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The Relationship of Potato Bacterial Soft Rot Disease with Reduced Sugar Content of Potato Tubers and Calcium

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ABSTRACT

Bacterial soft rot caused by *Pectobacterium carotovorum* subspecies *carotovorum* is among the important diseases of potato causing economic losses. A study was conducted in order to test the susceptibility of 5 different potato cultivars to the artificial inoculation with 10^7 Cfu/ml of the suspension of the causal agent *Pectobacterium carotovorum* subspecies *carotovorum* and the relationship of their susceptibility to reducing sugar and calcium contents of the tested potato cultivars tubers. The results of the study showed that all the tested potato cultivars were found to be susceptible to the artificial *inoculation* of the disease where Spunta was found to be the most susceptible cultivar, while Ajax was the lowest one. At the same time, a positive correlation was found between the reducing sugar content of the tested potato cultivars tubers and of the susceptibility to soft rot. Specifically, Spunta, the highest susceptible one, was found to have the highest reducing sugar content, in the meantime Ajax the lowest susceptible one had the lowest reducing sugar content. On the other hand, a negative correlation was found in relation to calcium peel content, where the results showed that Spunta the highest susceptible one had with the lowest calcium content and Ajax the lowest susceptible one had the highest calcium content.

Keywords: *Pectobacterium carotovorum* subspecies *carotovorum*, resistant, susceptibility.

INTRODUCTION

Potato (Solanum *tuberosum* L.) is one of the most important economic vegetables listed among the five principle crops grown in the world (Kandil *et al*., 2011) as well as in Jordan (Rajeh and Khlaif, 2000). Potato ranks the second among the economic crops in Jordan. In 2016, the area planted with potato in Jordan was 50.860 hectares with a total production of 158573 tons (Annual Statistical Report, 2016). Potato is planted in the Jordan Valley as well as in the Uplands, and grown during fall and summer, where 82% of this area was planted in uplands.

Different bacterial diseases have been reported to attack potato around the world leading to economic losses in yield and quality under favorable environmental conditions of: Brown rot (*Ralstonia solanacearum*), Common scab (Streptomyces *scabies*), Ring rot (*Clavibacter micheganensis*), Black leg (*Pectobacterium carotovora* subspecies *atrosepticum*), and soft rot (*Pectobacterium carotovorum* subspecies *carotovorum)* (Liao and Wells, 1987, Bishop and Davis 1990, Agrios, 2005 and Schroeder *et al.*, 2009).

However, potato soft rot is one of the important diseases of potato causing great reduction in yield, resulting in economic losses in the field during transit and storage causing losses up to 60%. (Abo- Elyousr *et al.*,

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2010; Toth *et al.*, 2011; Ngadze *et al*., 2012 a; Mantsebo *et. al.*, 2014). Soft rot was reported to be caused by different bacterial species; *Bacillus* sp., Fluorescent *Pseudomonas* and *Erwinia* species.

Soft rot disease caused by *Erwinia carotovora* subspecies *carotovora (Ecc)* is recently known as (*Pectobacterium carotovorum* subspecies *carotovorum*) (Dye, 1969). *Ecc* was reported as the most common causal agent of bacterial soft rot and is listed among the top ten plant pathogenic bacteria (Mansfield *et al.*, 2012) with a wide host range in tropical and sub-tropical regions, infecting vegetable species belonging to different major plant families (Rajeh and Khlaif, 2000; Bhat *et al*., 2010).

Soft rot bacteria are opportunistic pathogens which produce cell wall degrading enzymes in large amounts, outcompeting other pathogens. They are pectinolytic and they produce a wide range of enzymes which include proteases, cellulases, pectinases, and xylanases (Perombelon, 2002).

The main enterobacteria that cause tuber soft rot are *Pectobacterium carotovorum* subspecies *carotovorum* (*Pcc*), *Pectobacterium carotovorum* subspecies *atrosepticum* (*Pca*), *Pectobacterium carotovorum* subspecies *brasiliense* (*Pcb*), and *Dickeya* species (Lojkowska and Kelman, 1994; Ngadze *et al*., 2012 a; Mantsebo *et. al.*, 2014). Tubers can be contaminated by more than one bacterial pathogen and contamination is unavoidable (Perombelon, 2000).

The tubers susceptibility to bacterial soft rot is influenced by: tuber water potential, membrane permeability, intercellular concentration of reducing sugars, polyphenol oxidase, oxygen level, and other factors. Low oxygen concentration increases susceptibility to tuber soft rot, resulting in extensive tissue degradation (McGuire and Kelman, 1983; Ngadze *et. al.*, 2012b).

Pectobacterium species produce pectolytic enzymes (polygalacturonase and pectin lyase) which macerate tuber tissue and induce electrolyte leakage and cell death (McGuire and Kelman, 1986; Flego *et al*., 1997). Pectins are made up of chains of polygalacturonic acid residues with rhaminose insertions (Conway *et al*., 1992). In the potato medullary tissue, galacturonic acid precipitates calcium to form calcium pectate (McGuire and Kelman, 1986) and thus making the cell wall rigid (Gunter and Palta, 1998; Mantsebo *et. al.*, 2014).

Calcium is considered a determining factor in the resistance and susceptibility of potato tubers to bacterial soft rot (Miles *et al*., 2009; Abo-Elyousr *et al*. 2010; Mantsebo *et. al.*, 2014; Ngadze *et al.*, 2014). The use of plant nutrients to control diseases is an environmentally friendly practice and there is a need to provide a balanced nutrition so as to effectively control diseases (Dordas, 2008; Mantsebo *et. al.*, 2014; Ngadze *et al.*, 2014). McGuire and Kelman (1983) found tuber calcium concentration to be high in the cortex and periderm.

Results of various studies indicate the repression of pectic enzymes by sugar, especially glucose and sucrose, resulting in the retardation or inhibition of disease development in many host pathogen systems (Biehn *et al.*, 1972; Bugbees, 1973; Keen and Horton 1965; Patil and Dimond, 1968). Interestingly, Hubbard *et al.* (1978) showed a reversal of glucose repression of pectat lyase (PL) by adenosine 3’5’- cyclic mono3phosphate (CAMP) in *Erwinia corotovora*. Reducing sugar (RS) is important to the potato industry, because the processing of tubers with highly RS content produce dark chips and fries which are undesirable (Habib and Brown, 1957; Schwimmer *et al.*, 1957).

In Jordan, *Pcc* is considered the main causal organisms of soft rot in vegetables with a wide host range belonging to different plant families. Potato tuber soft rot is one of the most serious post-harvest problems in Jordan. Thus, in this study, we reported the most important characteristics of potato tuber of different cultivars after disease inoculation; calcium and reducing sugars content and an illustration of their role in soft rot resistance.

MATERIALS AND METHODS

Plant material

Healthy potato tubers of similar size for five tested cultivars, namely, Ajax, Diamont, Draga, Mondial, and Spunta were randomly collected from potato fields, which were harvested at same date of each cultivar. Then the following experiments were done for each cultivar.

Pathogen and inoculum preparation

A pathogenic isolate of *Pcc* was isolated from rotted potato (*Solanum tuberosum* L.) tubers showing bacterial soft rot and identified according to Schaad *et al*. (2001). Suspensions of 10^7 cfu/ml were prepared from 24hrs old *Pcc* bacterial culture and were used in this study for tuber inoculation.

Resistance of potato cultivars to soft rot

Laboratory experiments were conducted to test the reaction of the following local potato cultivars: Ajax, Diamont, Draga, Mondial, and Spunta against soft rot infection.

Sixteen tubers from each cultivar were randomly chosen, then surface sterilized with 0.5% sodium hypochloride solution for 2 minutes, rinsed three times with sterile distilled water (SDW) and air dried. A groove of 3mm in diameter at depth of 2cm was cut into the middle of each tubers by a sterile cork borer filled with a 10^7 CFU/ml bacterial suspension of *Pcc* prepared from 24hrs old culture. Inoculated tubers were separated into 4 replicates; each contained four tubers. Tubers of three replicates were inoculated with bacterial suspension and the remaining set of tubers was inoculated with sterile distilled water to serve as a check. The tubers sets (of four) from each cultivar were completely randomized. The inoculated tubers were placed separately into a moisten beaker covered with sterile aluminum foil, then distributed randomly and incubated at $27 \pm 2C^{\circ}$ for 72hrs. The rotting percentage for each inoculated tuber was calculated according to the Bartz formula (1994).

$$Rotting = \frac{Tuber\ wt\ (befor\ inoculation) - Tuber\ wt\ after\ removing\ rotting\ tissue}{Tuber\ wt (befor\ inoculation)} \times 100$$

The average percentage of rotting for each tested cultivar was computed.

Reducing sugar determination

Ten potato tubers from each cultivar were randomly chosen, then stored for 2 weeks at 4 C° in a refrigerator in the seed technology unit. Three grams of each potato tuber tissue were taken from the tubers of each cultivar and squeezed in a hand–held garlic press. Reducing sugar determination was conducted using the reducing sugar method in accordance with the Association of Official Analytical Chemists (AOAC) that measure total reducing sugar for each tuber. The average amounts of reducing sugar for each cultivar were also determined (Gunter and Palta, 2008 and Weaver *et al.*, 1978)

Calcium determination

Ten potato tubers from each cultivar were randomly chosen, these tubers were peeled out after perfect cleaning by distilled water. Calcium determination was conducted according to the method of Association of Analytical Micro and Macro Element (AOAM) for calcium determination in plant tissue for each tuber, and the average of calcium for all tubers of each cultivar was computed (Iritani and Weller, 1977, and McGuire and Kelman, 1983).

RESULTS

Resistance to soft rot

All inoculated or tested potato cultivars developed soft rot symptoms with various degrees. The rotting percentage ranged from 3.36 % up to 20.98 % (Table 1), where the lowest rotting percentage was found for Ajax and the highest was found for Spunta cultivar. Significant differences occurred between Spunta and the other four cultivars.

Reducing sugar content

It was found that there were variations in the amount of reducing sugars content between the tested potato cultivars (Table 2). These variations ranged from 14.4 ppm for Ajax cultivar to 30.4 ppm for Spunta.

Calcium content

Tubers peel and the suberization layer were found to have a higher percentage of calcium of the tested potato tuber, the quantities of calcium in tubers peels are shown in Table 3. Among the tested potato cultivars, the highest and the lowest calcium contents were found in the cultivars Ajax and Spunta that were 0.680, 0.159 w/w, respectively.

DISCUSSION

The results of this study showed correlations between rotting percentage of soft rot caused by *Pcc* and reducing sugar, calcium contents of the tested potato cultivars.

Five potato cultivars, namely, Ajax, Diamont, Draga, Mondial, and Spunta were used in this study. The results showed that there were variations between the cultivars resistance/susceptibility to soft rot in which Ajax cultivar was found to be the resistant one and Spunta was found to be the susceptible one to soft rot disease, while the other three cultivars could be classified as moderately susceptible. These findings are found to be in general agreement with Biehn *et al.* (1972 and Bugbee (1973), who reported that there were variations in the response of potato cultivars either to natural infection or to artificial inoculation. They tested fifty-nine of different potato cultivars and they found that some of these cultivars are resistant to the natural infection with soft rot disease of: Eajke, Cara, Carnea, Croft, Drayton, Feculam, Hasia, Koretta, and Nova.

The resistance/susceptibility of potato cultivars to soft rot was also found to be correlated with reducing sugar content. It was found that the Spunta cultivar had the highest reducing sugar content (RSC), while the Ajax cultivar had the lowest RSC among the tested cultivars. In addition, there was a positive correlation between RSC and rotting percentage of the tested cultivars; the RSC were 30.4 and 14.4, and the rotting percentage was 20.98 and 3.36 for the cultivars Spunta and Ajax, respectively. Furthermore, it was found that a reduction in the RSC of the other three cultivars by about 5ppm (Table 2) had led to a reduction of about 50% in rotting percentage compared with Spunta. These results are in agreement with the results of Iritani and Weller (1977). Furthermore, Tishel and Mazelis (1966) reported that the RSC in potato tubers varied in different cultivars, but storage factors, mainly temperature, may greatly alter sugar content. Cool storage usually causes accumulation of RS, resulting in higher RS levels. Additionally, Watada and Kunkel (1955) reported that RSC differed between potato cultivars. Levels of RS also differ between the stem and bud end of the tubers (Iritani *et al.*, 1973; Weaver *et al.*, 1978).

Furthermore, it was found that there is an inverse correlation between the rotting percentage of potato tuber and calcium content, the highest rotting percentage was 20.98 for Spunta cultivar, which is correlated with the lowest calcium content of 0.159 w/w, it was also obvious in Ajax cultivar where their lowest rotting percentage of 3.36 was correlated with the highest calcium content, i.e. 0.68 w/w. Thus, the results showed that the Spunta is considered as a susceptible cultivar, while Ajax is regarded as a resistant one. For other cultivars, i.e. Diamond, Draga and Mondial, it was also found that an increase in calcium contents to about 3 times compared with Spunta (Table 3) resulted in a reduction in rotting percentage to about 50%, which illustrates the role of calcium in the disease resistance. The role of calcium in increasing tuber resistance to soft rot was found to be in general agreement with (Kratzke and Palta, 1986; Palta, 2010; Mantsebo *et. al.*, 2014; Ngadze *et. al.*, 2014), who reported that increasing calcium content in potato tubers reduces the incidence of tuber soft rot during storage, thereby increasing shelf life. The results also revealed that calcium content of potato tuber increased the level of resistance to soft rot (Abo-Elyousr *et al*., 2010; Mantsebo *et. al.*, 2014; Ngadze *et. al.*, 2014). In addition, it was found that the susceptibility of tubers to rotting differs in terms of varieties and the rate of wound healing (Murant and Wood, 1957) as well as tuber calcium content (Locascio *et al*., 1992; Czajkowski *et al*., 2011).

The differences in calcium content between the tested cultivars was found to be in general agreement with Gunter and Palta (1998), who found that the accumulation of calcium in tubers varied among cultivars and seasons, and concluded that different cultivars have different calcium uptake thresholds due to their genetic makeup, implying that the improvement of tuber calcium can be done through plant breeding.

The role of calcium in soft rot resistance has been illustrated by Ozgen *et al*. (2002), who reported that calcium is a secondary messenger in plant cells, contributing to the maintenance of cell membrane stability and cell wall structure. Moreover, McGuire and Kelman (1986) reported that calcium bridging of the plasma membrane components reduced electrolyte leakage and maceration by pectolytic enzymes. This can be interpreted if we take into account that extracellular calcium is thought to help in maintaining the selective permeability of plasma membranes because of the bridging of calcium ions on phosphate and carboxylate groups of phospholipid head groups at the membrane surface (Gunter and Palta, 2008; Geary *et al*., 2010).

Moreover, calcium prevents polygalacturonases (PGs) from interacting with the pectin polymer and blocks the diffusion of PGs through the cell wall (Goodwin *et al*., 1997; Huber and Jones, 2012). Tubers with high calcium have an improved structural integrity of both the plasmalemma and cell wall materials compared to tubers with low calcium content. This is thought to inhibit the multiplication and spread of the bacterial pathogen throughout the tissue (McGuire and Kelman, 1986).

The mineral nutrition, such as calcium, nitrogen, magnesium, potassium, and phosphorus, during the growth period of a potato plant can influence the occurrence of tuber soft rot in storage (McGuire and Kelman, 1983; Ngadze *et. al.,* 2014). It is believed that increasing the susceptibility of plants to diseases is due to the lack or deficiency of essential elements, such as calcium and magnesium (Czajkowski *et al*., 2011).

The application of calcium fertilizer during the growing season reduced bacterial soft rot incidence from 43 to 4% in 3 years. As calcium increased, disease severity decreased because an increase in tuber calcium most likely led to an increase in the cross-linkages of pectate chains, thereby reducing susceptibility to tuber soft rot (Locascio *et al*., 1992). Ngadze *et al.* (2014) also reported that nutrients, such as boron, nitrogen, and calcium stimulate the production of phenols. They also investigated the role of calcium in increasing phenol metabolism in potato peels and the ensuing tuber resistance to soft rot pathogens.

CONCLUSION

Potato soft rot caused by the bacterium *Pcc* is among the most important bacterial diseases of potato. Our findings revealed that this disease is largely affected by the potato cultivar in which the rotting percentage of the infected potato tubers is mainly related to the grown cultivar.

Furthermore, our findings revealed that there were variations in the composition of studied potato cultivars of certain components. There is also a strong correlation between these components and the cultivar susceptibility/resistance to the soft rot. The studied components, i.e. reducing sugar and the calcium were found to be of high importance in the cultivar susceptibility to the disease.

Moreover, calcium content is of major importance in increasing potato cultivar resistance to bacterial soft rot, whereas the reducing sugar has a role in increasing the susceptibility to the disease. Thus, our findings revealed the resistance of Ajax cultivar and the susceptibility of Spunta.

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Table 1: Average rotting percentage of the different potato cultivars artificially inoculated with the soft rot pathogen suspension in the laboratory

Cultivar	Average rotting percentage
Ajax	3.36a
Diamont	10.23a
Draga	7.09a
Mondial	9.12a
Spunta	20.98b
L.S.D $P \leq 0,05$	7.2

Means followed by the same letter are not significantly different at $p \leq 0.05$

Table 2: Reducing sugar contents in potato tuber of different cultivars

Cultivar	RSC ppm
Ajax	14.4
Diamont	25.6
Draga	26.4
Mondial	24
Spunta	30.4

Table 3: Calcium content in potato tubers peel of different cultivars

Cultivar	Calcium w/w
Ajax	0.680
Diamont	o.405
Draga	0.546
Mondial	0.534
Spunta	0.159

علاقة مرض العفن الطري بمحتوى درنات البطاطا من السكر المختزل والكالسيوم

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ملخص

يعتبر مرض العفن الطري البكتيري والمتسبب عن البكتيريا *Pectobacterium carotovorum* subspecies *carotovorum* من اهم الامراض التي تصيب البطاطا مؤديا الى خسائر اقتصادية. اختبرت هذه الدراسة اصنافا مختلفة من البطاطا لمعرفة مدى قابليتها للاصابة بالعفن الطري و ذلك عن طريق العدوى الصناعية لدرنات هذه الاصناف بمعلق المسبب المرضي (10^7 cfu/ml) كما درست علاقة قابلية هذه الاصناف للاصابة بمحتوى درناتها من السكر المختزل والكالسيوم. بينت الدراسة بان جميع الاصناف المختبرة كانت قابلة للاصابة بمرض العفن الطري البكتيري بدرجات مختلفة، حيث كان صنف Spunta اكثرها قابلية للاصابة والصنف Ajax اقلها قابلية وكما وجد ان هناك علاقة بين محتوى الدرنات من السكر المختزل وقابليتها للاصابة، حيث كان صنف Spunta اعلى الاصناف المختبرة في محتواها من السكر المختزل واقلها الصنف Ajax وكانت العلاقة عكسية ما بين القابلية للاصابة و محتوى الدرنات من الكالسيوم، حيث وجد ان الصنف Spunta الاكثر قابلية للاصابة بالمرض واقلها من حيث محتواه من الكالسيوم . وعلى النقيض فان الصنف Ajax اقلها قابلية للاصابة وكان اكثر الاصناف من حيث محتواه من الكالسيوم.

الكلمات الدالة: *Pectobacterium carotovorum* subspecies *carotovorum*، المقاومة، الحساسية.

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