Variability and genetic diversity among *Streptomyces scabiei* infected potato cultivars with common scab from Egypt

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Abstract

Twelve isolates of the *Streptomyces scabiei* causing the common scab in potato were isolated from potato plants originated from different governorates in Egypt. In order to study genetic variation among these isolates a total of 5 random ten-mer primers were used to perform the Random amplified Polymorphic DNA RAPD-PCR profiles for these 12 isolates. The results revealed that these primers were able to produce different bands. The number of amplified bands varied between 7 to 14, and the size ranged from 247 to 2226 bp. RAPD molecular technique showed that these isolates could be grouped in two clusters. First cluster included the highest value of similarity (81\%) between strain No. 9 and strain No. 12; while in second cluster isolates No.1, 2, 3, 4, 5, 6 and 7 were pertained to Aswan, Elminia and Assiut regions with similarity value of 40 \%. Under open greenhouse conditions, reaction of certain potato cultivars to common scab infection was evaluated. Lady Rosetta cultivar exhibited the lowest susceptibility to disease by 62.67 followed by Cara cultivar with 58.33. While Diamont, Mondial and Spunta cultivar showed the least degree of scab symptoms. Cara contained the highest concentration of total sugar. Lady Rosseta contained the highest concentration of pectin substances 0.684. Diamont cultivar contained the highest concentration of calcium (0.105 mg/L), and Spunta contained the highest concentration of potassium (2.33 mg/L), while Mondial, contained the highest concentration of sodium (1.28 mg/L). These results showed that potato cultivars varied in their susceptibility to common scab disease and further studies are required to determine the factors responsible for resistance or susceptibility.

*Keywords: Streptomyces scabiei, potato cultivars, Lady Rosette, Cara, Diamont, Mondial, Spunta, RAPD-PCR.*
1. Introduction

Potatoes (*Solanum tuberosum* L.) is an important crop contains important minerals and vitamins and grown in different climate systems (Bastin, 1997). Potato is being infected with many fungal, bacterial, viral and nematodes pathogens. Among these diseases, potatoes common scab considered as an important disease infecting potato and cause a serious loss in potato quality (Hosny et al., 2016). Potato scabs caused by many species of *Streptomyces* e.g. *Streptomyces scabiei* the casual pathogen of common scab, netted scab caused by *S. acidiscabies*, and *S. turgidiscabies* is the bacterial species of russet scab (Kers et al., 2005). The known plant pathogenic *Streptomyces* include more than eleven species (Lambert and Loria, 1989; Wen et al., 2012). Differences and relationship of *S. scabiei* Strains studied by molecular technique such as Random Amplified Polymorphic DNAs (RAPDs) used to find out the possible presence of variation between the isolates. RAPDs technique is a useful technique for random DNA segments using single primers of arbitrary nucleotide sequence (Williams et al., 1990). Total soluble proteins using SDS-PAGE analysis have been used to determine the differences in protein characteristics (Bradford, 1976). *S. scabiei* strains were recently placed into three distinct genomic species based on DNA relatedness and 16s rDNA sequence, and assigned as either *S. scabiei*, *S. europaeiscabiei* and *S. stelliscabiei* (Bukhalid et al., 2002). The *necl* gene encodes a secreted necrogenic protein with an uncharacterized plant cell target and was shown to confer a pathogenic phenotype on the nonpathogen *S. lividans* (Bukhalid et al., 1998). The G + C content (54%) of *necl* is a typical relative to high G + C coding regions characteristic of *Streptomyces* strains, suggesting it was acquired through horizontal transfer from another taxon. The *necl* gene is also structurally and functionally conserved among unrelated pathogenic *Streptomyces* species and absent in nonpathogens from a wide geographic origin (Bukhalid et al., 2002; Park et al., 2003). In addition, there exists a strong correlation between thaxtomin A production and the presence of *necl* in pathogenic strains of *S. scabiei*, *S. acidiscabies* and *S. turgidiscabies* (Bukhalid et al., 1998). However, the *necl* protein is not required for thaxtomin production and therefore represents an independent virulence factor (Kers et al., 2005). It has been showed that there is a relationship between levels of some tuber’s constituents and their susceptibility to common scab disease. Many researchers suggested that these constituents such as: calcium, potassium, manganese (Davis et al., 1976) or reducing sugars (Goto, 1981) may play an important role in disease resistance. This study was designed to study the variation between the isolates of the pathogen, molecular comparison between Isolates of common scab, molecular confirmation of causal pathogens of common scab diseases. As well as reaction of some potato cultivars against common scab, and the role of constituents on their resistance to bacterial common scab.
2. Materials and methods

2.1 Isolation of the common scab pathogen

Potato tubers with common scab disease symptoms appearance samples were collected from different potato cultivated locations in Elminia, Assiut, Sohag, Aswan and New Valley governorates, Egypt during 2017. Isolation trials were carried out from infected potato tubers with well developed deep and superficial scab symptoms. Whole tubers were washed with tap water; surface sterilized by soaking in 1% sodium hypochlorite solution (NaOCl) for 10 min, rinsed twice in sterile distilled water, and gently blotted dry on sterilized tissue paper. Samples taken from corky layer of each lesion were lifted aseptically and about 5 mm$^3$ of straw-colored tissues were macerated directly in 5 ml sterile distilled water in a sterile mortar. After approximately 10 min, a loopful of the resulting suspension was streaked over the surface of Yeast Malt Extract Agar (YMEA) medium which consist of; 4 g yeast extract, 10 g malt extract, 4 g glucose, 2 g CaCO$_3$, 15 g agar and distilled water up to 1 liter, that poured in Petri plates. Colonies observed after 7-14 days of incubation at 28°C were isolated and purified through the colony isolation technique (Bouchek-Mechiche et al. 2000; Wanner, 2009). Pure cultures of the obtained S. scabiei isolates were kept on YMEA slants.

2.2 Pathogenicity tests

2.2.1 Inoculation of mini potato tubers

Forty days old tubers were thoroughly washed with sterile water then surface sterilized with 1% sodium hypochlorite solution. Bacterial isolates were grown on OMA oatmeal agar medium and incubated at 28°C for 14 days. Then, bacterial growth was removed by sterilized needle and mixed well in sterile distilled water. Tubers were immersed in the bacterial suspension ($10^8$ CFU/ml) for 15 min then placed in a closed sterilized beaker containing cotton and filter paper wetted with sterile distilled water to provide high humidity, then incubated at 28°C for 7 days (Babcock et al., 1993; Bukhalid et al., 1998; Mckenna et al., 2001).

2.2.2 Inoculation of potato tubers under greenhouse conditions

Pathogenicity of obtained isolates of Streptomyces spp. was tested on potato tubers according to the following disease index reported by Hao et al. (2009).

2.3 Identification of the causal pathogen

2.3.1 Morphological, physiological and biochemical tests

The isolated pathogenic bacteria were identified using cultural, morphological and biochemical tests. Colony shape and conidial colour on YMEA medium, substrate mycelium, and chain morphology, melanin production on tyrosine agar medium and bacterial growth on 3 different pLI’s (3, 4, 6 and 8) were studied. Gram staining, catalase test, starch and casein hydrolysis, growth on 3 concentrations (5, 6 and 7%) of NaCl,
growth under 4 different temperatures (4, 25, 27 and 40°C) and carbohydrate fermentation on different source of sugars (glucose, fructose, maltose, raffinose and mannitol) were also recorded using Bergeys Manual of Systematic Bacteriology for Methods (Holt et al., 1994). The isolated bacterial isolates proved to be pathogenic and cause common scab to potato tubers were identified according to their morphological, cultural and physiological characteristics as recommended by Schaad (1992); Manual of systematic Bacteriology, Determinative Bacteriology 9th edition (Holt et al., 1994).

2.4 Molecular comparison between pathogenic isolates

2.4.1 Determination of the changes between 12 S. scabiei isolates using Random Amplified Polymorphic DNA (RAPD) technique

Isolation of DNA was performed according to Saghai-Marooof et al. (1984).

2.4.2 Characterization of the isolates using RAPD-PCR

To estimate the molecular variability among the isolates, RAPD-PCR technique were employed using Five ten-ner random oligonucleotide primers. The RAPD assays were based on the polymerase chain reaction (PCR) amplification of random sites spread all over the genomic DNA. The used DNA amplification protocol was performed as described by Williams et al. (1990) with some modifications.

2.4.3 Primers used in RAPD analyses

Five random Oligonucleotide primer sequences selected from a set of Operon kits (OPA-12, OPE-5, OPM-13, OPN-3, and OPP-2), Operon Technologies Inc., Alameda, CA) were used in the present study. Their codes and sequences are shown in Table (1).

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Primer codes</th>
<th>Sequence (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OPA-12</td>
<td>TCGCGATAG</td>
</tr>
<tr>
<td>2</td>
<td>OPE-5</td>
<td>TCAAGGGAGGT</td>
</tr>
<tr>
<td>3</td>
<td>OPM-13</td>
<td>GGTGGTCAAG</td>
</tr>
<tr>
<td>4</td>
<td>OPN-3</td>
<td>GGTACTCCCC</td>
</tr>
<tr>
<td>5</td>
<td>OPP-2</td>
<td>TCGGCACGCA</td>
</tr>
</tbody>
</table>

2.4.4 Preparation of PCR reactions

Agarose gel photos were scanned by the Gene Profiler 4.03 computer software program, that uses automatic lane and peak finding for detecting the presence of banding patterns. To calculate the bands molecular weight, and a binary data matrix recording the presence (1) or the

2.4.5 Data analysis
absence (0) of bands was made. Then, the software package MVSP (Multi-Variate Statistical Package) was used to calculate the genetic similarities using the Dice coefficient of similarity of Nei and Li (1979). The cluster analysis was carried out based on calculated similarity estimates using the unweighted pair-group method with arithmetic average (UPGMA) software. The results were then represented as a dendrogram for all over primers.

2.5 Reaction of certain potato cultivars to common scab disease

Five potato cultivars namely, Cara, Spunta, Diamant, Mondial and Lady Rossetta were tested for their susceptibility to common scab disease under greenhouse conditions. Healthy potato tubers were planted in 30-cm²-diameter sterilized pots containing sterile 1:1 soil/sand mixture weight 7 kg. Potato tubers were surface disinfection by soaking tubers in 0.5 % sodium hypochlorite for 5 minutes then rinsed twice in sterilized water directly before planting. Fifty ml 10⁸ CFU/ml of ten days old culture from isolate No. (6) were added to each pot before planting. Five replicate pots for each isolate were used. Pots were arranged in a complete randomized block design with five replicates. Control plants were treated similarly by tap water.

2.5.1 Pectin contents

The anthrone sulphuric acid methods were used in determining the pectin content of potato tubers (Badour, 1959).

2.5.2 Sugar contents

Sugar contents was measured according to Thomas and Dutcher (1924). Sodium and potassium were measured by flame photometer (CL378- ELICO) while calcium was measured by Atomic Absorption Spectrophotometer according to Jackson (1973).

3. Results

3.1 Isolation and pathogenicity test of the causal pathogen

Twelve isolates presented most strong symptoms were obtained from naturally diseased potato tubers showing typical symptoms of common scab disease collected from different localities of Assiut, New Valley, Sohag, Aswan Behira and Elminia governorates, Egypt. Results in Table (3) showed that twelve isolates gave the symptoms of common scab disease on potato tubers. Isolates No. 2 and 6 showed the highest disease severity followed by isolates No. 11 and 12 exhibited the lowest disease severity respectively.

3.2 Pathogenicity tests of isolates of Streptomyces scabiei on potato Lady Rosseta cultivar under open greenhouse conditions

Results in Table (4) showed that isolates No. 6 and 8 gave the highest disease severity followed by isolates No. 2, 4, 11 and 12 whereas isolates No. 1 and 5
exhibited the lowest disease severity respectively.

Table (3): Disease incidence tests of isolates of *Streptomyces scabiei* on potato mini tubers.

<table>
<thead>
<tr>
<th>Bacterial isolates No.</th>
<th>Locality</th>
<th>DI of potato tubers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Assiut</td>
<td>12.7</td>
</tr>
<tr>
<td>2</td>
<td>Assiut</td>
<td>16.0</td>
</tr>
<tr>
<td>3</td>
<td>ElMenia</td>
<td>10.3</td>
</tr>
<tr>
<td>4</td>
<td>Aswan</td>
<td>11.0</td>
</tr>
<tr>
<td>5</td>
<td>Aswan</td>
<td>10.7</td>
</tr>
<tr>
<td>6</td>
<td>Assiut</td>
<td>15.3</td>
</tr>
<tr>
<td>7</td>
<td>Aswan</td>
<td>10.3</td>
</tr>
<tr>
<td>8</td>
<td>New Valley</td>
<td>9.3</td>
</tr>
<tr>
<td>9</td>
<td>Sohag</td>
<td>8.3</td>
</tr>
<tr>
<td>10</td>
<td>Sohag</td>
<td>10.0</td>
</tr>
<tr>
<td>11</td>
<td>New Valley</td>
<td>11.0</td>
</tr>
<tr>
<td>12</td>
<td>New Valley</td>
<td>10.3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>L.S.D at 5%</td>
<td></td>
<td>2.04</td>
</tr>
</tbody>
</table>

Table (4): Pathogenicity tests of isolates of *Streptomyces scabiei* on Lady Rosetta cultivar of potato under open greenhouse conditions.

<table>
<thead>
<tr>
<th>Bacterial isolates No.</th>
<th>Disease severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32.33</td>
</tr>
<tr>
<td>2</td>
<td>69.33</td>
</tr>
<tr>
<td>3</td>
<td>44.67</td>
</tr>
<tr>
<td>4</td>
<td>52.67</td>
</tr>
<tr>
<td>5</td>
<td>31.67</td>
</tr>
<tr>
<td>6</td>
<td>73.33</td>
</tr>
<tr>
<td>7</td>
<td>40.67</td>
</tr>
<tr>
<td>8</td>
<td>72.00</td>
</tr>
<tr>
<td>10</td>
<td>38.67</td>
</tr>
<tr>
<td>11</td>
<td>35.33</td>
</tr>
<tr>
<td>12</td>
<td>46.67</td>
</tr>
<tr>
<td>13</td>
<td>46.00</td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
</tr>
<tr>
<td>L.S.D at 5%</td>
<td>6.2</td>
</tr>
</tbody>
</table>

3.3 Identification of the causal pathogen: Morphological, Physiological and biochemical studies

Data indicated that all tested isolates were filamentous (or spiral) shape, non-motile, sporing, Gram positive, gelatin liquefaction positive, starch hydrolysis positive, urease negative, catalase test positive, esculin hydrolysis positive antibiotic positive, no growth at both 4°C and 40°C, H$_2$S production negative, levan production negative, methyl-red test negative, phenyl alanine deaminase test negative and casein hydrolysis positive. On the basis of morphological, cultural, physiological, and pathological characteristics of the isolated bacteria it
was concluded that all tested isolates could be identified as *Streptomyces scabiei*.

### 3.4 Molecular comparison between pathogenic isolates

RAPD-analysis was performed to determinate the variation at molecular level among all the collected isolates. A total of 5 random ten-mer primers were used to perform the RAPD-PCR profiles. Results in Table (5) and Figures (1 and 2) obtained from RAPD analysis on the 12 isolates reveled that all of the used primers were able to produce bands and the total number of the amplified bands is 49, and that the number of the polymorphic bands is 45, thus the polymorphism percentage in all the primers is 91.8 %. The number of amplified bands varied between 7 to 14, and the size ranged from 247 to 2226 bp. OPA-12 primer reacted with all the isolates and produced the largest number of bands compared the other used primers. The total number of bands generated by this primer was of 14 and they were all polymorphic bands, which varied in length from 247 to 2013 bp, and the number of bands among the strains ranged between 2 and 6.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Total number of bands (a)</th>
<th>Number of polymorphic bands (b)</th>
<th>Polymorphism b/a × 100 (%)</th>
<th>Size range (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPA-12</td>
<td>14</td>
<td>14</td>
<td>100</td>
<td>247-2013</td>
</tr>
<tr>
<td>OPE-5</td>
<td>11</td>
<td>9</td>
<td>81.8</td>
<td>287-2226</td>
</tr>
<tr>
<td>OPM-13</td>
<td>7</td>
<td>6</td>
<td>85.7</td>
<td>407-1675</td>
</tr>
<tr>
<td>OPN-3</td>
<td>8</td>
<td>8</td>
<td>100</td>
<td>302-1640</td>
</tr>
<tr>
<td>OPP-2</td>
<td>9</td>
<td>8</td>
<td>88.8</td>
<td>412-2057</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>45</td>
<td>91.8</td>
<td>247-2226</td>
</tr>
</tbody>
</table>

Figure (1): Dendrogram demonstrating the relationship among the 12 isolates based on data recorded from polymorphism of RAPD markers.
Results in Table (5) shows that the fragments with molecular weight of 2013, 407 and 247 appeared only in isolates no. 9 and 10 respectively, so they can be used as positive marker for these isolates and if we check the regional distribution found strains ranged between 2 and 6 in Assiut and Aswan while 9 and 10 in Sohag governorate. Data in Table (5) illustrate results of RAPD-analysis obtained by OPE-5 primer. It was found that the total number of amplified fragments which generated by this primer was of 11. The polymorphism percentage
was (81.80 %) since bands sized 540bp and 383bp were produced in all the isolates. The number of the produced bands ranged between 2 and 7 among the isolates. This primer reacted with all the isolates and produced bands varied among the strains from 287 to 2229 bp. Isolate No. 3 produced 2 unique fragments with molecular weight of 2226, and 2049. While fragments with molecular weight of 439 and 287 bp appeared only in isolate No. 1 and 4 respectively, so they can be used as positive marker for these isolates which they indicate to the highest severe isolates. OPM-13 primer reacted with all the isolates. The total number of bands generated by this primer was of 7, which varied among the isolates from 2 to 6 “which they all in Aswan region” and ranged in length between 407 to 1675 bp as shown in Table (5).data also indicated that the polymorphic percentage generated by this primer was (85.7 %). The fragment which has the molecular weight of 929 was unique for isolate no. 6. The only negative marker (non-amplified fragment) among all the primers appeared when this primer was used. The negative marker band showed up with the molecular weight of 407 bp with isolate no. 9 which can be a regional marker for Sohag region. OPN-3 primer reacted with only 11 isolates and did not amplify any bands in isolate No. 11 generating a polymorphism percentage with a value of 100 %. RAPD-analysis using this primer was illustrated in Table (5). The primer formed 8 fragments ranged in size between 302 to 1640 bp. The number of generated bands varied from 0 to 4. The fragments which have molecular weight of 1031 and 576 bp were unique bands for isolates No. 4 and 5 respectively and they are the highest severe isolates from Aswan region. OPP-2 primer generated 9 different bands shown in Table (5), one of the bands (568 bp) appeared in all the isolates generating a polymorphism percentage of 88.8 %. The bands varied in length from 413 to 2057 bp, and the number of bands produced by the isolates ranged between 3. This primer did not generate any positive or negative markers with these isolates which can be also a regional marker for the Moderate severe isolates from Elminia region. Results of the presence / absence of DNA fragments (markers) phenotypically were analyzed using MVSP program of Nie and Li (1979), and pair-wise comparisons between the tested the 12 different isolates were used to calculate the genetic similarity Table (5). Then, based on the calculated genetic similarity, an estimation of the relationship between the different strains could be concluded. The 12 isolates of were grouped in 2 main clusters based on genetic similarities given in Table (5), and dendrogram as in Figure (1). The first cluster included the highest value of similarity within the isolates was of 81.1 % between isolate No. 9 and isolate No. 12, from Sohag and New valeey while the lowest genetic similarity within isolates was observed between isolate no.
9 belonging to Sohag Region and isolates whereas No.1, 2, 3, 4, 5, 6 and 7 belonging to Aswan, Elminia and Assiut region from incidence isolates with a value of 40 %. The values of similarity illustrated in Figure (1).

3.5 Reaction of certain potato cultivars to common scab disease under open greenhouse conditions

Data of this experiment were presented in Table (6) indicated that the tested potato cultivars varied for *S. scabiei* isolate No. 8 of their susceptibility Lady Rosetta cultivar exhibited the highest susceptible (62.67) followed by Cara (58.33). While Diamant, Mondial and Spunta cultivars showed the least degree of common scab symptoms.

Table (6): Reaction of certain potato cultivars to common scab disease under open greenhouse conditions.

<table>
<thead>
<tr>
<th>Potato cultivars</th>
<th>Disease severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cara</td>
<td>58.33</td>
</tr>
<tr>
<td>Spunta</td>
<td>30.67</td>
</tr>
<tr>
<td>Diamant</td>
<td>33.00</td>
</tr>
<tr>
<td>Lady Rosetta</td>
<td>62.67</td>
</tr>
<tr>
<td>Mondial</td>
<td>31.00</td>
</tr>
<tr>
<td>L.S.D at 5%</td>
<td>4.8</td>
</tr>
</tbody>
</table>

3.6 Role of certain potato tuber constituents in their resistance to bacterial common scab

Common scab disease susceptibility was investigated by role of total sugar, pectin, calcium, sodium, and potassium contents in tubers of potato.

3.6.1 Total sugars and pectin

Data in Table (7) showed that Cara, Diamant and lady Rosseta cultivars contained the highest concentration of total sugar by 3.036, 2.743 and 1.95 mg/kg respectively followed by Mondial with 1.65 (mg/kg). Whereas Spunta cultivar contained 1.613 (mg/kg) as the lowest total sugar contents. Data in Table (7) indicated that pectin substances varied in different cultivar that Lady Rosseta cultivar 0.68 % and Cara with 0.672 % as the highest concentration of pectin substances followed by Diamant with 0.493 % and Mondial with 0.466 %. While Spunta gave the lowest percentage of pectin substance with 0.433% as pectin percentage.

Table (7): Certain potato cultivars contents of sugar and pectin (mg/kg) (total tubers).

<table>
<thead>
<tr>
<th>Potato cultivars</th>
<th>Sugar (mg/kg)</th>
<th>Pectin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cara</td>
<td>3.063</td>
<td>0.672</td>
</tr>
<tr>
<td>Spunta</td>
<td>1.613</td>
<td>0.433</td>
</tr>
<tr>
<td>Diamant</td>
<td>2.743</td>
<td>0.493</td>
</tr>
<tr>
<td>Lady Rosetta</td>
<td>1.95</td>
<td>0.680</td>
</tr>
<tr>
<td>Mondial</td>
<td>1.65</td>
<td>0.466</td>
</tr>
<tr>
<td>L.S.D at 5%</td>
<td>0.107</td>
<td>0.044</td>
</tr>
</tbody>
</table>
3.6.2 Calcium, potassium and sodium contents

Data in Table (8) showed that different concentrations of calcium, sodium and potassium ions in potato cultivars Diamant cultivar contained the highest concentration of calcium mg/L with 0.105 mg/L followed by Lady Rosseta with 0.093, and Cara 0.090. Potassium ions contents in tested potato cultivars showed that Spunta, Lady Rosetta and Diamant cultivars contained the highest concentration with 2.333, 2.073 and 2.000 (mg/L) of potassium ions. While Mondial and Cara cultivars showed the lowest concentration of potassium ions with 1.543 and 1.330 mg/L. sodium ions concentration showed 1.280 in Mondial, 1.193 in Lady Rosseta and 1.173 in Spunta appeared as highest concentration, whereas Diamant showed 0.633, and Cara 0.455 mg/L to contain the lowest concentration of sodium ions.

Table (8): Certain potato cultivars contents of Ca**, K+ and Na+ (mg/L) (total tubers).

<table>
<thead>
<tr>
<th>Ions (mg/L)</th>
<th>Potato cultivars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cara</td>
</tr>
<tr>
<td>Ca++</td>
<td>0.090</td>
</tr>
<tr>
<td>K+</td>
<td>2.333</td>
</tr>
<tr>
<td>Na+</td>
<td>1.173</td>
</tr>
</tbody>
</table>

4. Discussion

Potato is one of most important cultivated crops worldwide, common scab disease of it is tuber can make unaffordable loss that affecting on the quality and market value of potato. In this study, will undertaken the variation between the isolates of the pathogen and reaction of some potato cultivars against common scab, role of certain potato tuber constituents on their resistance to bacterial common scab. Twelve isolates of S. scabiei collected from naturally infected potato tubers and from the infested soils which is the causal agent of common potato scab disease. The characteristics of causal pathogen isolates shown that all the obtained isolates were belonging to S. scabiei (Bencheikh and Setti, 2007; Lerat et al., 2009; Manome et al., 2008; Wanner, 2004). Pathogenicity of S. scabiei isolates carried out on mini potato tuber in the laboratory by inoculation of mini potato tubers indicated that all isolates were able to induce scab symptoms and on mini potato tubers after 14 days of inoculation by ability to produce necrosis on tuber potato. Isolates 2, 6, and 8 were highly virulent. While isolates 9 and 10 were moderately virulent. The results obtained are in line with (Babcock et al. 1993; Bukhalid et al., 1998; Dees et al., 2012; Goyer and Beaulieu, 1997; Leiner et al. 1996; Lindholm et al. 1997; Shin et al., 2002; Qu et al., 2008). Mini potato tubers tested by S. scabiei isolates, indicated that isolates of S. scabiei were able develop common scab symptoms on mini potato tubers, 14 days after inoculation (Couillerot et al., 2009; Singhai et al., 2011). Random amplified polymorphic DNA (RAPD) is an efficient method for discriminating and studying genetic variations in potato.
diversity of Streptomyces isolates using random primers (Malkawi et al., 1999). The obtained results of using four primers of arbitrary nucleotide sequences to amplify DNA segments for the genomic DNA of seven *S. scabiei* isolates showed different levels of polymorphism. 645 amplicons were produced by all primers. The number of amplified products produced was varied. These results were agreed with data of many researchers (Cullen et al., 2000; Kong et al., 2001; Malkawi et al., 1999). Tubers of Lady Rosetta cultivar appeared to be the highest susceptible cultivar followed by Cara, Diamont, Mondial and Spunta. Wanner (2009) reported that potato cultivars gave variable response to *S. scabiei*. Results showed that different contents of sugar and pectin affect the incidence of common scab disease by *S. scabiei* that in line with that reported by other researchers (Dastager et al., 2006; Faucher et al., 1995; Paradis et al., 1994). Data indicated that there is correlation between tuber contents of total sugars and common scab severity. That match with Goto (1981) who reported that tubers of scab-susceptible cultivars had more scab and a higher reducing sugar content than those of resistant cultivars. Calcium contents in potato cultivars affected the incidence of common scab disease (Conn et al., 1998; Funa et al., 2005; Gottlieb, 1961; Lindholm et al., 1997; Wanner, 2004). Results indicated that there is a positive correlation between tuber contents of potassium and calcium and common scab severity. Whereas there is no correlation between tuber contents of sodium and common scab severity. These results were disagreed with those reported by Davis et al. (1976) who reported that there is a negative correlation between potassium and scab severity and match with Davis et al. (1976) who reported that nutrient analysis of petioles collected early in season showed a highly significant positive linear correlation between calcium and potassium with common scab severity. Also, Horsfall et al. (1954) and Goto (1985) mentioned that increased tuber calcium was positively correlated with scab severity.

**References**


Bencheikh, M. and Setti, B. (2007), "Characterization of Streptomyces scabies isolated from common scab lesions on potato tubers by morphological, biochemical and pathogenicity tests in Chlef region in Western Algeria", *Sciences &


streptomycetes causing potato-
common scab in Korea", Plant Diseases, Vol. 87, pp. 1290–1296.

Qu, X., Wanner, L. A. and Christ, B. J. (2008), "Using the txtAB operon
Quantify pathogenic Streptomyces

Saghai-Maroof, M. A., Soliman, K. M.,
Jorgensen, R. A. and Allard, R. W.
L. (1984), "Ribosomal DNA spacer-
length polymorphisms in barley:
Mendelian inheritance, chromosomal location, and
population dynamics", Proceedings of the National Academy of
Sciences, Vol. 81 No. 24, 8014–8018.

Schaad, N. W. (1992), Laboratory guide
for identification of plant pathogen
bacteria, 2nd ed., International Book
Distributing Co., Lucknow, India, pp. 44–58.

Shin, P. G., Kim, J. S. and Hahm, Y. I.
(2002), "Rapid identification of
potato scab causing Streptomyces
spp. using pathogenicity specific
primers", Plant Pathology Journal,
Vol. 18, pp. 338–341.

Singhai, P. K., Sarma, B. K. and
Srivastava, J. S. (2011), "Biological
management of common scab of
potato through Pseudomonas species
and vermicompost", Biological

Thomas, W. and Dutcher R.A. (1924),
The determination of carbohydrate
in plants by picric acid reduction
method, The estimation of reducing
sugar and sucrose", Journal of
American Chemistry Science, Vol.
46 No. 6, pp. 162–166.

Wanner, L. A. (2004), "Field isolates of
Streptomyces differ in pathogenicity
and virulence on radish", Plant

Wanner, L. A. (2009), "A Patchwork of
Streptomyces species isolated from
potato common scab lesions in
North America", American Journal
247–264.

Wen, C., Zheng, D., Shen, S., Chen, J.,
Liu, W. and Liu, T. (2012),
"Streptomyces scabiei subsp.
xuchangensis, A novel Streptomyce
isolate for staurosporine
production and a wheat take-all
control agent", International Journal
of Microbiology Research, Vol. 4
No. 7, pp. 282.

Williams, J. G. K., Kubelik, A. R., Livak,
J., Rafalski, J. A. and Tingey, S. V.
(1990), "DNA polymorphisms
amplified by arbitrary primers are
useful as genetic markers", Nucleic
Acids Research, Vol. 18, pp. 6531–
6535.