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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, 7 were pertained to Aswan, Elminia and Assiut regions with similarity value of 40 %. The response of various potato cultivars to common scab infection was studied at greenhouse condition. 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. Total sugar was recorded the highest concentration by Cara cultivar. The highest concentration of pectin components was found in Lady Rosseta with 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.

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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 pathoge *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). Based on DNA relatedness and the 16s rDNA sequence, *S. scabiei* were recently designated to three different genomic species *S. europaeiscabiei,* and *S. stelliscabiei.* (Bukhalid *et al*., 2002). Bukhalid *et al.* (1998) mentioned that the necl gene codes for a secreted necrogenic protein with an unknown plant cell target that has been found to impose a pathogenic phenotype on the nonpathogenic *S. lividans*. necl's G + C content (54%) is typical of high G + C coding regions found in Streptomyces strains, implying that it was obtained through horizontal transfer from another taxon. The necl gene also is physically and functionally consistent among unrelated pathogen Streptomyces species, but lacking in nonpathogenic Streptomyces species from different geographic area (Bukhalid *et al*., 2002; Park *et al*., 2003). Furthermore, in pathogen strains of *S. scabiei, S. acidiscabies,* and *S. turgidiscabies*, there is a considerable correlation with thaxtomin A synthesis and the presence of necl (Bukhalid *et al*., 1998). The necl protein, on the other hand, is not required for thaxtomin formation and hence functions as a standalone virulence factor (Kers *et al*., 2005). According to research, there is an association seen between amounts of particular tuber ingredients and their susceptibility to common scab disease. Calcium, potassium, and manganese, among other constituents, have been suggested by many researchers (Davis *et al*., 1976), also Goto (1981) found that reducing sugars could help with disease resistance. This study was designed to investigate the differences between pathogen isolates, molecular comparison between Isolates of common scab, molecular confirmation of causal pathogens of common scab diseases. As well as reaction of constituents on the resistance of various potato cultivars to bacterial causal pathogen of common scab disease.

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 pathogenic bacteria that promote common scab on potato tubers were identified using Schaad's proposed morphological, cultural, and physiological features (1992) and 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-Maroof *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-mer random Oligonucleotide primers. The RAPD assays used the polymerase chain reaction (PCR) to amplify random sites throughout the genomic DNA. The DNA amplification procedure utilised as modified from Williams *et al.* (1990).

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 employed in this research. Table (1) shows their codes and sequences.

Table (1): Primer sequences and codes were utilised to investigate the differences between isolates.

Serial No.	Primer codes	Sequence (5’ to 3’)
1	OPA-12	TCGGCGATAG
2	OPE-5	TCAGGGAGGT
3	OPM-13	GGTGGTCAAG
4	OPN-3	GGTACTCCCC
5	OPP-2	TCGGCACGCA

2.4.4 Preparation of PCR reactions

To perform the RAPD-PCR analysis, the PCR reaction and program were performed as shown in Table (2).

2.4.5 Data analysis

The Gene Profiler 4.03 computer software application was used to scan agarose gel pictures, which uses automatic lane and peak discovery to detect the existence of banding patterns. A binary data matrix recording the presence (1) or absence (0) of bands was created to compute the bands molecular weight. Then, using the Dice coefficient of similarity of Nei and Li, the software application MVSP

(Multi-Variate Statistical Package) was utilised to calculate the genetic similarities (Nei and Li, 1979). The cluster analysis was performed using the unweighted pair-group method with arithmetic average (UPGMA) software and derived similarity estimates. For allover primers, the results were then shown as a dendrogram.

2.5 Reaction of certain potato cultivars to common scab disease

Under greenhouse conditions, five potato cultivars were tested for susceptibility to common scab disease: Cara, Spunta, Diamant, Mondial, and Lady Rosetta. Healthy potato tubers were planted in sterilized 30-cm2-diameter pots holding a 7-kg sterile 1:1 soil/sand combination. Before planting, potato tubers were surface disinfected by soaking them in 0.5 percent sodium hypochlorite for 5 minutes and then rinsing them twice in sterilized water. Fifty ml 10^8 Before planting, each pot received 50 ml of 108 CFU/ml of ten-day-old culture from isolate No. (6). For each isolate, five replicate pots were employed. Five replicates of a complete randomized block design were used to place the pots. With five replicates, pots were put in a totally randomized block configuration. Tap water was used to treat the control plants in the same way.

2.5.1 Pectin contents

According to Badour, the pectin content in potato tubers was determined using anthrone sulphuric acid techniques (1959).

2.5.2 Sugar contents

Sugar contents was measured according to Thomas and Dutcher, (1924). According to Jackson, sodium and potassium were measured using a flame photometer (CL378-ELICO), whereas calcium was measured using an Atomic Absorption Spectrophotometer (1973).

Table (2): The used components and program to perform the RAPD-PCR analysis.

PCR reaction		RAPD-PCR Program	
Component	Amount		
dH_2O	9.5 µl	95°C, 3 minutes	
10X buffer	3.0 µl	30×	95°C, 30 seconds
dNTP's	3.0 µl		30°C, 30 seconds
Primer	2.5 µl		72°C, 2 minutes
Taq polymerase	0.5 µl	72°C, 5 minutes	
$MgCl_2$	4.0 µl	4°C, ∞	
Template DNA	2.5 µl		
Total volume	25.0 µl		

3. Results

3.1 Isolation and pathogenicity test of the causal pathogen

Naturally diseased potato tubers with typical signs of common scab disease were collected from various locations in the governorates of Assiut, New Valley, Sohag, Aswan, Behira, and Elminia providing twelve isolates with the severe symptoms. 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 most higher 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.

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

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

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

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

All of the isolates tested were filamentous (or spiral) in 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 4ºC and 40ºC, H_2S production negative, levan production negative, methyl-red test negative, phenyl alanine deaminase test negative, and casein hydrolysis. The isolated bacteria's morphological, cultural, physiological, and pathological properties led to the conclusion that all of the isolates tested were *S. scabiei*.

3.4 Molecular comparison between pathogenic isolates

RAPD-analysis were performed to determinate the variation at molecular level among all the collected isolates. Total of 5 random ten-mer primers were used to perform the RAPD-PCR profiles. Results in Table (5) and Figure (1, 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 bandsis 45, thus the polymorphism percentage in all the primers is 91.8 %. The amount of bands that were amplified 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. This primer produced 14 polymorphic bands in total, which varied in length from 247 to 2013 bp, the number of bands varied amongst the strains, ranging from 2 to 6.

Table (5): Polymorphism obtained by RAPD-analysis among all the strains.

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

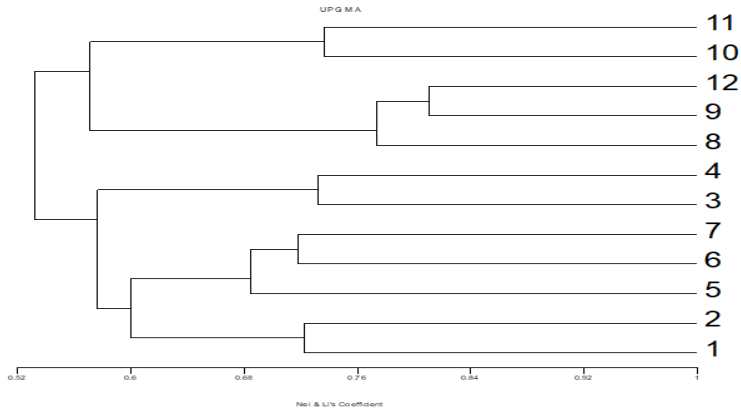

Figure (1): Dendrogram demonstrating the relationship among the 12 isolates based on data recorded from polymorphism of RAPD markers.

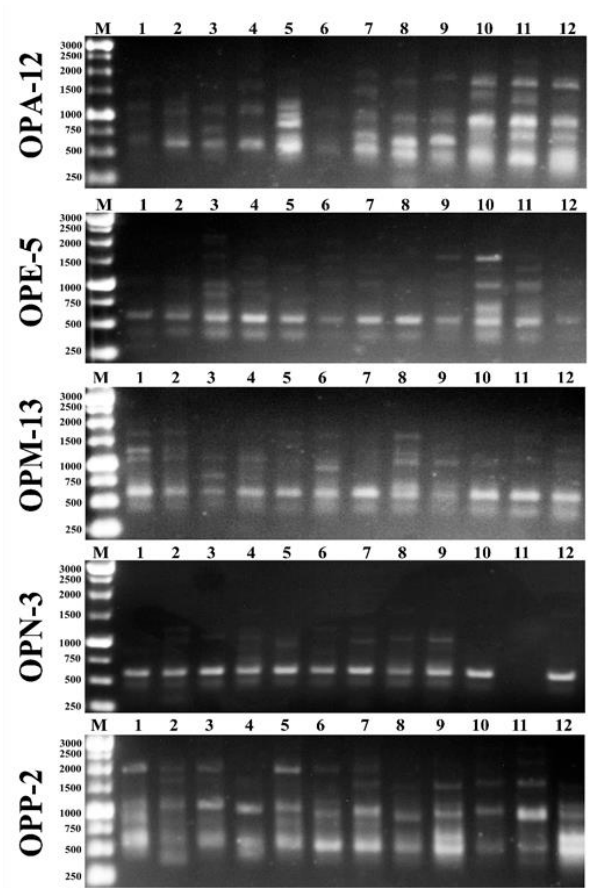

Figure (2): Agarose gel electrophoresis of the RAPD-PCR bands produced by the 5 different primers on all the bacterial isolates.

Results in Table (5) shows that the fragments with molecular weight of 2013, 407and 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, Egypt. Data in Table (5) show the results of the RAPD analysis that was carried by OPE-5 primer. It was found that the number of total amplified fragments which primer's output was of 11. The polymorphism percentage was (81.80 %) since bands sized 540 bp and 383 bp were produced in all the isolates. Among the isolates, the number of generated bands varied between 2 and 7. All of the isolates responded with this primer 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 produced by this primer was seven, 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 407bp 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 size ranged 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 bands number 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. The MVSP program (Nie and Li, 1979) was used to examine the results of the presence / absence of DNA fragments (markers) phenotypically, and pair-wise comparisons between the 12 distinct isolates were utilized to calculate the

genetic similarity (Table 5). The relationship between the different strains might then be estimated based on the determined genetic similarity. 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 similarity highest value within the isolates was of 81.1 % between isolate no. 9 and isolate no. 12, from Sohag and New Valley 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 The reaction of some potato cultivars to the common scab disease under greenhouse conditions

The results of this experiment were provided in Table (6), which showed that the susceptibility of the tested potato cultivars to *S. scabiei* isolate No. 8 varied. The cultivar Lady Rosetta was the most sensitive (62.67), followed by Cara (58.33). The cultivars Diamant, Mondial, and Spunta had the least amount of common scab symptoms.

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

Potato cultivars	Disease severity (%)
Cara	58.33
Spunta	30.67
Diamant	33.00
Lady Rosetta	62.67
Mondial	31.00
L.S.D at 5%	4.8

3.6 The role of various potato tuber components in bacterial common scab resistance

The influence of total sugar, pectin, calcium, sodium, and potassium concentrations in potato tubers in susceptibility to common scab disease was examined.

3.6.1 Total sugars and pectin

Data in Table (7) showed that Cara, Diamant and lady Rosseta cultivars The maximum concentration of total sugar was found 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.

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

Potato cultivars	Sugar (mg/kg)	Pectin (%)
Cara	3.063	0.672
Spunta	1.613	0.433
Diamant	2.743	0.493
Lady Rosetta	1.95	0.680
Mondial	1.65	0.466
L.S.D at 5%	0.107	0.044

According to the data in Table (7), pectin components differed by cultivar, with Lady Rosseta cultivar 0.68 percent and Cara cultivar 0.672 percent having the highest concentrations, followed by Diamant 0.493 percent and Mondial 0.466 percent. Spunta, on the other hand, has the lowest percentage of pectin component, with 0.433 percent.

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 Rosetta 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).

Potato cultivars / Ions (mg/L)	Cara	Spunta	Diamant	Lady Rosetta	Mondial	L.S.D
Ca++	0.090	0.080	0.105	0.093	0.078	0.01
K+	1.330	2.333	2.000	2.073	1.543	0.248
Na+	0.455	1.173	0.633	1.193	1.280	0.109

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. The variation between pathogen isolates and the reactivity of specific potato cultivars to common scab, as well as the involvement of particular potato tuber ingredients in their resistance to bacterial common scab, investigated in this study. Twelve isolates of *S. scabiei* collected from naturally infected potato tubers and from the infested soils which is the pathogenic causal 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 and after 14 days of inoculation, all isolates were able to generate scab signs on small potato tubers by ability to produce necrosis on tuber potato. Isolates 2, 6, and 8 were highly virulent. Isolates 9 and 10 on the other hand, were moderately virulent. The findings are consistent 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). Using random primers, random amplified polymorphic DNA (RAPD) is an effective approach for discriminating and investigating the genetic diversity of Streptomyces isolates (Malkawi *et al.,* 1999). The results of amplification of DNA segments for the genomic DNA of seven *S. scabiei* isolates employing four primers of arbitrary nucleotide sequences revealed varying levels of polymorphism. All primers yielded a total of 645 amplicons. The quantity of amplified products that were created varied. Many researchers' data corroborated these findings (Cullen *et. al*., 2000; Kong *et al.,* 2001; Malkawi *et al.,* 1999). Lady Rosetta tubers appeared to be the most vulnerable, followed by Cara, Diamont, Mondial, and Spunta tubers. Wanner (2009) found that different potato cultivars had different responses 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 the amount of total sugars in the tuber and the severity of common scab were found to be related. This agrees with Goto (1981), who found that tubers from scab-prone cultivars had more scab and a higher reducing sugar concentration than tubers from 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 discovered that there a correlation between potassium and calcium concentration in tubers and the severity of common scab. There is no correlation between sodium content in tubers and the severity of common scab. These findings were in contrast to those of Davis *et al*. (1976) who found a negative connection between potassium and scab severity and match with Davis *et al.* (1976) who observed that nutrient analysis of petioles obtained early in the season revealed a highly significant positive linear connection between calcium and potassium and the severity of common scab. Also, Goto (1985) increased tuber calcium was significantly associated with the severity of scab.

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