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Resistance Induced in Potato Tubers to Soft Rot Caused by *Erwinia carotovora* Subsp. *carotovora* by Treatments with Salicylic Acid and Acetylsalicylic Acid.

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Key words. Potato cultivars, disease index, soaking time, storage period, bacterial isolates, SA and ASA concentrations.

Abstract

Nine bacterial isolates obtained from naturally rotted potato tubers collected from different localities of Assiut and EL-Minia Governorates,. proved to be pathogenic and cause soft rot disease to potato tubers. They were identified as *Erwinia carotovora* subsp. *carotovora.*

The effects of salicylic acid (SA) and acetylsalicylic acid (ASA) at concentrations of 0.0125%, 0.05% and 0.25% significantly reduced soft rot development in wounded entire potato tubers cvs. Diamant, Lady Rosetta, and Argos as compared with control. Such reduction in disease development by tested treatments were significantly decreased with the increasing soaking time. Data also showed that the tested treatments of acetylsalicylic acid proved to be superior than salicylic acid in reduction of soft rot.

Generally, resistance induced in potato tubers by tested chemicals varied upon tested potato cultivars. Potato tuber cv. Lady Rosetta showed the highest reduction in soft rot disease index followed by cv. Diamant and finally cv. Argos.

Increasing time of storage periods up to 9 days after soaking potato tubers in salicylic acid and acetylsalicylic significantly increased the reduction of soft rot development.

Introduction

Bacterial soft rot disease is one of the most important and widespread bacterial diseases to a wide variety of plants either in the field or transit and storage causing great losses in plant growth and crop yield. (**Pérombelon and Kelman, 1980 and Wafaa, 1996).** *Erwinia carotovora* subsp. *carotovora* is the causal agent of soft rot disease in many solanaceous plants, especially potato (*Solanum tuberosum* L.) and tomato. The disease is recognized by tissue maceration due to cell wall degradation enzyme secreted by the pathogen (**Collmer and Keen, 1986**).

Induced resistance in plants can be triggered either by

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localized infection with pathogens or by treatment with certain chemicals such as salicylic acid (SA) and acetylsalicylic acid (ASA). SA plays an important signalling role in plant defence against pathogens, inducing systemic acquired resistance (SAR) and SAR gene expression in most systems studied (Malamy and Klessing 1992; Kessmann *et al*., 1994; Sticher *et al*., 1997).

Lopez *et al*. (2001) reported that the efficiency of acetylsalicylic acid (ASA) in inducing localized acquired resistance against infection by *E. carotovora* subsp. *carotovora*. They concluded that immersion potato tubers in ASA solution induced a significant reduction in soft rot incidence. Wounding of the tubers was the most effective inoculation method and ASA at concentration of 0.0125% (w/v). No phytotoxicity of such treatment was observed.

The present investigation was conducted to reveal the effect of different concentrations of SA and ASA on potato soft rot caused by *E. carotovora* subsp. *carotovora* under storage condition.

Materials and Methods

Isolation of causal pathogen

Isolation was carried out from naturally infected potato tubers showing soft rot symptoms. Potato tubers were collected from different localities of Assiut and El-Minia Governorates during 2007-2008 seasons. Diseased potato tubers were washed with tab water several times, surface sterilized by soaking in 1% sodium hypochlorite solution for 2 minutes, rinsed twice in sterilized water, then small portion of the diseased tissues were macerated with 5 ml of sterilized 0.05 M potassium phosphate buffer, after 10 minutes a loopful of the resulting suspension was streaked onto nutrient sucrose agar medium (NSA) (Dowson, 1957) and selective medium Pectate Tergitol (PT) as recommended by Burr and Schroth (1977), Liao and Scollenberger (2003).

Plates were incubated at 28°C for 48 h, and then examined for bacterial growth development. The single colony technique was used to obtain pure culture. Single colony of the isolates was sub-cultured onto the above mentioned media on tubes and maintained at 4^{o}C for further studies. Also the stock cultures of the isolates, were stored in sterilized distilled water at 4^{o}C.

Pathogenicity test

Ability of isolated bacteria to cause soft rot to potato slices and tubers was examined as follows: surface sterilized potato slices (one centimeter thick) were kept in Petri-dishes containing damp sterilized filter paper, a loopful of growth of the tested isolates (24 hr. old) was streaked over the surface of such slices and incubated at 27°C and examined daily for rotting over a period of three days.

Also, potato tubers surface sterilized with 1% sodium hypo-

chloride solution were used to prove pathogenic capability of the tested isolates. A cavity (about 1 cm in depth and 0.5 cm in width) was made in each tuber by a cork-borer. Each isolate was streaked on (PT) agar medium in Petri-dishes and incubated at 27°C for 48 h. A single colony of the isolates was selected and grown in 250 ml Erlenmeyer flasks containing 100 ml of nutrient sucrose broth (NSB) and incubated at 27±2°C for 48 h on a rotary shaker at 150 rpm. Bacterial cell suspension was centrifuged (8 min. at 10,000 g), the cells resuspended in tap water and cell density adjusted to be $5x10^8$ CFU/ml using a spectrophotometer at wavelength of 620 nm (McGuire and Kelman, 1984). The adjusted bacterial suspensions were used for inoculations. The tubers were inoculated by placing two drops of bacterial suspension prepared from 24hr. old culture of each of potato isolated bacteria on bottom of each cavity then coverd with removed potato plugs. Treated tubers were kept in clean sterilized plastic containers each supplemented with a sterilized moist cotton and incubated at 27°C for 4 days. After incubation, inoculated tubers were cut into halves to observe rotting. Four replicates were used for each tested isolate (De Boer and Kelman,1978).

Disease assessment:

Severity of disease was recorded using the method of Saleh *et al*., 1996 and Yaganza *et al*. (2004) as follow:

Disease severity index was calculated by following equation:

$$DSI = \frac{A - B}{A} \times 100$$

A = Tuber weight with rotting.

B = Tuber weight without rotting.

Effect of salicylic acid and acetylsalicylic acid on inducing localized acquired resistance against bacterial soft rot of potato tubers

In this experiment, the effect of treating potato tubers with SA and ASA on soft rot disease severity was studied.

Potato tubers of three potato cultivars (Diamant, Lady Rosetta and Argos) were surface sterilized by dipping in 1% sodium hypochloride solution for three minutes followed by rinsing in five changes of sterilized tap water. Tubers were allowed to dry at room temperature (about 30°C). After drying the tubers were artificially treated by submerging in different concentrations of 0.0125%, 0.05% and 0.25% of SA and ASA for the periods of 0.5, 1 and 2 hours. They were examined for their susceptibility to rotting after 0, 3, 6 and 9 days storage period. A cavity 1 cm in depth and 0.5 cm in width was made in tubers by a cork-borer, then potato tubers were inoculated with two drops of bacterial suspension ($5x10^8$ CFU/ml) as mentioned before. Treated tubers were kept in a clean sterilized plastic containers, each supple-

mented with a sterilized moist cotton and incubated at 27°C for 4 days. After incubation, tubers were cut into halves to observe rotting. The percent disease index (PDI) was estimated using the formula that mentioned before. Four replicates were used for each treatment.

Statstical analysis:

All experiments were set up in a complete randomized design. Data were subjected to analysis of variance (ANOVA), using the statistical analysis system (SAS Institute Inc., 1996). Means were compared with L.S.D. test at $P \leq 0.05$ levels.

Results

Isolation of bacterial isolates and pathogenicity tests

Nine bacterial isolates were obtained from naturally diseased potato plants showing symptoms of soft rot disease collected from different localities of Assiut and El-Minia Governorates. Results in Table (1) show that the nine tested isolates were pathogenic and produced symptoms of soft rot on potato plants. Isolate No. EC3 gave the highest disease index (20.1%) followed by isolate EC9 (14.7%) and then isolate EC5 which (12.1%). and then isolate EC6 (6.3%). Whereas isolate No. EC1, EC2, EC4, EC5 and EC8 caused the least disease index (3.1- 4.2%).

Table (1): Source and pathogenic capability of *Erwinia carotovora* subsp. *carotovora* isolates obtained from naturally diseased potato tubers:

Bacterial isolates	Locality	Disease index
EC1	Assiut	4.0
EC2	Assiut	3.8
EC3	Assiut	20.1
EC4	El-Minia	4.2
EC5	El-Minia	12.1
EC6	El-Minia	3.1
EC7	Assiut	6.3
EC8	El-Minia	3.5
EC9	El-Minia	14.7
Control		**0.0**

L.S.D $_{(0.05)}$: 1.08

Identification of the causal pathogen:

Morphological and physiological characteristics:

Identification of isolated pathogenic bacteria was carried out using the morphological and physiological characteristics.

Results indicated that all tested isolates were rod-shape, motile, non-sporing, gram negative, gelatin liquefaction positive,

starch hydrolysis negative, urease negative, catalase test and Esculin hydrolysis positive, no growth at both 4°C and 40°C, oxidase test and H_2S production negative, levan production positive, methyl red test (M.R) positive, voges proskauer test (V.P) negative, litmus milk positive, phenyl alanine deminase test negative, casein hydrolysis and nitrate reduction positive, phosphatase test negative.

Also the tested isolates produced acid and gas from: sucrose, glucose, fructose, lactose, glactose, maltose, mannose, manitol, trehalose, cellobiose and rhminose, and did not produce acid from starch, dextrin.

On the basis of the obtained data and those reported by Billing *et al*. (1961), Dye (1968), Krieg and Holt (1984), Schaad (1988), Holt *et al*. (1994) and Staley *et al*. (2005), it could be stated that all tested isolates are identified as *Erwinia carotovora* subsp. *corotovora*.

Effect of salicylic acid and acetylsalicylic acid in inducing localized acquired resistance against bacterial soft rot on potato tubers:

Data in Tables (2, 3 and 4) indicate that soaking tubers of Diamant, Lady Rosetta and Argos cultivars in all tested concentrations (0.0125%, 0.05% and 0.25%) of SA and ASA have significantly reduced soft rot development in wounded entire potato tubers as compared with control. The reduction of soft rot severity increased with increasing time of soaking tubers. Tubers of the tested cultivars soaked in both tested chemicals and concentrations for 2h exhibited the highest reduction on disease severity followed by soaking tubers for 1h and then soaking for 0.5h as combared with the control (non-treated). Data also revealed that treated potato tubers of the tested cvs. with all tested conc. of SA and ASA and stored for 9 days showed the highest decrease in disease severity followed by those stored for 6 days and then 3 days as compared with those of 0 time.

Results also indicated that soaking potato tubers of the tested cultivars in ASA in all tested concentrations caused higher decrease in soft rot severity than treatment with SA.

In general, potato cv. Lady Rosetta showed the highest resistance to the disease followed by cv. Diamant then cv. Argos.

Table(2): Effect of submerging potato tubers in compounds inducing localized acquired resistance against bacterial soft rot of potato tubers cv. Diamant.

Treatment			Storage period (days)				Mean	
Compound	Conc.	Time	0	3	6	9		
Non treated			**25.1***	**23.2**	**26.2**	**27.2**	**25.4**	
SA	**0.25%**	**0.5 h**	25.1	23.2	26.2	27.2	25.4	
		1 h	22.8	20.9	17.7	12.7	18.5	
		2 h	17.4	17.6	11.6	10.5	14.3	
		mean	21.8	20.6	18.5	16.8	19.4	
	0.05%	**0.5 h**	23.3	21.7	18.6	15.4	19.8	
		1 h	21.9	19.7	16.7	13.7	18.0	
		2 h	21.3	18.5	14.2	12.7	16.7	
		mean	22.2	20.0	16.5	13.9	18.1	**19.5**
	0.0125%	**0.5 h**	33.1	22.7	21.5	21.0	24.6	
		1 h	24.7	20.2	19.2	15.1	19.8	
		2 h	24.6	19.3	15.7	14.1	18.4	
		mean	27.5	20.7	18.8	16.7	20.9	
ASA	**0.25%**	**0.5 h**	14.1	15.2	12.6	12.0	13.5	
		1 h	13.4	14.7	7.8	8.7	11.2	
		2 h	11.0	13.2	4.8	5.8	8.7	
		mean	12.8	14.4	8.4	8.8	11.1	
	0.05%	**0.5 h**	18.9	16.2	14.2	15.0	16.1	
		1 h	16.7	15.1	11.9	10.9	13.7	
		2 h	13.4	14.3	8.5	7.9	11.0	**13.5**
		mean	16.3	15.2	11.5	11.3	13.6	
	0.0125%	**0.5 h**	22.2	17.3	19.5	15.0	18.5	
		1 h	18.9	16.2	14.3	13.4	15.7	
		2 h	16.0	15.5	11.4	11.4	13.6	
		mean	19.0	16.3	15.1	13.3	15.9	
Mean			**20.7**	**18.6**	**16.4**	**18.6**	**17.8**	

***Disease index:**

L.S.D $_{(0.05)}$:

A(Treatment):	**0.28**	**B** x **C:**	**0.48**
B(Concentration):	**0.28**	**B** x **D:**	**0.56**
C(Submerge time):	**0.27**	**C** x **D:**	**0.56**
D(Storage periods):	**0.32**	**A** x **B** x **C:**	**0.84**
A x **B:**	**0.48**	**A** x **B** x **D:**	**0.97**
A x **C:**	**0.48**	**B** x **C** x **D:**	**0.97**
A x **D:**	**0.56**	**A** x **B** x **C** x **D:**	**1.68**

Table (3): Effect of submerging potato tubers in compounds inducing localized acquired resistance against bacterial soft rot potato tubers cv. Lady Rosetta.

Treatment			Storage period (days)				Mean	
Compound	Conc.	Time	0	3	6	9		
Non treated			18.7*	17.2	19.7	20.4	19.0	
SA	0.25%	0.5 h	12.6	10.2	9.4	7.7	10.0	11.4
		1 h	11.4	9.4	6.8	4.2	8.0	
		2 h	9.9	8.4	3.1	1.6	5.8	
		mean	11.3	9.3	6.4	4.5	7.9	
	0.05%	0.5 h	14.3	33.3	10.7	9.9	17.1	
		1 h	13.5	30.2	9.4	6.0	14.8	
		2 h	10.6	28.4	6.2	4.5	12.4	
		mean	12.8	30.6	8.8	6.8	14.8	
	0.0125%	0.5 h	17.0	12.3	11.7	12.5	13.4	
		1 h	15.1	11.2	11.0	7.8	11.3	
		2 h	11.6	10.4	8.6	7.0	9.4	
		mean	14.6	11.3	10.4	9.1	11.4	
ASA	0.25%	0.5 h	8.6	5.1	5.4	4.7	6.0	6.5
		1 h	6.0	4.1	3.1	2.1	3.8	
		2 h	4.5	3.4	2.6	1.7	3.1	
		mean	6.4	4.2	3.7	2.8	4.3	
	0.05%	0.5 h	11.7	6.1	7.8	7.7	8.3	
		1 h	10.8	5.4	5.6	4.7	6.6	
		2 h	7.6	4.4	5.1	3.1	5.1	
		mean	10.0	5.3	6.2	5.2	6.7	
	0.0125%	0.5 h	13.0	7.5	9.9	11.2	10.4	
		1 h	12.1	6.5	8.5	7.0	8.5	
		2 h	8.8	5.7	7.0	4.8	6.6	
		mean	11.3	6.6	8.5	7.7	8.5	
Mean			12.2	12.1	9.1	8.1	10.4	

***Disease index:**

L.S.D $_{(0.05)}$:

A(Treatment):	**0.22**	**B** x **C:**	**0.37**
B(Concentration):	**0.21**	**B** x **D:**	**0.43**
C(Submerge time):	**0.22**	**C** x **D:**	**0.43**
D(Storage periods):	**0.25**	**A** x **B** x **C:**	**0.64**
A x **B:**	**0.37**	**A** x **B** x **D:**	**0.74**
A x **C:**	**0.37**	**B** x **C** x **D:**	**0.74**
A x **D:**	**0.43**	**A** x **B** x **C** x **D:**	**1.29**

Table (4): Effect of submerging potato tubers in compounds inducing localized acquired resistance against bacterial soft rot potato tubers. cv. Argos.

Treatment			Storage period (days)				Mean	
Compound	Conc.	Time	0	3	6	9		
Non treated			33.5*	29.7	34.5	35.3	33.3	
SA	0.25%	0.5 h	24.8	22.8	18.1	17.1	20.7	20.4
		1 h	20.8	21.2	13.3	12.2	16.9	
		2 h	17.8	20.8	12.0	11.5	15.5	
		mean	21.1	21.6	14.5	13.6	17.7	
	0.05%	0.5 h	25.6	23.1	20.2	17.8	21.7	
		1 h	25.6	22.5	16.7	17.0	20.5	
		2 h	22.9	21.4	15.4	21.2	20.2	
		mean	24.7	22.3	17.4	18.7	20.8	
	0.0125%	0.5 h	29.2	24.4	23.2	21.2	24.5	
		1 h	28.1	23.1	21.7	17.7	22.7	
		2 h	26.8	22.5	16.6	16.9	20.7	
		mean	28.0	23.3	20.5	18.6	22.6	
ASA	0.25%	0.5 h	18.2	17.3	13.6	14.1	15.8	15.4
		1 h	15.4	16.3	11.6	12.0	13.8	
		2 h	11.8	15.2	8.1	8.4	10.9	
		mean	15.1	16.3	11.1	11.5	13.5	
	0.05%	0.5 h	22.0	18.7	14.3	16.1	17.8	
		1 h	17.0	17.9	12.8	15.4	15.8	
		2 h	14.6	16.1	10.7	11.5	13.2	
		mean	17.9	17.6	12.6	14.3	15.6	
	0.0125%	0.5 h	23.9	17.1	20.5	17.9	19.9	
		1 h	19.7	16.9	15.7	14.0	16.6	
		2 h	16.0	17.2	12.4	14.3	15.0	
		mean	19.9	17.1	16.2	15.4	17.1	
Mean			22.9	21.1	18.1	18.2	20.0	

***Disease index:**

L.S.D $_{(0.05)}$:

A(Treatment):	**0.30**	**B** x **C:**	**0.52**
B(Concentration):	**0.30**	**B** x **D:**	**0.60**
C(Submerge time):	**0.30**	**C** x **D:**	**0.60**
D(Storage periods):	**0.34**	**A** x **B** x **C:**	**0.90**
A x **B:**	**0.52**	**A** x **B** x **D:**	**1.04**
A x **C:**	**0.52**	**B** x **C** x **D:**	**1.04**
A x **D:**	**0.60**	**A** x **B** x **C** x **D:**	**1.80**

Discussion

Results reported herein indicate that the nine bacterial isolates obtained from naturally diseased potato tubers collected from different localities of Assiut

and EL-Minia Governorates proved to be pathogenic and able to infect potato tubers causing soft rot symptoms. They were identified as *E carotovora* subsp. *carotovora* These results are in agreement with those reported by many workers (Zayed and maayouf, 1989; Choi *et al*., 1990; Togashi and Nami, 1991; Arsenijevic *et al*., 1996 and Clark *et al*., 1998)

The effect of SA and ASA treatments on soft rot incidence in potato tubers inoculated with *E. carotovora* subsp. *carotovora* under different storage periods was investigated. Results showed that SA and ASA at concentrations of 0.0125%, 0.05% and 0.25% significantly reduced soft rot development in wonded potato tubers in tested potato cvs. (Diamant, Lady Rosetta, Argos). Potato tubers cv. Lady Rosetta showed the highest reduction in soft rot disease index followed by Diamant and finally Argos.

Such results are in agreement with those reported by Waffa (1996) and Lopez lopez *et al*. (2006). who demonstrated that acetylsalicylic acid (aspirin) at concentration of 0.0125, 0.025, 0.03, 0.04 and 0.05% (w/v) did not inhibit bacterial growth of *Erwinia carotovora* subsp. *carotovora in vitro*, High concentration of ASA (2 and 5mM) were not effective in reducing disease severity either wounded or intier potato tubers or slices.

Results also showed that potato tubers treated with ASA of all tested potato cultivars caused greater reduction in disease severity than SA treatment.

Increasing storage periods after submerging in SA and ASA were significantly reduced soft rot developments on potato tubers of tested potato cvs. These results are agree with results of Ward *et al.,* (1991), Ukness *et al.,* (1992) and Wafaa (1996) who reported that increasing intervals time of inoculation with pathogen after aspirin application enhanced resistance induction against bacterial soft rot.

Several authors have demonstrated that SA plays an important role as a signal in the mechanism of systyemic acquired resistance (Raskin, 1992; Sticher *et al*., 1997).

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حث المقاومة لمرض العفن الطري في درنات البطاطس المتسبب عن بكتريا إيروينيا كاروتوفورا تحت النوع كاروتوفورا بواسطة المعاملة بحمض السلسليك و حمض الأستيل سلسليك

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تم الحصول علي 9 عزلات بكتيرية ممرضه تسبب مرض العفن الطري البكتيرى لدرنات البطاطس وذلك من عينات مرضيه مصابه تم تجميعها من مناطق مختلفه من محافظتى اسيوط والمنيا. وقد عرفت هذه العزلات طبقا لصفاتها المورفولوجيه والفسيولوجية علي إنها البكتريا إيروينيا كاروتوفورا تحت النوع كاروتوفورا *Erwinia carotovora* subsp. *carotovora*

ولقد أظهرت دراسة تأثير حمض الأستيل سلسليك وحمض السلسليك ما يلي:

1- أدى غمر درنات البطاطس في التركيزات (0,0125% ، 0,05 %، 0,25 %) لكل من حمض الاستيل سلسليك وحمض السلسليك الي خفض الاصابه بالمرض في اصناف البطاطس المستخدمه في الدراسه (صنف الدايمونت ، صنف الليدى روزتا ، صنف الأرجوس) .

2- أظهرت الدراسة أن تأثير حمض الاستيل سلسليك كان أكثر فعاليه من حمض السلسليك في تقليل الإصابه بالمرض.

3- أدى تخزين درنات البطاطس للأصناف المختبره المعامله بحمض السلسليك وحمض الاستيل سلسليك لمدة 9 أيام إلي أكبر خفض للإصابه بالعفن الطري يليه الدرنات المخزنه لمدة 6 أيام وأخيرا الدرنات المخزنه لمدة 3 أيام.

4- إتضح من الدراسة تباين درنات أصناف البطاطس المختبره في مدى قابليتها للإصابة بالعفن الطري البكتيرى إذ تبين أن درنات صنف الليدى روزتا أقل قابليه للإصابة بالمرض يليه صنف الدايمونت ثم صنف الأرجوس.