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DETERMINATION OF INCIDENCE OF POTATO SOFT ROT IN MAJOR POTATO GROWING AREAS OF PUNJAB, PAKISTAN

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ABSTRACT

Bacterial soft rot caused by *Erwinia carotovora* is a serious disease and can cause substantial post-harvest losses to potato. Its presence has been reported from Punjab, but current prevalence is not reported. Current study was initiated to assess the incidence of bacterial rot disease in Punjab. Survey of potato growing areas of Punjab (which include fields and markets) was conducted twice to assess the disease incidence and severity. Major districts included Lahore, Okara, Faisalabad, Sahiwal, Sialkot and Taxila. Several fields from each district were randomly visited and the samples of plants, tubers and soil were collected. Crystal violet pectate (CVP) medium was used for isolation of *E. carotovora*. Bacterial soft rot was prevalent in the fields and incidence ranged from 2-7% and highest incidence was found 7% in Sialkot. Market survey revealed the incidence of soft rot relatively higher as compared to field. The rot suspected samples were plated on CVP media and the characteristic pit formation was observed. Eight (8) isolates were obtained with pit formation feature and preliminary identification was based on the growth on CVP media at 27, 33.5 and 37 °C. Three strains were found associated to rot as *E. carotovora* subsp. *atroseptica*, *E. carotovora* subsp. *carotovora* and *E. chrysanthemi*. The isolates were further confirmed using biochemical tests. Pathogenicity was tested on tuber slices which exhibited the rot symptoms.

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most important vegetable crop of the world including Pakistan. The climate of Pakistan, especially of Punjab, is very suitable for the production of potatoes. Punjab is the chief potato producer of Pakistan and more than 80% of the potato comes from it. Availability of healthy potato tubers (seeds) is a problem. The available potato tubers (seed) contain latent infections which result in low yield and poor quality. Among potato tuber pathogens, the bacterial pathogens occur in many forms like brown rot, soft rot and ring rot of potatoes.

More or less 22% of potatoes are lost per year due to viral, bacterial, fungal, and pest attack to potato tuber and potato plant, incurring an annual loss of over 65 million tons (Czajkowski et al., 2011). In Pakistan, potato soft rot disease was first time reported in 1984 from Swat valley (Khan et al., 1985). From hilly areas and plains of Punjab, it was reported in 1985 and 1986 respectively by (Turkensteen, 1986) and gained a higher incidence particularly in potential potato growing areas with an incidence ranging between 0.2-2.9% (Hafiz, 1986). (Turkensteen, 1986) concluded that bacterial wilt caused

by *Ralstonia solanacearum* was the most important bacterial disease in Punjab causing losses over 30% followed by *Erwinia carotovora* subsp*. atroseptica* (potato blackleg) causing losses up to 30% and by *Erwinia carotovora* subsp*. carotovora* (potato soft rot) causing losses up to 10%. Soft rot disease of potato is caused by bacterium *Erwinia carotovora* subsp. *carotovora* (newly named as *Pectobacterium carotovorum* subsp. c*arotovorum)*. It is a Gram-negative, facultatively anaerobic, rod-shaped bacterium having a number of flagella that produces deep pits on crystal-violet-pectate (CVP) medium. Ecc strains (causing potato soft rot and, in some cases blackleg too) have extensive spread in temperate zones as well as in tropical zones, confirming that their host ranges are wider as compared to other subspecies (Wells and Moline, 1991).

Previous crop infected residues and tuber rotting during the season are among the essential sources of inoculum, latent infections in seed tuber offer the chief source of initial infection during potato production (Hannukkala and Segerstedt, 2004). This pathogen can rot tubers in store or in the field where the early decay of seed tuber pieces can result in non-emergence or blacking (Perombelon, 2002). When the rotted mother tubers could emerge, infection of the stems can occur (Pérombelon et al., 1987). The extent of decay can lead to a very serious economic loss (Agrios, 2005).

Bacterial soft rot is one of the most important diseases causing post-harvest losses of potatoes in storage (Wells and Butterfield, 1997). When tubers are naturally contaminated with a pathogen, under optimal storage conditions these bacteria remain latent but when finds favourable conditions, they proliferate and cause decay of tubers. This decay is due to release of pectolytic enzymes that break the walls of cells releasing the cell contents and cytosol in the surroundings causing softness and watery appearance. The disease may first appear in the field on plants grown from previously infected seed pieces or tubers. The bacteria may also survive on certain weeds. Once the bacteria ingresses the tissue, it starts decaying and produce large amounts of pectolytic enzymes i.e. polygalacturonases, pectin methyl esterase, pectin lyase and several isoenzymes of pectate lyase (Pérombelon, 1992). The released bacteria can then infect other plants as well.

In the context of abovementioned facts, the present study was conducted to determine the incidence of soft rot in the potato growing areas of Punjab so that a strategy can be suggested to improve the protection of the crop.

MATERIALS AND METHODS

Survey for incidence and prevalence: Survey was conducted in major potato growing areas of Punjab (Table 1). From each location, 3 fields were selected, and 3 spots were randomly selected from each field.

Table 1. List of areas and locations visited during the survey.

Sr. No.	Area	Locations Surveyed
1	Faisalabad	Sadhar. Pansara, Chaba, Tandianwala, Bangla, Koraywala, Buraywala
2	Sahiwal	Chak 86/6r, Chak 95/6r, Chak 30/14L
3	Taxila	Taxila fields, Hazro
4	Okara	Baman Shah, Burj Jeeway Khan, Moza Ameer Aman, Salwal, Qadirabad
5	Lahore	Mujaki, Mangaal, Lakhodair, Ganja, Sindhuwa, Awan, Dihiwala, Gurki
6	Sialkot	Claswala, Dhilam, Gunah, Chicharwali, Ghoinkey, Sehjokala

On the basis of symptoms, two rows of selected spot were observed. The wilted plants were uprooted, and the samples were preserved in polythene bags, taken to the laboratory and stored at 4 °C for further processing. Disease incidence and prevalence were determined by the following formula:

$$Disease\ Incidence\ (\%) = \frac{No.\ of\ plants\ wilted}{Total\ No.\ of\ plants\ uprooted} \times 100$$

$$Disease\ Prevalence\ (\%) = \frac{No.\ of\ fields\ infected}{Total\ No.\ of\ fields\ surveyed} \times 100$$

Vegetable markets were also visited and rotten potato tubers exhibiting the soft rot symptoms were collected, preserved in polythene bags, brought to laboratory and stored in refrigerator at 4 °C for further isolation of the pathogen. The incidence was determined by the following formula.

$$Disease\ Incidence\ (\%) = \frac{No.\ of\ rotted\ tubers}{Total\ No.\ of\ tubers\ inspected} \times 100$$

Isolation of *Erwinia carotovora:* *E. carotovora* was isolated on crystal violet pectate (CVP) media from the infected tubers samples. The rotten portion of the tuber was cut, and surface disinfected with 1% NaOCl and

washed with distilled water and placed onto the medium. The plates were incubated at 27, 33.5 and 37°C for 2-3 days. On CVP, only soft rot Erwinias can form characteristic cavities after 48 h, *E. carotovora* subsp. *atroseptica* (Eca) generally forms cavities at 27 ºC only, *E. carotovora* subsp. *carotovora* (Ecc) and *E. chrysanthemi* (Ech) also do so up to temperatures of 33.5 and 37 ºC respectively (Perombelon and Hyman, 1986).

Pathogenicity of *E. carotovora:* All the isolates were checked for their ability to cause soft rot on potato tubers following the procedure of (Lelliott et al., 1966). Healthy tubers of potato were sterilized with 70% ethyl alcohol, then rinsed with sterile distilled water, and aseptically cut into slices having a thickness of 1-2 cm by using a sterile knife. The potato slices were put in Petri dishes containing sterilized filter paper impregnated with 2 ml of sterile distilled water. The potato slices were wounded through toothpick in order to inoculate them with the pathogen and incubated at 30°C for 2 days in moistened Petri dishes. Potatoes without pathogen on sterilized distilled water served as control. Inoculated tuber slices were carefully examined for soft rot appearance. Disease severity was assessed by the visual scale developed by (Ahmad et al., 1995).

1 = No or few symptoms
2 = 1-10 % tuber area affected
3 = 11-20% tuber area affected
4 = 21-30% tuber area affected
5 = 31-40% tuber area affected
6 = 41-50% tuber area affected
7 = 51% or more tuber area affected

Biochemical tests for *E. carotovora:* Biochemical tests of *E. carotovora* were performed that included the production of indole, phosphatase and lecithinase, growth at 5% NaCl, acid production from lactose, maltose, trehalose, α-methyl-D-glycoside. These tests also differentiate between Eca, Ecc and Ech based on the response to each test (Goszczynska et al., 2000).

RESULTS

Incidence and prevalence of potato soft rot: The survey of potato growing areas in Punjab revealed that bacterial soft rot was not a major problem in any field. Nearly 90 fields (total 30 locations from six areas and 3 fields from each location) were visited from several districts and many of the fields in these areas were found free from soft rot symptoms. The severity of disease in each location was variable and the killed plants were also observed. It was observed that the incidence of soft rot was higher in potatoes collected from Markets of field areas (Table2 and 3).

Table 2. Incidence (%) of soft rot disease in potato fields of Punjab.

Area	Location	Soft rot		Area	Location	Soft rot	Area
		DI (%)	DP (%)			DI (%)	DP (%)
Okara	Baman Shah	4	33	Faisalabad	Sadhar	7	66
	Burj Jeeway Khan	3	33		Pansara	0	0
	Moza Ameer Aman	0	0		Chaba	2	33
	Salwal	0	0		Tandianwala	4	33
	Qadirabad	5	66		Bangla	0	0
					Koraywala	0	0
					Buraywala	0	0
	Average	2.4			Average	1.85	
Sahiwal	Chak 86/6r	5	33	Taxila	Taxila fields	1	33
	Chak 95/6r	0	0		Hazro	0	0
	Chak 30/14L	0	0				
	Average	1.66			Average	0.5	
Lahore	Mujaki	6	33	Sialkots	Claswala	4	33
	Mangaal	0	0		Dhilam	0	0
	Lakhodair	0	0		Gunah	7	66
	Ganja Sindhuwa	4	66		Chicharwali	4	66
	Awan	0	0		Ghoinkey	0	0
	Dihiwala	2	33		Sehjokala	0	0
	Gurki	3	33				
	Average	2.14			Average	3.75	

The readings are average of two surveys, DI= Disease Incidence; DP= Disease prevalence.

Table 3. Incidence of soft rot potatoes collected from markets.

Sr. No.	Area	Incidence (%)
1	Okara	4-6
2	Sahiwal	5-9
3	Lahore	4-7
4	Faisalabad	7-9
5	Taxila	5-8
6	Sialkot	4-7

Isolation of *E. carotovora:* The isolation on different temperatures helped to differentiate the Erwinias. Growth was observed in all the plates incubated at different temperatures. Total 8 isolates were obtained from the infected samples which were purified by continuous streaking until the pure colonies were obtained. The characteristic pit formation was observed by the isolates. Out of 8 isolates, 4 isolates (LSR1, LSR3, FSR5 and SSR1) produced pits at 27°C, three isolates (FSR3, FSR4 and SSR2) at 33.5°C and one isolate (OSR2) at 37°C indicating them as *E. carotovora* subsp. *atroseptica*, *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* respectively (Table 4).

Pathogenicity of *E. carotovora:* Tuber slice assay showed that isolates were pathogenic and soft rot symptoms appeared on the slices of potato. The control was treated only with water and the test isolates were compared to the control slices.

Table 4: List of soft rot isolates.

Sr. No.	Isolate	Pits at 27°C	Pits at 33.5 °C	Pits at 37°C
1	LSR1	+	-	-
2	LSR3	+	-	-
3	FSR3	-	+	-
4	FSR4	-	+	-
5	FSR5	+	-	-
6	SSR2	-	+	-
7	SSR1	+	-	-
8	OSR2	-	-	+

Biochemical Tests of *E. carotovora:* Biochemical characterization of the isolates was done which confirmed the isolates to be Eca, Ecc and Ech (Table 5). All the results of biochemical tests used in the study were confirmed with the previous studies and it was confirmed that all the isolates were *E. carotovora*.

Table 5. Biochemical characterization of soft rot isolates.

Isolate	Indole	*P	lecithinase	Growth at 5% NaCl	lactose	Maltose	Trehalose	α-methyl-glycoside
LSR1	-	-	-	+	+	+	+	+
LSR3	-	-	-	+	+	+	+	+
FSR3	-	-	-	+	+	-	+	-
FSR4	-	-	-	+	+	-	+	-
FSR5	-	-	-	+	+	+	+	+
SSR2	-	-	-	+	+	-	+	-
SSR1	-	-	-	+	+	+	+	+
OSR2	+	+	+	-	-	-	-	-

*P= phosphatase.

DISCUSSION

The incidence of bacterial rot disease recorded in the current study has shown that this disease is not a major problem in areas of Pakistan. (Ahmad et al., 1995) made detailed survey of Pakistan's major potato growing areas of Punjab and recorded twenty diseases of potato. He also reported the presence of bacterial soft rot pathogen in central Punjab, but its incidence was reported as minor.

Current study focusing on the bacterial soft rot disease of potato explained the pathogen having a slightly increased incidence in these areas as compared to previously reported data. Soft rot disease was reported for the first time in Pakistan in 1984 from Swat valley (Khan et al., 1985). From hilly areas and plains of Punjab, (Turkensteen, 1986) reported it in 1985 and 1986, respectively. The disease was more frequent in the districts of Sialkot, Gujranwala and Faisalabad with an incidence ranging between 0.2-2.9% (Hafiz, 1986). Our study has updated the information regarding the incidence of this disease and its highest average incidence was found at Sialkot which was observed to be 3.75% and at individual area maximum 7% was found in Sialkot. However, the incidence in the market was slightly higher and maximum 9% was found.

E. carotovora is known to cause soft rot in many vegetables. Several strains of this bacterium are involved in rot like *atroseptica* and *carotovora*. These are prevalent in every region and are found associated with soft rot. It was earlier thought that soft rot is caused by only one type of bacterium but later on, it was revealed that *E. carotovora* has several sub species which are causing the rots. Among erwinias, bacteria differ greatly for optimum temperature for their growth. Based on the growth on media at different temperatures, it was made possible to differentiate them. On CVP, only soft rot erwinias can form characteristic cavities after 48 h, *E. carotovora* subsp. *atroseptica* generally forms cavities at 27 ºC only, *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* also do so up to temperatures of 33.5 and 37 ºC respectively (Perombelon and Hyman, 1986). Further several biochemical tests also made it easy to differentiate the erwinias. Ecc can be differentiated from all other *E. carotovora* strains solely on the basis of acid production from α-methylglycoside, production of reducing substances from sucrose, and inability to grow at 37°C (Graham, 1972). Our results are in accordance to earlier observations. There is difference on the utilization of different carbon sources in the literature Eca can utilize maltose as carbon source while Ecc is unable to utilize maltose as sole source of carbon (Malcolmson, 1959). Our study has shown that Ecc do not use maltose as a sole carbon source.

CONCLUSION

The results updated the incidence of bacterial soft rot in the major potato growing areas of Punjab, Pakistan. The incidence of soft rot is also present in the field which exacerbate during storing conditions which is evident from the survey of the market from different areas and higher incidence was observed. Still the incidence of soft rot since previous reports means that infested material is still present which is causing recurring disease. Strong need is there to minimize these losses by proper quarantine measures and also proper seed certification programs so that it may be controlled.

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