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Court of Infection with *Pseudomonas syringae* Pv. *Lachrymans* in Cucumber

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Abstract: *Pseudomonas syringae* pv. *lachrymans* were isolated from cucumber (*Cucumis sativus*) seedlings raised from seeds which were previously treated with Thiram or Captan. Five isolates were identified and used in pathogenicity test. Leaf inoculation gave the highest percentage of infection, followed by stem and the lowest percentage. The results highlight to some extent that the court of infection with *P. syringae* pv. *lachrymans* is the cucumber leaf. Further investigations are needed to study the interrelation between the location of infection and the translocation of the pathogen in the plant to confirm this results.

Keywords: Court of infection, *Pseudomonas syringae* pv. Lachrymans, cucumber

Introduction

Cucumber (*Cucumis sativus* L.) is one of the most important vegetable crops in Egypt for both domestic consumption and exportation. The total area planted with the crop in the year 1999 was 58249 acre with a yield amounted 433242 ton. Cucumber is also grown in winter in Egypt under low tunnels and greenhouses for domestic consumption and exportation. The seeds are usually imported from certified sources in USA and Europe; and are being documented against seeds-borne diseases.

Angular leaf spot caused by *P. syringae* pv. *lachrymans* is an important cucumber seed-borne pathogen (Seymour *et al.*, 1957; Helmers, 1958; Naumann, 1961; Gustafson and Gustafson, 1980; Kritzman and Zutra, 1983;Gomah, 1997). The first record of the disease in Egypt was made in 1989 in El-Minia Governorate in cucumber greenhouses (El-Sadek *et al.*, 1992). The disease importance has been increasingly growing under greenhouse conditions where relative humidity and temperature are quite favourable for severe outbreaks.

This work was undertaken to study the effect of the court of infection on disease development in cucumber plant.

Materials and Methods

Seed sources: Cucumber seed samples, origin, year of harvest and chemical treatments are shown in the following table:

Origin	No. of samples	Cultivar	Harvest	Chemical treatment
France	4	Beta alpha	1993	TMTD
USA	14	Beta alpha	1992	Captan
		Amira II		A
		Madena		A
		Basha F1		A
		Celebrity		A
		Sultan F1		A
Holland	10	Beta alpha	1993	A
England	1	Beta alpha F1	1993	A
Israel	1	Beta alpha F1	1993	A
Denmark	2	Beta alpha	1993	A
Japan	3	Sweet cranch	1993	A

isolation: Lesions developed on the seedlings grown for seed health testing (Shakaya and Chung, 1983) were used in isolation. The seed samples were thoroughly washed in water for five minutes, placed in a folder filter paper (4.5 x 4.5 cm5) and arranged in a cassette holder. The holder was placed in a glass jar with 2-3 cm tap water depth. After 8-10 days of incubation at 20-30EC, infected seedlings with lesions developed on cotyledonary leaves were used for isolation. The selected seedlings were gently washed three times in sterile tap water. The infected cotyledonary leaves was then crushed in sterile Petri dish containing 2 ml sterile water and kept for 30 minutes, the obtained suspension was streaked on the surface of sucrose nutrient agar plates and incubated at 28EC for 3 days (Volcani, 1966). Plates of King=s medium B were also streaked and incubated at 28EC for 48 hours.

Pathogenicity tests: The inoculum was prepared from 48 hours old cultures on King=s medium B suspended in sterilized water. The turbidity of the microbial suspension of the inoculum was adjusted to 10^7-10^8 cells/ml using SPEKOL 11 CARL ZEISS JENA. Cucumber plants in plastic pots (15 cm in diam.) were sprayed with the bacterial suspension (s), using a hand atomizer, then covered with polyethylene bags and kept in the greenhouse for 3 days at 25EC. Lesions developed on the leaves were used in re-isolation trails.

Identification of the isolated organisms: Pathogenic bacteria originally isolated from diseased materials were identified according to methods of Lelliott and Stead (1987).

Morphological characteristics: Air dried films from 48 hrs old cultures were Gram stained, as modified by Kopeloff and Beerman (Cruickshank *et al.*, 1975), using acetone alcohol as decolourizer. Demonstration of capsules was made in wet India ink preparations. Motility was detected according to Dowson (1957). Colony morphology and other growth characteristics were described on glucose nutrient agar.

Biochemical characteristics:

Utilization of glucose (O/F) test: Screw capped tubes of O/F medium (Fahy and Persley, 1983) were inoculated. The change of colour was recorded after 3 days.

Production of acids from carbohydrate: Peptone free basal medium (Dowson, 1957) was used in this investigation. All carbon sources were sterilized separately and added to the medium to give 1% concentration (Cruicshank *et al.*, 1975). Acid production was recorded after three days.

Gelatin liquefaction: Deep inoculations were made in tubes containing gelatin agar medium (Lelliott and Stead, 1987). After three days of incubation at 30EC hydrolyzed gelatin remained after chilling in ice water was recorded.

Starch hydrolysis: Spot inoculation was made on nutrient agar containing 0.2% soluble starch. After 48 hrs of incubation at 30EC, the plates were flooded with iodine solution and the results were recorded (Lelliott and Stead, 1987).

Catalase test: A loopful of growth from 24-48 hours agar slopes was smeared on a slide and covered with a drop of 20 volume hydrogen peroxide. Gas bubbling was recorded as positive (Lelliott and Stead, 1987).

KOH test: Two drops of a 3% (w/v) solution of potassium hydroxide was placed on a clean slide. Bacterial cells were transferred from the culture and mixed with drops of KOH at rapid, circular agitation. After 5-8 seconds, the loop was alternately raised and lowered just off the surface of the slide to detect stringing effect. The stringing effect was considered positive if it appears within 15 seconds (Suslow *et al.*, 1982).

Fruits inoculation: It is a rapid method for *Pseudomonas lachrymans* detection. Cucumber and squash fruits, larger than 80-100 g for cucumber and 150-200 g for squash were selected. The fruits were dipped in alcohol and flamed twice to surface sterilize. As aseptical as possible the fruits were sliced into about 1 cm thick, then placed in sterilized Petri dish containing two filter papers. The bottom of the Petri dish was flooded with sterile water to keep the slice surface moist. The slices were inoculated with bacterial suspension in a density of 10^7-10^8 cells/ml. The plates were incubated at 24EC for 3-5 days. The characteristic growth of *P. Lachrymans* was recorded (Ohuchi *et al.*, 1980a)

Confirmed tests for identification of *Pseudomonas lachrymans*:
Levan production test: Plates of nutrient agar containing 5% sucrose were used for levan production. Large, white, domed, mucoid colonies were assumed positive for levan production (Lelliott *et al.*, 1966).

Oxidase test: Kovac=s (1956) method was used. The development of distinct purple colour in 10 seconds was recorded as positive.

Potato soft rot: Whole potatoes were dipped in alcohol and flamed twice for surface sterilization. As aseptical as possible the potato was cut into about 1 cm thick slices, and placed in Petri dishes. The bottom of the Petri dish was covered with sterile water to keep the potato moist. A loopfull of the culture was placed in the center of the potato slice and was incubated at about 28EC for 48 hours (Lelliott and Stead, 1987).

Arginine dihydrolase: Formation of alkali in arginine medium, in sealed tubes, was regarded as positive (Thornley, 1960).

Tobacco hypersensitivity: Tobacco plants, *Nicotiana tabacum* L. cv. White burley, were used in this experiments. The bacterial isolates were suspended in sterile tap water and adjusted to 10^7-10^8 cells/ml. The suspension was injected into the intercellular space of the tobacco leaves. Plants were incubated at 20-25EC for 24 hrs, (Klement *et al.*, 1964).

Inoculation of plant organs:
Leaf: Leaf inoculation was carried out by dusting the leaves with carborandom and rubbing with a piece of cheesecloth previously dipped into the bacterial suspension at the mentioned adjusted density. Check plants devoiding the bacteria were prepared similarly. The plants were covered for 48 hours, with polyethylene bags, to maintain high relative humidity, and kept in the greenhouse at 20-25EC. The inoculated plants were observed for the development of disease symptoms. The number of plants showed a dark green water soaked area after 7 days of inoculation were counted as a positive reaction. The method of determination of leaf infection was followed after El-Sadek *et al.* (1992).

Bud: Bud inoculation was made by placing a drop of a bacterial suspension at the same mentioned density with care not to touch the bud or cotyledons (De Lange and Leben, 1970).

Stem: Stem inoculation was made by placing a droplet of the bacterial suspension on the axis of node and then puncturing the stem through the inoculum with a sterile needle. Check plants were inoculated with sterile water (El-Sadek *et al.*, 1992).

Results and Discussion

Isolation and pathogenicity: Infected seedlings developed during the course of seed health testing were used in isolation. Table (1) shows that 520 seedlings developed leaf lesions 10 days after sowing.
Isolation made from different infected leaves revealed heterogenous bacterial colonies. Gram negative colonies were selected, propagated on NA and tested for pathogenicity to cucumber plants. The total number of selected G-ve bacterial isolates were 76 ones. Only 58% of them were found pathogenic to cucumber plants.

Identification: Data in Table (2) show the results of identification of the most predominant pathogenic bacteria isolated from cucumber seedlings. The tested isolates were Gram negative nonsporulating rods. Aerobic with oxidative metabolism of glucose. Catalase positive, oxidase negative gelatin liquefied and starch was not hydrolyzed. The tested isolates did not induce soft rot in potato slices and produced hypersensitive reaction in tobacco leaves. Inoculated cucumber slices showed growth characteristics of *Ps. Lachrymans*.
Acid was produced from glucose, galactose, fructose, manose, arabinose, xylose, sucrose and mannitol. Maltose, lactose, rhamnose and solicin were not utilized.
The described characteristics of the isolated bacteria conform with those reported for *Pseudomonas syringae lachrymans*, and group Ib fluorescent *Pseudomonas* (Lelliott and Stead, 1987).

Inoculation of plant organs: Table (3) shows the results of inoculating buds, stems and leaves of cucumber on the development of angular leaf spot disease. The hybrid Beta Alpha and Sweet Cranch inoculated plants developed less infection compared to the Beta alpha non hybrid plants. The recorded percentages of infected seedlings were 57.8%, 66.6% and 72.2% for the mentioned cultivars, respectively. The highest percentage of infected seedlings, however, was recorded following leaf inoculation being 90%, 78.4% and 86.6% for the cultivars Beta alpha and Beta alpha hybrid and Sweet Cranch, respectively. Bud inoculation developed low percentage of infection being 46.6%, 30.0% and 36.8% for the same cultivars, respectively. Stem inoculation on the other hand was intermediate. In this regard, severity of infection and differences in symptoms developed were varied according to the organ affected. Young leaves generally showed a much greater susceptibility to the disease than did the mature ones (Saleh and Korobko, 1981; Umekawa and Watanabe, 1982;

Table 1: Percentage of infection of cucumber samples and number of pathogenic isolates

No. of samples	No. of seedlings	Infected seedlings	% of infection	Total G-ve colonies	Pathogenic isolates	% of pathogenic isolates
35	11904	520	4.4	76	44	58

Table 2: Identification test of *Pseudomonas syringae* pv. Lachrymans* isolated from cucumber

Test	Isolate number				
	103	517	604	2903	3303
Gram stain	-	-	-	-	-
Spores	-	-	-	-	-
Flouresens	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-
Catalase	+	+	+	+	+
KOH	+	+	+	+	+
O/F	O	O	O	O	O
Levan (L)	+	+	+	+	+
Oxidase (O)	-	-	-	-	-
Potato soft-rot (P)	-	-	-	-	-
Arginine (A)	-	-	-	-	-
Tobacco H.R. (T)	+	+	+	+	+
Glucose	A	A	A	A	A
Galactose	A	A	A	A	A
Fructose	A	A	A	A	A
Manose	A	A	A	A	A
Arabinose	A	A	A	A	A
Xylose	A	A	A	A	A
Sucrose	A	A	A	A	A
Mannitol	A	A	A	A	A
Maltose	-	-	-	-	-
Lactose	-	-	-	-	-
Rhamnose	-	-	-	-	-
Salicin	-	-	-	-	-

* Fluorescent Pseudomonas group Ib. O = oxidative, A = Acid, + = positive, - = negative

Tablel 3: Response of cucumber cultivars in infection by *P. lachrymans* isolates using different methods of inoculation

Treatments		% of infected seedlings Isolate number					Mean
		1	2	3	4	5	
Beta alpha	Bud	50.0	33.0	42.0	50.0	58.0	46.6
	Stem	83.0	67.0	67.0	92.0	92.0	80.2
	Leaf	92.0	75.0	83.0	100.0	100.0	90.0
	Mean	75.0	58.3	64.0	80.6	83.3	72.2
Beta alpha hybrid	Bud	33.0	17.0	25.0	33.0	42.0	30.0
	Stem	67.0	58.0	50.0	75.0	75.0	65.0
	Leaf	83.0	67.0	67.0	83.0	92.0	78.4
	Mean	61.0	47.3	47.3	63.6	69.6	57.8
Sweet Cranch	Bud	42.0	25.0	25.0	42.0	50.0	36.8
	Stem	83.0	67.0	58.0	92.0	83.0	76.6
	Leaf	83.0	75.0	75.0	100.0	100.0	86.6
	Mean	69.6	55.6	52.6	78.0	77.6	66.6

LSD at 5% level = 3.214

Komoto and Kimura, 1983a; Mortensen, 1992 and El-Sadek *et al.*, 1992).

Stem inoculation induced a water-soaked symptoms and in some cases developed canker at the inoculation sites (Saleh and Korobko, 1981; El-Sadek *et al.*, 1992). It was also found that *P. lachrymans* in addition of causing symptoms of angular leaf spots, it could also induce wilting in the vegetative plant parts parallel to increase in ambient temperature.

On the other hand, the results showed also, that the isolates 4 and 5 were more pathogenic than the others. Differences were also reported by Hopkins and Schenck (1972), Pohronezny *et al.* (1977), Saleh and Korobko (1981), El-Sadek *et al.* (1992) and Mortensen (1992).

Data presented here suggest that the court of infection is

probably the cucumber leaves.

References

Crulckshank, R., J.P. Duguid, B.P. Marmion and R.H.A. Swain, 1975. Medical Microbiology, 12th Ed., vol. 2, The practice of medical microbiology, Churchill Livingstone, Edinburgh.

De Lange, A. and C. Leben, 1970. Colonization cucumber buds by *Pseudomonas lachrymans* in relation to leaf symptoms. Phytopathol., 60: 1865-1866.

Dowson, W.G., 1957. Plant diseases due to bacteria. Cambridge University Press, pp: 232.

El-Sadek, S.A.M., N.R. Abdel-Latif, T.I. Abdel Gawad and N.A. Hussein, 1992. Occurrence of angular leaf spot disease in greenhouse cucumbers in Egypt. Egypt J. Microbiol., 27: 157-175.

Fahy, P.C. and G.F. Persley, 1983. Plant bacterial diseases. A diagnostic guide, Academic Press, 393 pp.

Gomah, A.A., 1997. Location and transmission of economic bacterial diseases of cucurbits. Ph.D. Thesis, Faculty of Agric., Mansoura University, Egypt.

Gustafson, M. and I. Gustafson, 1980. Bladbakterios pa frilandgurka. Forsok och foradlings under aren 1977-1990. Sveriges Utsadesforenings Tidskrift 90, 197-209. (Cited by Mortensen, C. Nieves, 1992.

Hellmers, E., 1958. Bakteriesygdomme hos danske kulturplanter. Den kgl. vet-og Landbohojskole, Kobenhavn, 120 pp.

Hopkins, D.L. and N.C. Schenck, 1972. Bacterial leaf spot of watermelon caused by *Pseudomonas lachrymans*. Phytopathol., 62: 542-545

Klement, Z., G.L. Farkas and L. Lovrekovich, 1964. Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathol., 54: 474-477.

Komoto, Y. and T. Kimura, 1983a. The process of seed transmission in angular leaf spot of cucumber. Bulletin of the Chugoku National Agricultural Experiment Station. E21: 1-20. c.f. Rev.Pl.Pathol., 63: p. 137.

Kovacs, N., 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature, London, 178, 703. (cited by Mortensen C. Nieves, 1992b).

Kritzman, G. and D. Zutra, 1983. Systemic movement of Pseudomonas *syringae* pv. *lachrymans* in the stem, leaves, fruits and seed of cucumber. Cana. J. Plant Pathol., 273-278.

Lelliott, R.A., E. Billing and A.C. Hayward, 1966. A determinative scheme for the fluorescent plant pathogenic *Pseudomonas*. J. Appl. Bact., 29: 470.

Lelliot, R.A. and D.E. Stead, 1987. Methods for the diagnosis of bacterial diseases of plants. Methods in plant Pathology, vol. 2 Editor T.F. Preece. Blackwell Scientific Publications, pp: 216.

Mortensen, C. N., 1992. Seed-borne bacterial diseases. Danish Government Institute of Seed Pathology for Developing countries.

Naumann, V. K., 1961. Die eckige blattflecken-Krankheit der Gurke (*Pseudomonas lachrymasn* [Smith et Bryan] Carsner). Bachrichtenblatt fur dern Deutschen Planzenschutzdienst, vol., 15: 81-91. (cited by Mortensen C. Nieves, 1992).

Ohuchi, A., Y. Shimazaki and A. Ezuka, 1980. A rapid method for detecting *Pseudomonas lachrymans* by ooze-exudation of inoculated cucumber fruit. Bulletin of the National Institute of Agricultural Sciences, C 34: 29-49. (c.f. Rev. Pl. Path., 60: p. 204).

Parashar, R.D. and C. Leben, 1972. Detection of *Pseudomonas glycinae* in soybean seed lots. Phytopathology, 62: 1075.

Pohronezny, k., C. Leben and P.O. Larsen, 1977. Systemic invasion of cucumber by *Pseudomonas lachrymans*. Phytopathology, 67: 730-734.

Saleh, Y.E. and A.P. Korobko, 1981. Some biological aspects of bacterial diseases of melon and watermelon in Ukrainian SSR. I. *Pseudomonas lachrymans* (Smith and Bryan) carsner. Egyptian J. Bot., 24: 219-229.

Seymour, D. Van Gundy and J.C. Walker, 1957. Seed transmission, over-wintering and host range of the cucurbit-angular-leaf spot pathogen. Plant Disease Reptr., 41: 137-140.

Shakya, D.D. and H.S. Chung, 1983. Detection of *Pseudomonas avenae* in rice seed. Seed Science and technology, 11: 583-587.

Suslow, T.W., M.N. Schroth and M. Isaka, 1982. Application of a rapid method from gram differentiation of plant pathogenic and saprophytic bacteria without staining. Phytopathology, 72: 917-918.

Thornley, M.J., 1960. The differentiation of *Pseudomonas* from other gram negative bacteria on the basis of arginine metabolism. J. Appl. Bacteriol., 1: 37-52.

Umekawa, M. and Y. Watanabe, 1982. Relation of temperature and humidity to the occurrence of angular leaf spot of cucumber grown under plastic house. Annals of the Phytopathological Society of Japan, 48: 301-307.

Volcani, Z., 1966. a quantitative method for assaying cucumber seed infection caused by *Pseudomonas lachrymans*. Isr. J. Bot., 15: 192-197.

Wiles, A.B., 1951. Studied on Pseudomonas lachrymans in cucumber. Phytopathology, 41, 38 (c.f. El-Sadek *et al.*, 1992).