* . The current article is the first report of bacterial gall disease of chinaberry in Iran

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Keywords: Chinaberry, Gall, *Pseudomonas meliae*

INTRODUCTION

Chinaberry (*Melia azedarach* L.) is a beautiful tree indigenous to the Himalayas, which grows as a native plant in Iran (14). The tree has beautiful and aromatic flowers and is planted as an ornamental plant in gardens and urban landscapes (13). Also, chinaberry trees are used for controlling soil erosion and for controlling *Meloidogyne* spp biologically. (15). Several fungal diseases of chinaberry such as septoriosis (*Septoria* sp.), cercosporiosis (*Cercospora* sp), root rot (*Rosellinia necaterix*) and white wood rot (*Helicobasidium purpureum*) have been reported (3). Bacterial gall of chinaberry caused by *Pseudomonas meliae* is the most important disease of chinaberry and was first reported by Ogimi from Japan (16). *Pseudomonas meliae* is a gram negative, non-fluorescent pseudomonad and based on DNA/DNA hybridization was placed in the same group with *P. meliae* together with *P. amygdali, P. savastanoi, P. ficuserectae* and 16 pathovars of *P. syringae* (5). Based on 16S rRNA gene analysis *P. meliae* has been placed in the *P. syringae* group (2).

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Similar to bacterial gall, chinaberry gall disease was first reported from Shiraz, Iran (7). The diseased trees were observed with many galls on stems and shoots. The symptoms of the disease in chinaberry trees are paranchymatic galls that appear on the stems and shoots of many trees. The galls on the stems and shoots of trees are small but become large and woody as the disease progresses. Chinaberry trees with gall symptoms show slow growth and dieback of shoots and stems (Fig. 1). Since chinaberry trees are important in the landscape in Fars province of Iran, identification of the agent causing chinaberry gall disease is important.

Fig. 1. Galls on shoot and stem of chinaberry from Shiraz, Iran.

MATERIALS AND METHODS

Isolation of Bacteria

Samples of shoots and stems of chinaberry with gall symptoms were collected from landscapes of Shiraz, Fars province in the south of Iran. The fresh and white galls were washed with water and surface-disinfected with 0.5% sodium hypochlorite solution and crushed in sterile distilled water. The resulting suspensions were streaked on nutrient agar (NA) medium. After incubation at 25°C for 48 h, representative single bacterial colonies were sub cultured on NA. A total of 50 strains were isolated from galls and maintained for further study.

Physiological and Biochemical Tests

The determinative tests were conducted as described previously: Gram staining and flagella staining, acetoin production, gelatin liquefaction, hydrolysis of casein, pigment production, starch hydrolysis, growth at 5 and 35°C in yeast salts broth in a rotary shaker, growth in 2 and 4% NaCl, catalase, arginine dehydrolase, levan production on sucrose nutrient agar (SNA), organic acid and amino acid utilization (4, 17), colony morphology on nutrient agar (NA), glucose oxidation or fermentation, lecithinase, indol production with Kovac's reagent, H_2S production from L-cysteine, urease, nitrate reduction (4), oxidase test, potato soft rot (12), hypersensitive reaction on tobacco (10). All carbohydrates used in the tests for acid production were filter-sterilized before being added to the basal agar medium (4, 117).

Pathogenicity Test

One-year-old chinaberry, winter jasmine, olive and neem tree (*Melia indica*) plants and seedlings of tomato, sunflower and pepper were used for inoculation. One loop of fresh bacterial culture on an NA medium (about 10^8 CFU) was placed into the wounded stems of plants and the hole was protected with Para film for four days. The control plants were inoculated by sterile distilled water. A total of 10 chinaberry plants were inoculated and the experiment was repeated at least two times. The plants were kept in a greenhouse at 26 $^{\circ}$ C and 75-80% RH. Symptom development was observed for up to 4 months after inoculation.

Protein Profiles

Chinaberry isolates (15 isolates) together with *P. syringae, P. viridiflava, P. fluorescens* from culture collection of the Department of Plant Protection, Shiraz University and *P. savastanoi* isolated from winter jasmine in Shiraz, Iran were analyzed by electrophoresis of the whole cell proteins. Bacterial strains were grown for 24 h on NA. The suspension of bacterial cells in SDW was prepared and pelleted by centrifugation in an eppendorf microcentrifuge tube for 15 min at 10000g. The pellet was diluted with SDW to optical density of 1.5 at 600nm. To 1ml of each sample, 0.2 ml of mix B (containing Tris buffer 0.5M, glycerol, brome phenol blue, 2-mercaptoethanol and SDS) was added and after shaking, the sample was boiled for 2.5 min (1, 11). Protein profiles were determined in a denaturing discontinuous electrophoresis system (10% polyacrylamid separation gel and 5% stacking gel).

RESULTS AND DISCUSSION

Bacterial Identification

The bacteria isolated from chinaberry gall were all identical in morphological and biochemical characteristics. The colonies of the isolates were white to cream in color, circular with entire margins on nutrient agar medium. The isolates were gram and oxidase negative, rod-shaped, aerobic and did not produce fluorescent pigment on King's B medium. All strains were negative for levan production on SNA, potato rot and nitrate reduction, but were positive for arginine and Tween80 hydrolysis and hypersensitive reaction on tobacco. Other characteristics of the isolates are listed in Table 1. The isolates showed homogeneity in their physiological, biochemical and nutritional characteristics, but some of the isolates exhibited variation in a few properties such as H_2S from cysteine and utilization of mannose. The isolates were able to hydrolyse arginine, but did not produce levan and fluorescent pigments on King's B medium in contrast to *P. syringae* isolates that did (8). Also, the isolates differed from *P. savastanoi* in their production of fluorescent pigment on King's B medium and utilization of mannitol and arabinose (16, 17).

Pathogenicity Test

All isolates inoculated to healthy chinaberry induced galls on the stems after 3 weeks and the size of the galls were about 3 cm after one month (Fig. 2). The isolates were pathogenic only on chinaberry and did not produce any symptoms on neem tree *(Melia indica*), winter jasmine, olive, tomato, sunflower and pepper. The isolated bacteria from chinaberry were not pathogenic on neem tree (*Melia indica)* from the

same family, but *P. savastanoi* and *P. syringae* had a broad host range (16, 17). The results obtained from pathogenicity test were similar to those of Ogimi (16). Systemic movement of the isolates in the vascular system of chinaberry was shown by the formation of secondary gall above the inoculation site on stem. Systemic movement of *Agrobacterium tumefaciens* has also been reported in the vascular system of *Chrysanthemum morifolium* (9).

Table 1. Physiological, biochemical and nutritional characteristics of Chinaberry isolates from Shiraz, Iran

Characteristic	Chinaberry isolates	Characteristics	Chinaberry isolates
Gram stain	-[(a)]	Utilization of:	
Catalase	+	Cellobiose	-
Potato soft rot	-	Sorbitol	-
Oxidase	-	Trehalose	-
Gelatin liquefaction	-	Sucrose	+
Starch hydrolysis	-	Glucose	+
Lecithinase	-	Galactose	+
Fluorescent pigment on KB	-	Mannose	+/-
Aesculin	-	Fructose	+
Levan	-	Glycerol	+
Hydrolysis of Tween80	+	Ribose	+
Growth at 35°C	+	Citrate	+
Growth at 5°C	-	Malate	+
Growth in: 2% (W:V) NaCl	+	Xylose	-
4% (W:V) NaCl	-	L- arabinose	-
Arginine dihydrolyse	+	Lactose	-
Tobacco hypersensitivity	+	Maltose	-
H_2S production from cysteine	+/-	Dextrin	-
Nitrate reduction	-	Manitol	-
		Adonitol	-

[a] +, positive; -, negative;

Protein Profiles

The electrophoresis pattern of the whole cell proteins exhibited that chinaberry isolates were identical and were different from *P. syringae, P. savastanoi, P. viridiflava* and *P. fluorescens* (Fig. 3). The homogeneity based on protein profiles between the isolates confirmed their homogeneity in phenotypic and pathogenicity characteristics. The protein patterns of the chinaberry isolates could be differentiated from *P. syringae, P. savastanoi, P. viridiflava* and *P. fluorescens* at species level.

On the basis of morphological, biochemical, nutritional and pathogenicity characteristics, compared with *P. syringae* and *P. savastanoi* the isolated bacterium from chinaberry galls was identified as *P. meliae* (16, 17). Based on DNA-hybridization, 16 different pathovars of *P. syringae* and type strains of four related species, *P. savastanoi, P. ficuserectae, P. meliae,* and *P. amygdali,* showed 72-100% binding to the type strain of *P. savastanoi* and were placed in Genomospecies 2 (5).

Fig. 2. Formation of gall on stem of chinaberry artificially inoculated with *Pseudomonas meliae*

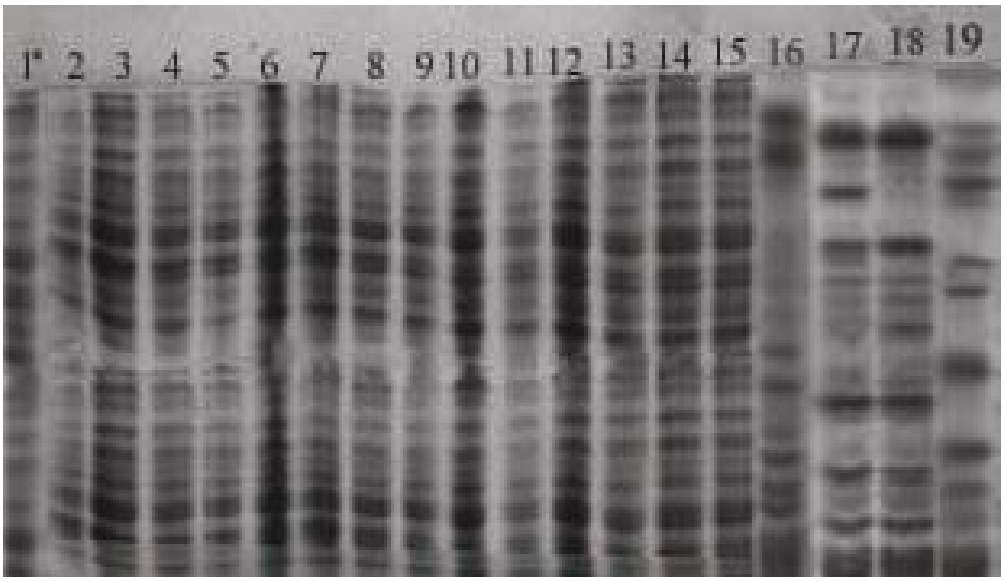

Fig. 3. Electrophoresis of whole cell proteins of *Pseudomonas melia* isolates from chinaberry. Lane 1-15 chinaberry isolates; lane 16, *P. savastanoi* (isolated from winter jasmine), lane 17, *P. syringae*, lane 18, *P. viridiflava*, lane 19, *P. fluoresce*

The homogeneity between the isolates based on phenotypic, nutritional, pathogenicity properties and whole cell protein profiles showed that *P. meliae* isolated from chinaberry in Shiraz, is a homogenous species, a result that corresponds to that of Ogimi (16). The symptoms of diseased trees include several galls and dieback on shoots and stems. The appearance of bacterial gall of chinaberry in Iran, its distribution and arrival to Iran is not recognized yet. To the authors' knowledge, bacterial gall of chinaberry is reported only from Japan (16) and Iran and there is no

information about the distribution and control of the disease, its importance and the molecular characteristics of *P. meliae* in the world.

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سبب شناسی بیماری گال باکتریایی زیتون تلخ در ایران

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چکیده- زیتون تلخ (سنجد تلخ) (*Melia azedarach* Lin.) درختی است زیبا، که موطن اصلی آن هیمالیا است و به صورت یک گونه بومی در ایران در آمده است. این درخت دارای گل های زیبا و معطری است و از آن به عنوان یک درخت تزئینی در باغ ها و بوستان هـــــای شهری استفاده می شود. طی سال های ۱۳۸۳- ۱۳۸۲ علائمی شبیه به گال باکتریایی روی درختان زیتون تلخ در فضای سبز شهر شیراز مشاهده گردید. از گال های جوان، ریز و نرم یک باکتری گرم منفی جدا سازی شد. جدایه های باکتری مذکور، اکسیداز منفی و قادر به تولید رنگ دانه فلورسنت روی محیط King's B ، تولید لوان، لهانیدن ورقه های سیب زمینی ، هیدرولیز ژلاتین و کازئین ، احیاء نیترات، تحمل نمک طعام چهاردرصد و رشد در 5 درجه سانتی گراد، تولید اوره آز، تولید ایندول نبود. جدایه ها قادر به هیدرولیز آرژنین، تحمل نمک طعام دو درصد و رشد در 35 درجه سانتی گراد و کاتالاز مثبت بودند و درگیاه توتون ایجاد فوق حساسیت نمودند. جدایه های مذکور پس از مایه زنی به نهال های بذری زیتون تلخ علائم گال را نشان دادند. حرکت سیستمیک جدایه ها روی گیاه زیتون تلخ به اثبات رسید. براساس آزمونهای بیوشیمیایی، فیزیولوژیکی و بیما ری زایی عامل بیماری به عنوان *Pseudomonas meliae* شناسایی شد. بررسی نقوش پروتئینی نشان داد که جدایه های مذکور همگن می باشند و با جدایه *های P. syringae ، P. viridiflava P. savastanoi* و *P. fluorescens* متفاوت بودند. این اولین گزارش از وجود بیماری گال باکتریایی درخت زیتون تلخ در ایران می باشد.

واژه های کلیدی: زیتون تلخ, گال، *Pseudomonas meliae*

* به ترتیب استاد و دانشجوی پیشین کارشناسی ارشد

** مکاتبه کننده