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OCCURRENCE OF VASCULAR STEM ROT DISEASE ON GLOBE ARTICHOKE CAUSED BY *ERWINIA CAROTOVORA* IN EGYPT.

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ABSTRACT

During the summer of 2001, a vascular stem rot of artichoke (*Cynara scolymus* L) was reported. Gram negative, non sporulating bacteria were isolated from the affected lesions. Colonies developed on nutrient glucose agar were greyish-white to creamy white, smooth, round, glistening, slightly raised and visible on isolation plates after 24h. On crystal violet pectate medium deep cup – like pits were produced 3 days after plating. Based on physiological, biochemical and pathogenicity tests, the bacterium was identified as a strain of *Erwinia carotovora* subsp. *carotovora*.

INTRODUCTION

Artichoke (*Cynara scolymus* L.) is a herbaceous perennial plant which is grown for its edible receptacles and scales, or bracts, of the blossom buds. The origin of the globe artichoke is believed to be North Africa and other Mediterranean regions. Worldwide, the major producers are Italy, Spain and France, with U.S production accounting for 4% of the world supply (Swiader *et al.*, 1992).

The first recorded bacterial disease of globe artichoke being the soft rot (*Erwinia carotovora* subsp. *carotovora*) although the first description of the disease was made on symptoms only (Elliot, 1951 and Fahy & Persley, 1983). Another soft rot disease caused by *Erwinia chrysanthemi* was reported in the Castroville region of California's central coast. Symptoms are most commonly observed following periods of warm weather. Inoculation in the greenhouse of wounded plants recovered *E. chrysanthemi* that produced stunt, wilt and crown rot symptoms (Colbert *et al.*, 1990). Also, *Erwinia nigrifluens* the cause of artichoke violet necrosis in Argentina was reported (Soto, 1997).

Another record of bacterial disease of artichoke was *Xanthomonas cynarae* sp. *nov.* the cause of bacterial bract spot of artichoke.The disease was observed for the first time in 1954 in Brittany and the Loire Valley, France. This disease causes water – soaked spots on bracts and depreciates marketability of the harvest. Ten strains of the pathogen causing bacterial spot of artichoke, were identified as a member of the genus *Xanthomonas,* (Trebaol *et al.*, 2000).

MATERIALS AND METHODS

1. Diseased samples

During the summer season, 2001 diseased globes of artichoke plants showing a vascular stem rot symptoms were collected from 5 fields at three

villages (Kerdasa, Nahia and El-Meaatemdia) of Imbaba district, Giza Governorate. Globes were sorted into different cultivars while the predominating cultivated cultivars were Balady, French and Imperial star.

2. Isolation

Infected stems showing internal rot, wilt and dark discoloration of the leaf petioles were sampled for isolation. Small cuttings of vascular bundles and pith of stems were surface disinfected, in 1% sodium hypochlorite, sectioned in sterile distilled water and streaked on nutrient glucose agar (NGA) medium, semi selective media of, Miller – Schroth, (MS) medium, 1972 and crystal violet pectate (CVP), (Cupples & Kelman, 1974).

3. Identification of the isolated bacteria

Tentative identification of 8 isolates was made by observing the colony morphology on NGA and CVP media. Air dried films of 24h old cultures were Gram stained as modified by Kopeloff and Beerman (Cruickshank *et al.*, 1975) using acetone alcohol as decolorizer. Confirmation of Gram reaction was made by the non-staining KOH method (Suslow *et al.*, 1982). Oxidase test (Kovacs, 1956), potato soft rot, nitrate reduction were made according to Lelliot *et al.* (1966).

Tests used for distinguishing the globe artichoke isolates versus the reference strains of soft rot Erwiniae *(E. carotovora* var. *atroseptica, E. carotovora* var *carotovora* and *E. chrysanthemi)* included formation of indole, anaerobic fermentation of glucose, growth at 36C, formation of reducing compounds from sucrose (Dye D.W. 1969) and pectate degradation (Hildebrand, 1971).

Production of acid from carbohydrates was tested in peptone free basal medium (Dowson, 1957). All carbon sources were sterilized separately as stock solution(s) and added to the test medium to give 1 % concentration (Cruicshank *et al.*, 1975). Records on acid prouction were made after three days and up to one month.

4. Pathogenicity

Eight weeks old plants of artichoke cv. French (*Cynara scolymus* L.) were used in this study. The isolated bacteria were inoculated by injecting the bacterial suspension (10^7 cfu/ml.) of each isolate, using sterile syringe, into the crown of the stem. Plants were kept to grow in the greenhouse at 28-30C. Check plants were injected with sterile distilled water. Three replicates per treatment were used. Sumptoms regulary observed throughout two weeks after inoculation.

RESULTS AND DISCUSSION

1. Field observations and disease symptoms :

Field observations made on the disease indicated that most of the infected plants showed internal rot symptoms in stems, that resulted in wilt and dark discoloration on leaf petioles at the crown area. The described

severe symptoms being late in the growing season may be due to the high temperature in summer which is favours to the disease development. This observation agrees with the findings of Colbert *et al.* (1990).

The disease incidence, howerver , ranged from 3-7% , Table (1) that may be considered as a minor disease in Kerdasa village, contrary to El-Meaatemdia and Nahia that showed greater incidence. Further surveys are needed at different districts to evaluate the disease widespread.

It seems probable that soil and climatic conditions are among the most important predisposing factors for soft rot development. Infected cuttings, corms and suckers used in vegetative propagtion might be the most efficient reasons for the increase of bacterial diseases in certain fields, and the major cause of dissemination of such diseases from one place to another (Powelson & Apple, 1984).

Table (1) Source and percentage of infection in tested samples

Village	Farm No.	Infection (%)	Samples ollected	Area inspected (Fed.)
El-Meaatemdia	1	7	17	11
	2	5	11	8
Kerdasa	3	3	8	5
Nahia	4	6	12	8
	5	6	12	6
Total	5	-	60	38
Mean	-	5.4	-	-

2. athogenicity test and symptoms:

All isolates tested were found pathogenic to the artificially inoculated artichoke plants (cv. French) in greenhouse and showed typical symptoms of soft rot caused by *Erwinia carotovora* while all check plants remained disease – free. Most of the inoculated plants died out within two weeks after inoculation. The survived inoculated plants showed discoloration and rot on stem and pith at the crown area. Infected plants were small in size compared with healthy check and showed necrotic vascular invasion mixed with soft rot. Brown to dark brown color was shown after cutting the infected stem. The pathogenic bacteria were re-isolated from artificially inoculated diseased plants in all cases and showed the same identification characteristics.

3. Morphological characteristics of the pathogen :

All isolates were morphologically similar. The cells were Gram negative rods, non-sporulating, motile with peritrichous flagella and no pigments was detectable in King's medium B. On CVP medium, all isolates produced translucent, iridescent colonies produced cup – like depression or pits. Colonies grown on (MS) semi - selective medium for two days at 28C showed white with yellow to orange centers and coroid margins resembling a fried egg.

4. Biochemical and physiological reactions :

All isolates were facultatively anaerobic, oxidase negative, reduced nitrate, caused soft rot on potato slices and fermented glucose. The results declared that the isolated bacteria are belonging to Erwinias soft rot group. The reference isolates were Erwinia carotovora subsp. atroseptica, E. *carotovora* subsp. carotovora and *E. chrysanthmi* (Table2).

Table (2): Morphological, physiological and biochemical characteristics of globe artichoke isolates

	Reference isolates			Globe artichoke isolates							
	E.c.a.	E.c.c.	E.chr	1	2	3	4	5	6	7	8
Gram stain	-	-	-	-	-	-	-	-	-	-	-
KOH	+	+	+	+	+	+	+	+	+	+	+
Spores	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+
Potato soft rot	+	+	+	+	+	+	+	+	+	+	+
Oxidase reaction	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+
O/F reaction	F	F	F	F	F	F	F	F	F	F	F
Indole formation	-	-	+	-	-	-	-	-	-	-	-
Growth at 36C	-	+	+	+	+	+	+	+	+	+	+
Pigments	-	-	-	-	-	-	-	-	-	-	-
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-	-
Acid prouduction from											
Lactose	+	+	-	+	+	+	+	+	+	+	+
Maltose	+	+	-	+	+	+	+	+	+	+	+
Melibiose	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	-	+	+	+	+	+	+	+	+
Cellobiose	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	+	+	+	+
Dextrin	-	-	-	-	-	-	-	-	-	-	-
Dulcitol	-	-	-	-	-	-	-	-	-	-	-

+ = positive reaction - = negative reaction F = fermintative
E.c.a. = *Erwinia carotovora* subsp. *atroseptica*
E.c.c.= *Erwinia carotovora* subsp. *carotovora*
E.chr = *Erwinia chrysanthmi*

The results presented in this study show that the isolate bacteria from naturally infected globe artichoke were identical to those described for *Erwinia carotovora* subsp. *carotovora*. On the other hand field symptoms, pathogenicity, physiological and biochemical tests of the bacterial isolates directed the auther to identify the isolated *Erwinina* as subspecies of *carotovora* which was designated earlier by Elliot (1951) and Fahy & Persley (1983) as *Erwinia carotovora* subsp. *carotovora*. According to the available data base, this is the first record of stem rot disease on globe artichoke in Egypt.

Several authors shown that *E. carotovora* subsp. *carotovora* may survive in the rhizosphere of non-host plants other than globe artichoke (Mc Intyre *et al,* 1978; Lapwood & Harris, 1980 and Smith & Bartz, 1990). Also, Thomson *et al.* (1981) reported that cracks and injuries were found in susceptible cultivars especially when growers used higher rates of nitrogn fertilizer. Such cracks favor the invation of the bacteria and increase the incidence of disease.

Further studies are needed to evaluate the resistance of the imported globe artichoke cultivars comparing to the domestic and old varieties. The control of this soft rot bacteria either biological or through improvement of farming practices is showed be considered too.

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مرض عفن الساق البكتيرى فى الخرشوف المتسبب عن البكتريا ايرونيا كاروتوفورا فى مصر

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شوهدت أعراض مرض العفن الطرى البكتيرى على سيقان الخرشوف وذلك أثناء موسم نمو ٢٠٠١، بمركز إمبابة محافظة الجيزة بمصر. وقد تم الحصول على العديد من العزلات حيث أظهرت جميعها أنها سالبة لجرام وإتصفت المستعمرات النامية على بيئة آجار الجلوكوز المغذى باللون الأبيض المائل للاصفرار مع نعومة المستعمرات وإستدارتها ولمعانها وإرتفاعها البسيط وذلك بعد ٢٤ ساعة من تلقيحها على البيئة. أما على بيئة الكريستال فيوليت بكتات فظهرت المستعمرات ذات تجاويف عميقة تشبه الفنجان وذلك بعد ثلاثة أيام من التحضين. وقد عرفت العزلات على أنها سلالات من البكتريا إيرونيا كاروتوفورا تحت النوع اروتوفورا استناداً إلى الاختبارات الفسيولوجية والكيميائية الحيوية والاختبارات المرضية كما أجرى الحصر الحقلى للمرض فى خمس مزارع بثلاث قرى بمحافظة الجيزة.