African Journal of Biotechnology Vol. 11(1), pp. 37-45, 3 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2709
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Molecular characterization of early blight disease resistant and susceptible potato cultivars using random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) markers

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Accepted 9 December, 2011

Potato early blight disease caused by *Alternaria solani* is one of the major factors limiting potato production worldwide. Developing highly resistant cultivars is the most effective way to control the disease. In this study, 20 random amplified polymorphic DNA (RAPD) and 6 simple sequence repeats (SSR) primers were applied to assess the genetic diversity and to identify molecular markers associated with resistance to early blight disease in resistant (Cara, Spunta and Valor), moderately resistant (Hermes and Atlantic) and susceptible (Atlas, Desiree and Lady-Rosetta) potato cultivars. The results showed that 296 and 29 clear fragments were amplified by RAPD and SSR, respectively. SSR revealed higher frequency of polymorphic bands (93.1%) than RAPD (57.4%). The clustering pattern obtained from the analyses of two marker systems showed a similar distribution of the tested potato cultivars. Interestingly, the resistant potato cultivars were grouped together in the dendrograms generated from RAPD and SSR clustering analyses. RAPD primers OPY-07, OPG-05, OPA-10 and OPA-18 generated five unique fragments (OPY-07$_{880bp}$, OPG-05$_{949bp}$, OPA-10$_{1000bp}$, OPA-18$_{2030bp}$ and OPA-18$_{1128bp}$) presented only in resistant cultivars. In addition, all resistant cultivars and moderate resistant cultivar (Hermes) shared two markers; one RAPD (OPA-18$_{1070bp}$) and the other SSR (STI57$_{209bp}$). On the other hand, RAPD primer UBC-17 generated specific fragment UBC-17$_{720bp}$ presented only in susceptible cultivars and moderate resistant cultivar (Atlantic). This study demonstrate that RAPD and SSR markers can be effectively used to assess the genetic variation among potato cultivars in order to develop molecular markers associated with early blight disease to improve potato breeding programs.

Key words: *Alternaria solani*, cluster analysis, resistance, *Solanum tuberosum.*

INTRODUCTION

Potato early blight caused by *Alternaria solani* Sorauer, is one of the most destructive fungal foliar diseases in many potato (*Solanum tuberosum*) growing regions (Pelletier and Fry, 1989; Shtienberg et al., 1990; Christ, 1991; Van der Waals et al., 2003). The disease causes severe yield-losses through defoliation of potato plants (Pelletier and Fry, 1989; Shtienberg et al., 1990; Christ, 1991; Van der Waals et al., 2003). Potato growers use fungicides to control potato early blight disease, but the increased environmental regulations and the development of fungicides-resistant fungal isolates are restricting the available chemicals to control the disease (Holm et al., 2003).

The use of resistant cultivars is considered the most efficient, cost effective and environmentally more

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acceptable means of controlling plant diseases (Dorrance and Inglis, 1997; Rodríguez et al., 2007). Based on the careful selection of parents and the improvement of heterosis utilization, development of potato varieties resistant to early blight remain a necessity to control this fungal devastative disease (Lambert et al., 2005; Pinto et al., 2002). Quantification of genetic diversity present within potato cultivars by molecular markers would be of great help to improve the inheritability and durability of disease resistance through selection of efficient and diverse combination of parents. Therefore, to accelerate the introgression of resistance into the potato genome, molecular markers tightly linked to resistance traits are needed (Bisognin and Douche, 2002a, 2002b; Pattanayak et al., 2002; Khampila et al., 2008; El_Komy, 2010). Among such molecular markers are the random amplified polymorphic DNA (RAPD) and microsatellites or simple sequence repeats (SSR) that have rapidly gained popularity in detecting polymorphisms among different potato genotypes. (Milbourne et al., 1997; Bisognin and Douches, 2002a, 2002b; Hong et al., 2006; Siri et al., 2009; Abou-Taleb et al., 2010; Kandemir et al., 2010).

The main objectives of the present study were to; (1) evaluate the genetic diversity of certain potato cultivars showing different levels of resistibility/susceptibility to early blight disease using two different PCR-based technologies, RAPD and SSR and (2) correlate the RAPD and SSR molecular markers to early blight resistibility/susceptibility treat. Such information could lead to the development of more reliable methods for improving the potato breeding programs against early blight disease.

MATERIALS AND METHODS

Evaluation of early blight resistance in certain potato cultivars

Plant material and growing conditions

Eight potato cultivars (Atlas, Atlantic, Cara, Desiree, Hermes, Lady-Rosetta, Spunta and Valor) were used in this study. These were obtained from the International Potato Center (CIP), Kafr El-zayat, Egypt. Potato seed tubers were grown in clay pots containing soil: sand: peat moss mixture at a ratio of 4:4:1, respectively. The plants were kept in a growth chamber at 18°C for 16 h photoperiod for six weeks prior flowering stage.

Fungal cultures and inoculum preparation

Two aggressive *A. solani* isolates (As-3 and As-5) were isolated from potato plants showing early blight symptoms. Fungal isolates were grown on potato dextrose agar (PDA) plates and incubated at 25°C for 7 to 10 days. Then, cultures were grown for 6 days at 25°C on PDA under near-ultraviolet light (310 to 400 nm) with 16 h day^{-1} light to induce sporulation. Conidia were collected by washing plates with sterile water and the resulting spore suspension was adjusted to concentration of 1×10^5 spores ml^{-1} using the haemocytometer (Hadis et al., 2011).

Plant infection

Early blight resistance in potato cultivars was evaluated using a droplet inoculation method under glasshouse conditions according to Rodriguez et al. (2006). Potato plants at the beginning of flowering stage (45 to 50 days old) were inoculated by placing 10 µL of conidial suspension (1×10^5 spores ml^{-1}) per leaflet. In the case of blank treatments, 10 µL of sterilized distilled water per potato leaflet were used. At least 50 leaflets (on nodes no. 5 to 8) representing three potato plants from each cultivar were used for each fungal isolate. To ensure the adherence of conidial suspension at the inoculation site on the leaflet surfaces, the conidial suspension was supplemented with 1% gelatin. After inoculation, plants were kept for 24 h in a moist chamber at 25°C at 12 h photoperiod. After this time, plants were transferred to the greenhouse. Disease development was estimated by measuring the diameter of lesion extension in cm (LE) 10 days after inoculation.

Genetic diversity among potato cultivars

Potato genomic DNA extraction

Genomic DNA was extracted from young leaves according to Doyle and Doyle (1987). Potato leaf tissue was processed by freezing with liquid nitrogen and ground into fine powder using a mortar and pestle. Approximately 100 mg of fine powder were transferred to 1.5 ml micro-centrifuge tube and 600 µL of 65°C-preheated modified CTAB extraction buffer [100 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 2% CTAB (hexadecyltrimethylammonium bromide), 20 mM EDTA (sodium salt, pH 8.0). Tubes were vortexed for 1 to 3 s and incubated for 60 to 90 min in water bath at 65°C. Afterward, the tubes were allowed to cool to room temperature for 5 min. A volume of 700 µL chloroform: isoamylalcohol (24:1) was added to each tube and then the tubes were gently mixed for 5 to 10 min. The mixtures were centrifuged at 4°C for 10 min at 8000 ×*g*. 600 µl of upper aqueous layer were transferred to a clean 1.5 ml micro-centrifuge tube. The DNA was then precipitated by adding 600 µL of isopropanol and the tubes either left for 15 min at room temperature or overnight at -20°C.

To pellet the DNA, the tubes were centrifuged at 5000 ×*g* for 2 min at 4°C. The supernatant was decanted and the DNA pellet was washed with 600 µL of 70% ethanol. The tubes were centrifuged at 3000 ×*g* for 2 min at 4°C.The ethanol was carefully aspirated and the DNA pellets were dried by leaving the tubes for 15 min at room temperature. DNA pellet was re-suspended in 100 µL TE [10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)] and stored at -20°C. The quality and quantity of the DNA were assessed using 1% agarose gels (Sambrook et al., 1989).

RAPD analysis

RAPD reactions were performed according to Williams et al. (1990). All PCR reactions were carried out in a final volume of 25 µL containing: 1µL 10 µM primer, 0.3 µL *Taq* DNA polymerase (5 U μL^{-1}), 2.5 µL 10× PCR buffer (containing 15 mM Mg Cl_2), 1 µL 10 mM $MgCl_2$, 1 µL 2 mM dNTPs (of each), 1 µL of template DNA (approximately 50 ng) and 18.2 µL sterilized deionized water. The sequences of the RAPD primers used in this study are shown in Table 1.

RAPD reactions were conducted in a Techne TC-412 thermocycler (Barloworld Scientific Ltd, United Kingdom). RAPD-PCR program was as follows: one cycle of 94°C for 5 min as initial denaturation, 40 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min and a final extension cycle at 72°C for 10 min. The PCR products were analyzed by electrophoretic separation in 1.5% agarose gels stained with 0.5 µg ml^{-1} ethidium bromide. Mid range

Table 1. RAPD primer sequences, total number of amplified bands, number of polymorphic bands and their percentages.

Primer name	Primer sequence	Total number of band	Number of polymorphic band	Polymorphism (%)
OPA-02	5'-TGCCGAGCTG-3'	14	9	64.3
OPA-03	5'-AGTCAGCCAC-3'	9	2	22.2
OPA-04	5'-AATCGGGCTG-3'	18	12	66.7
OPA-0 5	5'-AGGGGTCTTG-3'	13	5	38.5
OPA-10	5'-GTGATCGCAG-3'	13	9	69.2
OPA- 11	5'-CAATCGCCGT-3'	21	17	81.0
OPA-12	5'-CAGCACCCAC-3'	11	1	09.1
OPA-18	5' AGGTGACCGT-3'	15	7	46.7
OPB-05	5'-TGCGCCCTTC-3'	15	8	53.3
OPB-09	5'-TGGGGGACTC-3'	12	6	50.0
OPB-19	5'-ACCCCCGAAG-3'	26	20	76.9
OPH-01	5'-GGTCGGAGAA-3'	15	7	46.7
OPH-04	5'-GGAAGTCGCC-3'	13	6	46.2
OPH-09	5'-TGTAGCTGGG-3'	15	12	80.0
OPG-05	5'-CTGAGACGGA-3'	15	10	66.7
OPP-08	5'-ACATCGCCCA-3'	12	6	50.0
OPY-07	5'-AGAGCCGTCA-3'	12	5	41.7
OPZ-11	5'-CTCAGTCGCA-3'	21	14	66.7
UBC-04	5'-CCTGGGTGGA-3'	12	10	83.3
UBC-17	5'-CCTGGGCCTC-3'	12	4	33.3
Total		294	170	57.8
Mean		14.7	8.5	-

DNA Ladder (Jena Bioscience, place, Germany) with size marker ranging from 100 to 3000 bp was used as a molecular size standard.

SSR analysis

SSR reactions were generated according to Siri et al. (2009) with some modifications. The 25 µL reaction volume contained 1 µL 10 µM of each primer, 0.15 µL *Taq* DNA polymerase (5U μL^{-1}), 2.5 µL 10× PCR buffer, 1 µL 2 mM dNTPs (of each), 1 µL template DNA (approximately 50 ng) and 18.35 µL sterilized deionized water. The sequences and annealing temperatures of the SSR primers are shown in Table 2.

The SSR-PCR program was as follows 7 min as initial denaturation step at 94℃ followed by 40 cycles of 45 s. at 94℃, 45 s. at 47 to 60℃ (depending upon SSR primer-pair), and 90 s at 72℃ and a final extraction step of 5 min at 72℃. SSR-PCR reactions were carried out in Techne TC-412 thermocycler (Barloworld Scientific Ltd, United Kingdom). PCR products were analyzed on 3% agarose gels stained with 0.5 µg ml^{-1} ethidium bromide. Mid range DNA Ladder with size marker ranged from 100 to 3000 bp was used as a molecular size standard.

Data handling and cluster analysis

In order to test the reproducibility of the two marker systems, the RAPD and SSR reactions were repeated at least twice. Amplified fragments generated from RAPD or SSR fingerprints were scored as 1 for the presence of a band and 0 for its absence. It was assumed that the bands with the same size were identical. Pair-wise genetic comparisons based on RAPD and SSR fingerprints among potato genotypes were calculated using Jaccard's similarity coefficient embedded in NTSYS-pc Software (Rolf, 1993). Phylogenetic trees were constructed using unweighted pair-group method with arithmetical averages (UPGMA) embedded in NTSYS.

RESULTS

Evaluation of early blight resistance in certain potato cultivars

Two different isolates of *A. solani* were chosen for the evaluation of early bight resistance in eight potato cultivars (Atlas, Atlantic, Cara, Desiree, Hermes, Lady-Rosetta, Spunta and Valor) using a droplet inoculation method under glasshouse conditions. Disease development was estimated as diameter of lesion extension (cm) within 10 days after inoculation. Based on the lesion extension, Cara, Valor, and Spunta potato cultivars showed high resistance to the two isolates of *A. solani*. Potato cultivars that showed moderate resistance were Hermes and Atlantic with average lesion diameters 0.79. The lowest resistance was obtained in Atlas (1.41), Diseree (1.46) and Lady-Rosetta (1.51) (Table 3).

Genetic diversity among potato cultivars

RAPD analysis

RAPD-PCR was used to assess the genetic diversity

Table 2. SSR primer-pair sequences, annealing temperatures and PCR amplicons description.

SSR primer-pair	Primer sequence	Annealing temperature °C	Total number of band scored	Number of polymorphic bands	Polymorphism (%)
STM0007	5'-GACAAGCTGTGAAGTTTAT-3' 5'-AATTGAGAAAGAGTGTGTGTG-3'	55	4	3	75
STM0019	5'-AATAGGTGTACTGACTCTCAATG-3' 5'-TTGAAGTAAAAGTCCTAGTATGTG-3'	47	7	7	100
STM1052	5'-CAATTTCGTTTTTTCATGTGACAC-3' 5'-ATGCGCTAATTTGATTTAATACGTAA-3'	50	5	4	80
STI24	5'-CGCCATTCTCTCAGATCACTC-3' 5'-GCTGCAGCAGTTGTTGTTGT-3'	60	5	5	100
STI30	5'-TTGACCCTCCAACTATAGATTCTTC-3' 5'-TGACAACTTTAAAGCATATGTCAGC-3'	60	3	3	100
STI57	5'-CCTTGTAGAACAGCAGTGGTC-3' 5'-TCCGCCAAGACTGATGCA-3'	60	5	5	100
Total			29	27	93.10
Mean			4.8	3.85	-

Table 3. Diameter of radial growth in cm (lesion extension) related to leaflets inoculated with *A. solani* isolates on different potato cultivars ten days post-inoculation.

Fungal isolate	Potato cultivar[b]								Mean[c]
	1	2	3	4	5	6	7	8	
As-3	1.51*	0.79	0.46	0.51	0.79	1.48	0.41	1.37	0.92[A]
As-5	1.52	0.79	0.48	0.52	0.80	1.47	0.43	1.45	0.93[A]
Mean[d]	1.51[a]	0.79[d]	0.47[f]	0.51[e]	0.79[d]	1.47[b]	0.42[g]	1.41[c]	-
Infection reaction[e]	S	M	R	R	M	S	R	S	

Mean of 50 leaflet replicates. [b]Potato cultivars: 1, Lady-Rosetta; 2, Hermes; 3, Valor ; 4, Spunta ; 5, Atlantic ; 6, Desiree; 7, Cara; 8, Atlas; [c]main effect of each *A. solani* isolate; [d]main effect of potato cultivars; [e] infection reaction; R, resistant cultivar; M, moderately resistant cultivar; S, susceptible cultivar; L.S.$D_{0.05}$ for interaction= 0.053. Values followed by the same letter (s), are not significantly different at P=0.05.

among the eight potato cultivars, and the results indicated that these varied in their resistibility/susceptibility against the early blight pathogen. All RAPD-PCR reactions were repeated at least twice. The obtained results showed that each primer generated distinct RAPD patterns. The twenty RAPD primers used to fingerprint the eight potato cultivars generated 296 bands ranging from 152 to 2790 bp. Out of these 296 bands, 170 (57.4%) were polymorphic. The polymorphism ratios for the 20 decamer primers ranged from 9.1 to 83.3%. The primer UBC-04 had the highest polymorphism ratio, whereas the primer OPA-12 had the lowest polymorphism ratio (Table 1). RAPD primers OPY-07, OPG-05, OPA-10 and OPA-18 generated five unique fragments (OPY-07_{880bp}, OPG-05_{949bp}, OPA-10_{1000bp}, OPA-18_{2030bp}, and OPA-18_{1128bp}) that were present only in resistant cultivars (Figure 1a). The primer OPA-18 generated OPA-18_{1070bp} fragment detected in the resistant cultivars, as well as the moderate resistant cultivar Hermes. On the other hand, the primer UBC-17 produced a fragment (UBC-17_{720bp}) that was found in the susceptible potato cultivars (Lady- Rosetta, Desiree and Atlas), in addition to moderate potato cultivar Atlantic (Figure 1b).

Data in Table 4 show the pair-wise similarity matrix based on RAPD fingerprints using Jaccard's similarity coefficient. The genetic similarities among the eight potato cultivars ranged from 0.410 to 0.826 with an average of 0.708. The highest pair-wise genetic similarities were seen between the potato resistant cultivars. The cluster analysis showed that the eight potato cultivars were divided into two clusters (Figure 2). The

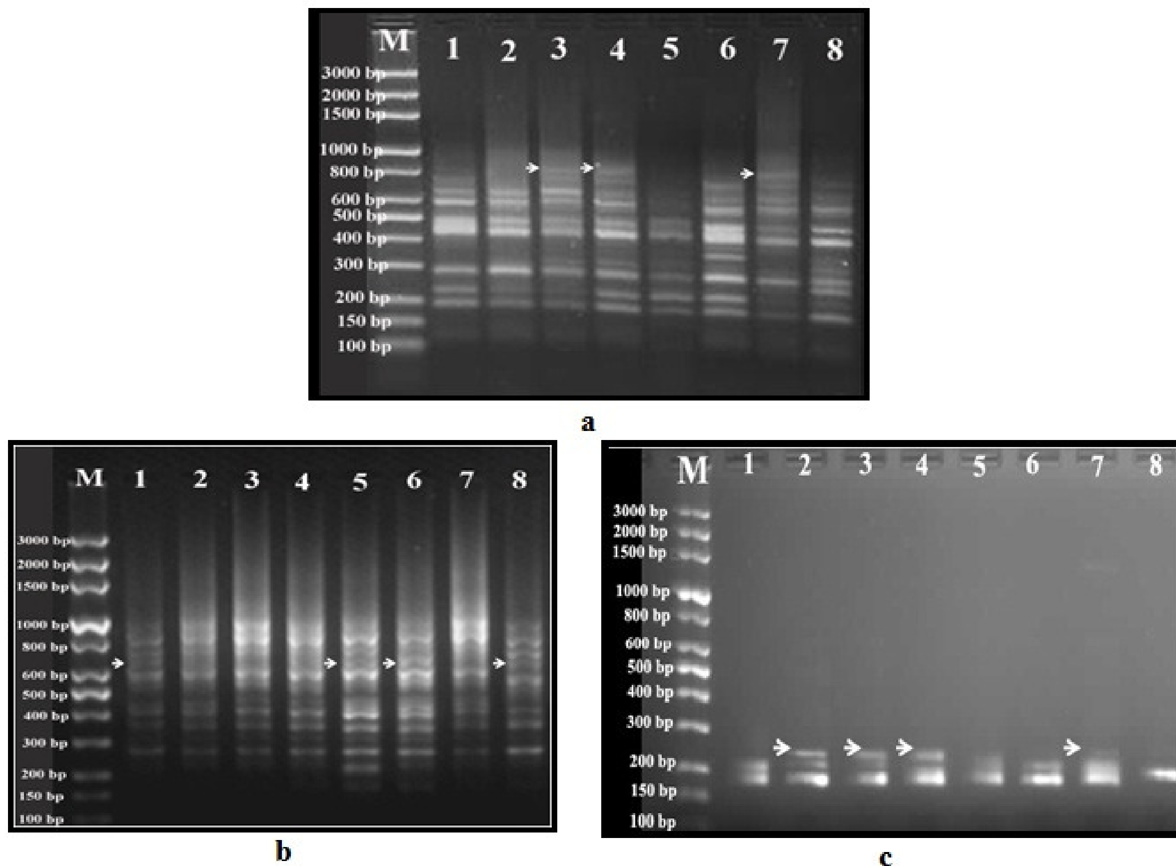

Figure 1. RAPD and SSR patterns for the eight potato cultivars varied in their resistibility/susceptibility against early blight pathogen, generated by 10-mer random primers OPY-7 (a); UBC-17 (b) and SSR primer STI57 (c). Potato cultivars: 1, Lady-Rosetta; 2, Hermes; 3, Valor; 4, Spunta ; 5, Atlantic; 6, Desiree; 7, Cara; 8, Atlas. White arrows indicate unique fragments OPY-7_{880bp} (a); UB C-17_{720bp} (b) and $STI57_{209bp}$ (c).

Table 4. Pair-wise genetic similarities based on RAPD markers among the potato cultivars that varied in their resistibility/susceptibility against early blight pathogen.

Potato cultivar[a]	1	2	3	4	5	6	7	8
1	1.0000							
2	0.7509	1.0000						
3	0.7414	0.8054	1.0000					
4	0.7537	0.7803	0.8257	1.0000				
5	0.6573	0.6370	0.6240	0.6937	1.0000			
6	0.7817	0.7442	0.7323	0.8223	0.7306	1.0000		
7	0.7354	0.7874	0.7816	0.7640	0.6406	0.7328	1.0000	
8	0.6454	0.6406	0.4122	0.6964	0.6973	0.7183	0.6760	1.0000

[a]Potato cultivars: 1, Lady-Rosetta; 2, Hermes; 3, Valor ; 4, Spunta ; 5, Atlantic; 6, Desiree; 7, Cara; 8, Atlas.

first cluster was subdivided into two groups (G1 and G2). Group 1 included only the early blight susceptible cultivars Lady-Rosetta and Desiree, while group 2 contained the moderate resistant cultivar, Hermes, and the early blight resistant cultivars, Cara, Spunta and Valor. The second cluster included the moderate resistant cultivar, Atlantic, and the susceptible potato, Atlas.

SSR analysis

Six SSR primers were also used to assess the genetic diversity among the eight potato cultivars. The total

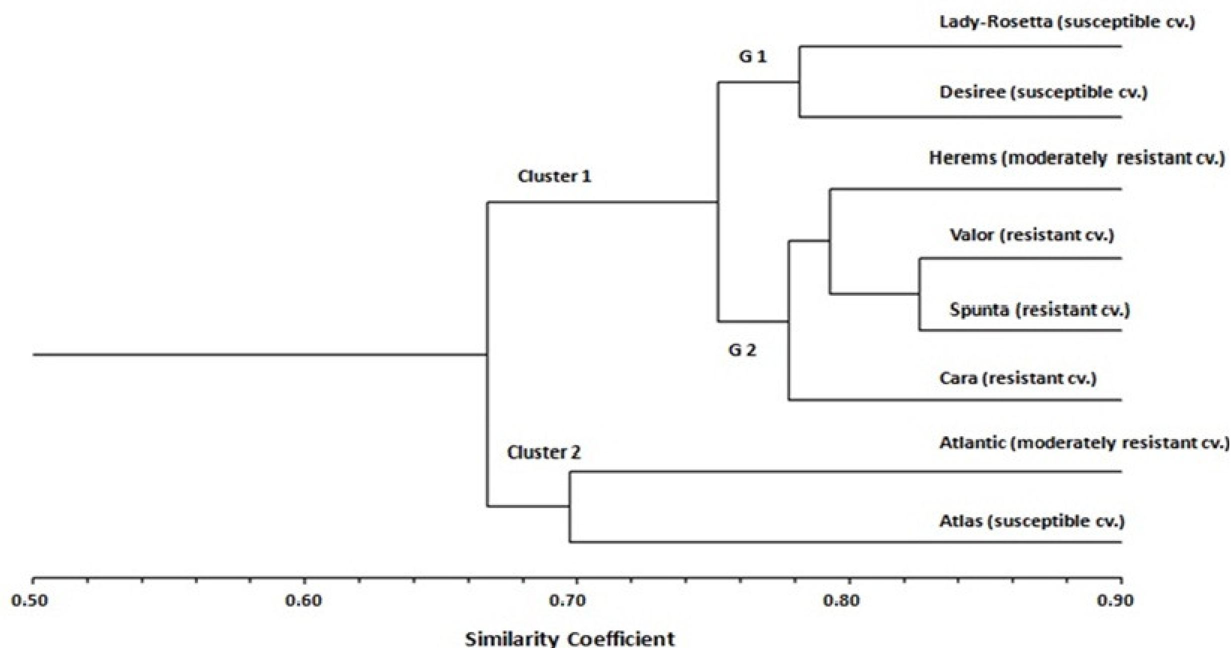

Figure 2. A dendrogram based on RAPD markers of the eight potato cultivars variation in their resistance to early blight pathogen.

Table 5. Pair-wise genetic similarities based on SSR markers among the potato cultivars that varied in their resistibility/susceptibility against early blight pathogen.

Potato cultivar[a]	1	2	3	4	5	6	7	8
1	1.0000							
2	0.2500	1.0000						
3	0.3000	0.8000	1.0000					
4	0.2273	0.6470	0.5263	1.0000				
5	0.5000	0.4117	0.4705	0.4444	1.0000			
6	0.4444	0.3684	0.4210	0.4737	0.6250	1.0000		
7	0.3333	0.4117	0.3888	0.5000	0.4375	0.4444	1.0000	
8	0.1428	0.3157	0.2380	0.3500	0.2000	0.3000	0.2632	1.0000

[a]Potato cultivars: 1, Lady-Rosetta; 2, Hermes; 3, Valor ; 4, Spunta ; 5, Atlantic; 6, Desiree; 7, Cara; 8, Atlas.

numbers of amplified bands produced by the six SSR primers were 29 (Table 2). Out of these 29 loci, 27 (93.1%) were polymorphic. The number of bands produced by each SSR primer varied from three to seven with an average of 3.85 band/primers. STM0019 primer-pair produced the highest number of bands. The polymorphism ratios of six SSR primer-pairs were between 75 and 100%. All the bands generated from STM0019, STI24, STI30 and STI57 primer-pairs were polymorphic (Table 2). SSR primer-pair STI57 generated a fragment $STI57_{209bp}$ detected in the resistant cultivars, in addition to moderate resistant cultivar Hermes (Figure 1c).

Furthermore, the pair-wise genetic similarities among the eight potato cultivars ranged from 0.143 to 0.800 with an average genetic similarity of 0.388 (Table 5). Higher genetic similarities were detected among the resistant cultivars than the moderate resistant or susceptible potato cultivars. Interestingly, the moderate resistant cultivar, Hermes, was genetically close to the resistant cultivars (Table 5). The UPGMA analysis showed that the eight potato cultivars were separated into two clusters (Figure 3). Cluster 1 was divided into two groups (G1 and G2). Group 1 included the susceptible cultivars Lady-Rosetta and Desiree, in addition to the moderately resistant cultivars Atlantic. On the other hand, the moderate resistant cultivar Hermes and the resistant cultivars Cara, Spunta and Valor were grouped in group 2. Cluster 2 included only the early blight susceptible cultivar Atlas.

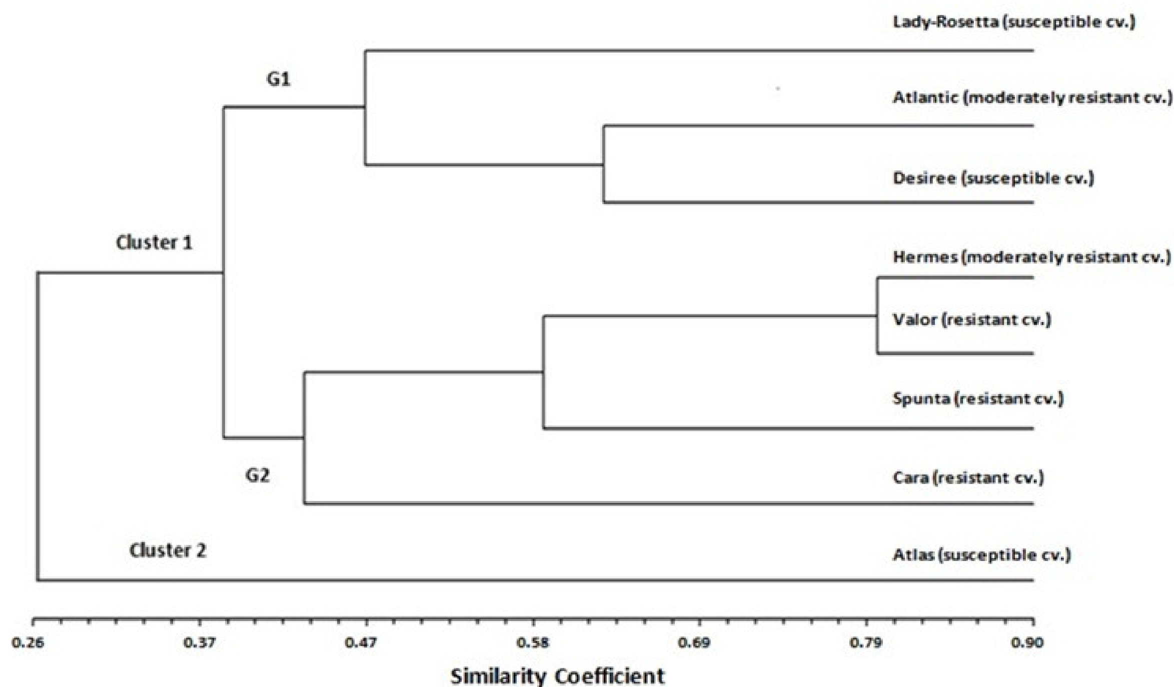

Figure 3. A dendrogram based on SSR markers of the eight potato cultivars variation in their resistance to early blight pathogen.

DISCUSSION

Potato early blight caused by *A. solani* is one of the most destructive fungal foliar diseases in many potato growing regions (Pelletier and Fry, 1989; Shtienberg et al., 1990; Christ, 1991; Van der Waals et al., 2003). Potato growers use fungicide treatments to control early blight disease, but the increased environmental regulations and development of fungicides-resistant fungal isolates are restricting the available chemicals to control the early blight disease (Holm et al., 2003). The use of resistant cultivars is considered the most efficient, cost effective and environmentally a more acceptable means of controlling plant diseases (Dorrance and Inglis, 1997; Rodríguez, et al., 2007). Therefore, information about the early blight resistibility/susceptibility of commercially important cultivars is needed by those making decisions regarding early blight management, which could help to optimize the success of integrated control strategies.

In this study, two different isolates of *A. solani* were chosen for the evaluation of early bight resistance in eight potato cultivars (Atlas, Atlantic, Cara, Desiree, Hermes, Lady-Rosetta, Spunta and Valor) using a droplet inoculation method in glasshouse experiment. Disease development was estimated as diameter of lesion extension (LE) within 10 days after inoculation. Obtained results showed positive correlation between the infected tissue area (lesion extension) and resistibility/ susceptibility of potato cultivars. Moreover, the cultivars were classified into three categories; resistant, moderate resistant and susceptible. Cara, Valor and Spunta were the highest significant resistant potato cultivars, while Lady-Rosetta, Desiree and Atlas were the lowest in this respect. These results indicate that, the resistibility/ susceptibility of the tested potato cultivars varied according to their response to infection by *A. solani* isolates (Dita et al., 2007). The low values of LE recorded in leaves of resistant cultivars suggested that resistance events that delay tissue colonization after fungus penetration play a role in *A. solani*-potato pathosystem (Gebhardt and Valkonen, 2001; Dita et al., 2006). Nash and Gardner (1988) reported that disease severity can be determined more precisely and objectively by measuring lesion extension when the inoculum was applied as single drops on leaflets. Also, Berger et al. (1997) reported that lesion expansion reflects the process of host colonization and has been used by plant pathologists to assess cultivar resistance. Additionally, lesion expansion can be a useful component to provide evidence about the mechanism of resistance (Parlevliet, 1979).

Developing resistant potato cultivars is one of the main objectives of potato breeding programs, so as to offer an effective and environment-friendly method to control early blight disease. These resistant cultivars reduce the application of fungicides that have negative impact on the ecosystem and eventually reduce their production (Christ and Haynes, 2001). However, developing new potato cultivars through breeding programs is time-consuming, laborious and needs high-trained personnel. These

problems can be better managed by identifying molecular markers that are associated with resistance sources (Tanksley et al., 1995). Moreover, molecular markers have also been used to estimate genetic diversity and assess genetic relationships among plant varieties. Among such molecular markers, RAPD and micro-satellites or SSR that have been used in potato and its wild relatives in order to study genetic diversity, poly-morphisms, phylogenetic relationships as well as genetic mapping and genotype identification (Milbourne et al., 1997; Bisognin and Douches, 2002a, 2002b; Hong et al., 2006; Siri et al., 2009; Abou-Taleb et al., 2010; Kandemir et al., 2010). In this study, 20 RAPD and six SSR primers were applied to assess genetic diversity and to identify molecular markers associated with resistance to early blight disease in certain potato cultivars showing different levels of resistibility/susceptibility to early blight disease. The obtained results showed that 296 and 29 PCR amplicons were amplified by RAPD and SSR, respec-tively. SSR markers revealed higher frequency of polymorphic bands (93.1%) than RAPD (57.4%). However, the average genetic similarities based on SSR markers was much lower (0.388) than that based on the RAPD markers (0.784). The high levels of DNA polymorphism shown by SSR markers could be due to the result of changes in the number of repeats in an array which is considered to be the result of slippage mutation of DNA polymerase that occurs during DNA replication (Milbourne et al., 1997).

Moreover, cluster analyses based on the RAPD and SSR markers showed that the obtained grouping pattern had a similar distribution of the eight potato cultivars used in this study. The dendrograms prepared based on RAPD and SSR analysis, grouped the resistant cultivars within one group. These results are in agreement with those of Burnham et al. (2002) who found that genetic relatedness among resistant soybean cultivars against *Phytophthora sojae* were more genetically closer to each other than the susceptible cultivars. Also, Hong et al. (2006) showed that resistant potato cultivars against potato virus Y (PVY) were genetically close. RAPD markers could be beneficial for revealing the genetic variability of different genotypes of potato in their resistibility/susceptibility to the late blight disease (Abou-Taleb et al., 2010). On the other hand, Pattanayak et al. (2002) found no clear correlation between RAPD fingerprints and resistibility of potato cultivars against late blight disease.

In an effort to identify molecular markers associated with early blight disease resistance, we found that RAPD primers OPY-07, OPG-05, OPA-10 and OPA-18 produced five unique fragments (OPY-07_{880bp}, OPG-05_{949bp}, OPA-10_{1000bp}, OPA-18_{2030bp} and OPA-18_{1128bp}) that were only present in the resistant cultivars. Two resistance-specific RAPD and SSR markers, OPA-18_{1070bp} and $STI57_{209bp}$, were also detected in the moderate resistant cultivar Hermes. On the other hand, the susceptible-specific RAPD UBC-17_{720bp} marker (found only susceptible cultivars) was also detected in moderate resistant cultivar Atlantic. Lee et al. (2005) used amplified fragment length polymorphism (AFLP) technology to identify AFLP markers that are associated with late blight resistance traits in different potato genotypes. They showed that a unique 136 bp AFLP marker was only observed in highly resistant potato lines. Ananga et al. (2006) also identified RAPD markers that were asso-ciated with resistance to blackleg disease in *Brassica* species. Wickeramasinghe et al. (2009) developed PCR-based markers linked to quantitative resistance to late blight disease in potato.

In conclusion, this study supports the idea of using the molecular markers to facilitate the development of resistant potato cultivars through breeding programs against one of the most serious potato fungal diseases, *Alternaria* late blight. Further studies can be carried out to molecularly characterize the RAPD and SSR unique mar-kers that are associated with early blight resistibility/sus-ceptibility described in this study. For example, these markers can be cloned and used as sequence characterized amplified region markers (SCARs) in order to select putative resistant potato cultivars to be used in the breeding programs against early blight disease. Moreover, these markers can be sequenced and used in genome mapping projects to identify resistibility/ susceptibility genes.

ACKNOWLEDGEMENTS

This research was supported by the King Saud University, Deanship of Scientific Research, College of Food and Agriculture Sciences, Research Center.

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