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ORIGINAL ARTICLE

Genetic diversity among late blight resistant and susceptible potato genotypes

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KEYWORDS

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Abstract RAPD polymerase chain reaction analysis was used to study the genetic diversity among a wild potato variety *Solanum demissum* (very resistant to late blight) and six potato cultivars (Hanna, Lady-Olympia, Lady-Rosetta, Spunta, Diamant and Cara) varied in their resistance to *Phytophthora infestans*. Cluster analysis of six potato genotypes showed that, all tested genotypes were separated into two clusters (1 and 2). Cluster 1, included only the wild potato variety (*S. demissum*), whereas cluster 2 divided into two groups (G1 and G2). Late blight high resistant cultivars Hanna and Cara were grouped in G1. Group 2 included the moderate resistant cultivar Spunta and the susceptible cultivars Diamant, Lady-Rosetta and Lady-Olympia. The potato cultivars that showed highest genetic similarity to the wild potato variety were the resistant cultivars Hanna and Cara. Lowest genetic similarity was obtained with the susceptible cultivars Lady-Rosetta, Diamant and Lady-Olympia. RAPD primer K17 yielded a band with molecular weight of 936 bp found in all susceptible potato cultivars (Lady-Rosetta, Lady-Olympia and Diamant). On the other hand, band with molecular weight of 765 bp were detected in the wild potato and the resistant cultivars Hanna and Cara. Results of this study suggested that, the RAPD marker technique could be beneficial for revealing the genetic variability of different genotypes of potato varied in their resistibility to late blight.

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1. Introduction

Two types of genetic resistance to *Phytophthora infestans* are available in wild and cultivated potato genotypes; race-specific resistance controlled by the dominant alleles of the R genes and general resistance or race-unspecific resistance governed by polygenes (Wastie, 1991). Both types of resistance are stated to be controlled by alleles at the same genetic locus or by related alleles of closely linked loci (Young, 1996). Incorporation and improving genetic resistance to *P. infestans* with careful

selection of parents and better utilization of heterosis is an increasingly important aspect of potato breeding. Quantification of genetic diversity present within the cultivars by molecular markers would be of great help to improve inheritable disease resistance in potato 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 are needed (Bisognin and Douches, 2002; Pattanayak et al., 2002).

Random Amplified Polymorphic DNA (RAPD) marker technique is easy and quick, and requires no prior sequence information (Williams et al., 1990). In tuber bearing *Solanum* species, RAPD markers have been seen to be sufficiently sensitive to detect genetic variation (Demeke et al., 1996; Sosinski and Douches, 1996; Milbourne et al., 1997; McGregor et al., 2000; Botez et al., 2004, 2005; Hong et al., 2006). RAPD markers have also been used successfully to detect segregation level in F2 populations (Quiros et al., 1993; Hosaka et al., 1994). The goal of the present study was an attempt to establish a genetic diversity among late blight resistant and susceptible potato genotypes based on molecular markers with the aid of the RAPD–PCR technique. Such information may lead to the development of more reliable methods for potato breeding programs against late blight disease.

2. Materials and methods

2.1. Plant material

Six potato cultivars (*Solanum tuberosum*) varied in their resistibility to late blight disease were used in this study (El-Komy, 2007); Hanna, Cara, (resistant) Spunta, (moderately resistant) Diamant, Lady-Rosetta and Lady-Olympia (susceptible). Potato cultivars were obtained from International Potato Center (CIP) Kafr El-zayat, Egypt.

Solanum demissum GLWKS 0216 (wild potato) was obtained from Dr. Klaus J. Dehmer (IPK Gatersleben, Genebank Department at the Foundation Institute of Plant Genetics and Crop Plant Research, Germany).

2.2. Genetic diversity among potato genotypes

2.2.1. Potato genomic DNA extraction

The DNA was isolated according to protocol of Griffith and Shaw (1998). Potato leaf tissue was processed by freezing with liquid nitrogen and was ground into a fine powder using a mortar and pestle. Approximately 100 mg of that powder was transferred to 1.5 ml micro-centrifuge tube and 600 µl of warm (65 °C) 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 vortexes for 1–3 s, and incubated for 60–90 min, in water bath at 65 °C. After that, the sample was allowed to cool to room temperature for 5 min.

A volume of 700 µl chloroform/octanol (24:1) was added, the solution was gently mixed for 5–10 min. The mixture was centrifuged for 10 min at 8000*g*. Six hundred micro-liters of upper, aqueous layer were transferred to clean 1.5 ml microcentrifuge tube, and a volume of 600 µl of cooled isopropanol was added to precipitate the DNA. The mixture was centrifuged at 5000*g* for 2 min at room temperature. The supernatant was decanted, and 600 µl of 70% ethanol was added at room temperature and gently inverted the tube several times to wash the DNA. The mixture was centrifuged at 3000*g* for 2 min at room temperature.

Carefully the ethanol was aspirated using a pipette. The tube was inverted in to clean absorbent paper and air dried the pellet for 15 min. 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. DNA concentration was determined using spectrophotometer (Beckman DU-65) and was adjusted to 50 ng μl^{-1}.

2.2.2. RAPD–PCR conditions

All PCR reactions were carried out in a final volume containing: 1 µl (50 pmol) of primer, 0.3 µl *Taq* DNA polymerase (5 U μl^{-1}), 2.5 µl PCR buffer, 1 µl 10 mM Mg Cl_2, 1 µl 2.0 mM dNTPs (for each), 1 µl of template DNA (approximately 50 ng) and 18.2 µl sterile distilled H_2O. The sequences of the RAPD primers used in this study are shown in Table 1. Thermocycling was conducted in a Biometra – UNO II at 94 °C for 5 min as initial denaturation and 35 cycles of 94 °C for 0.5 min, 55 °C for 0.5 min and 72 °C for 1.5 min. This was followed by a 10 min final extension at 72 °C. The PCR product was analyzed by electrophoretic separation in 1.5% gel. 1 kb DNA Ladder (New England Biolabs) with size marker, ranged from 500 to 10 000 bp was used as a molecular size standard (McGregor et al., 2000).

2.3. Data handling and cluster analysis

Data were scored for computer analysis on the basis of the presence and absence of the amplified products for each primer. If a product was present in genotype, it was designated '1', if absent it was designated '0' after excluding unreproducible bands. Pair-wise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to generate similarity coefficients which were used to construct a dendrogram by UPGMA (unweighted pair-group method with arithmetical averages) using NTSYS-pc Software, Rolf, 1993.

3. Experimental results

RAPD polymerase chain reaction was used to study the genetic diversity among late blight resistant and susceptible potato genotypes. The DNAs of six potato cultivars Hanna, Cara, (resistant) Spunta, (moderate resistant) Diamant, Lady-Rosetta and Lady-Olympia (susceptible) and a wild potato variety *S. demissum* (very resistant) were compared using 10 RAPD primers. All reactions were repeated at least twice. Representative results obtained with those primers are given in Fig. 1.

Table 1 Primer sequences used in this study.

Primer number	Sequence	Primer number	Sequence
K09	5′-TGGGGGACTC-3′	K14	5′-GGTCGGAGAA-3′
K10	5′-TGCGCCCTTC-3	K15	5′-AGGTGACCGT-3′
K11	5′-ACATCGCCCA-3′	K16	5′-CTGAGACGGA-3′
K12	5′-ACCCCCGAAG-3′	K17	5′-GGAAGTCGCC-3′
K13	5′-CTCAGTCGCA-3′	K18	5′-TGTAGCTGGG-3′

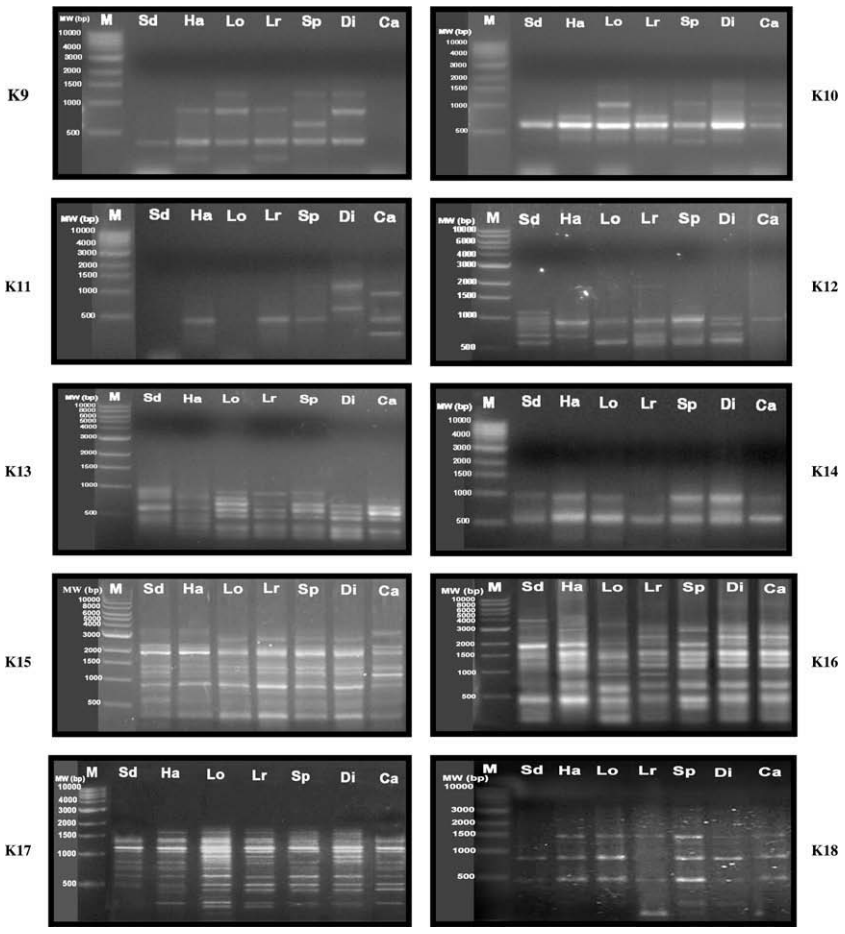

Figure 1 RAPD patterns for the tested wild potato *S. demissum* and six potato cultivars varied in their resistance to *P. infestans*, generated by 10-mer random primers (K9, K10, K11, K12, K13, K14, K15, K16, K17 and K18). M, DNA marker; Lr, *S. tuberosum* cv. Lady-Rosetta (susceptible); Sd, *S. demissum* (very resistant); Sp, *S. tuberosum* cv. Spunta (moderately resistant); Ha, *S. tuberosum* cv. Hanna (resistant); Di, *S. tuberosum* cv. Diamant (susceptible); Lo, *S. tuberosum* cv. Lady-Olympia (susceptible); Ca, *S. tuberosum* cv. Cara (resistant).

Table 2 Number of polymorphic and common d bands detected by each RAPD primer among wild potato *S. demissum* and six potato cultivars varied in their resistance to *P. infestans*.

Primers	Number of polymorphic bands	Number of common bands	Total number of bands
K09	5	0	5
K10	4	1	5
K11	5	0	5
K12	5	0	5
K13	6	2	8
K14	2	1	3
K15	5	8	13
K16	11	3	14
K17	9	6	15
K18	5	0	5
Total	57	21	78

The obtained results showed that, each primer generated distinct RAPD patterns differ than the others. RAPD primers generated a number of 78 bands ranging from 34 to 3690 bp. The appearance of specific amplified bands from each potato genotype was used as a measure of polymorphism, defined by amplified band of characteristic size appearing in at least one isolate. The obtained results in Table 2 showed that, out of the 57 polymorphic bands (polymorphism 73%), 21 bands generated by random primers were unique and were present in both the late blight resistant and susceptible potato cultivars.

Dendrogram of six potato cultivars and the wild potato variety using UPGMA analysis from pairwise comparison of RAPDs showed that all tested potato genotypes were separated into two clusters (1 and 2). Cluster 1, included only the wild potato variety whereas cluster 2 divided into two groups (G1 and G2). Late blight resistant cultivars Hanna and Cara were grouped in group 1. On the other hand, group 2 included the moderate resistant cultivar Spunta and the susceptible cultivars Diamant, Lady-Rosetta and Lady-Olympia (Fig. 2).

The closest genetic similarity (GS) was found between the moderate resistant cultivar Spunta and the susceptible cultivar Diamant (GS = 0.75).

Similarity coefficients (Table 3) reveal that the potato cultivars that showed highest genetic similarity to the wild potato variety were the resistant cultivars Hanna (GS = 0.60) and

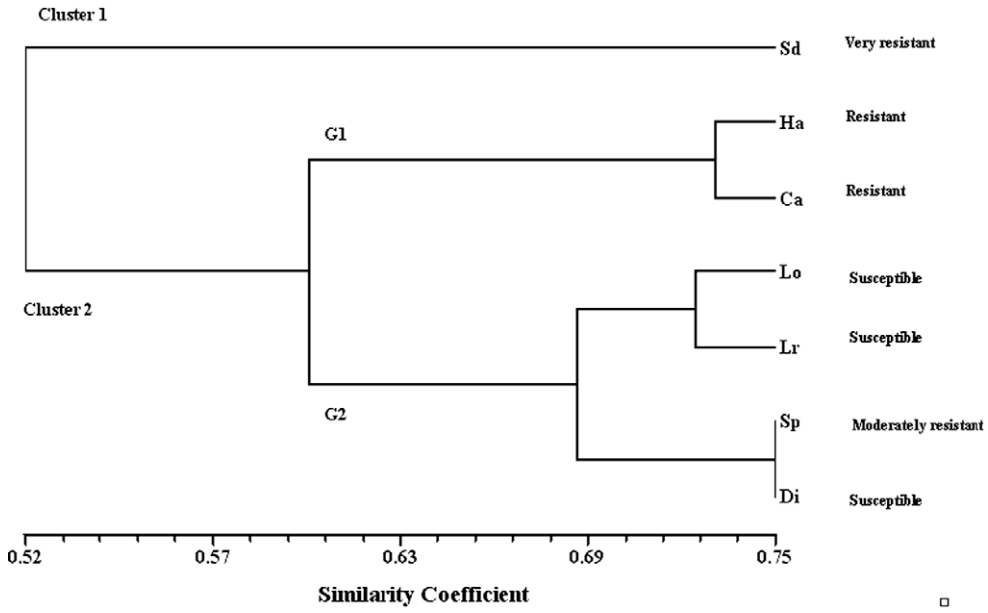

Figure 2 Dendreogram of wild potato *S. demissum* and six potato cultivars varied in their resistance to *P. infestans* by UPGMA analysis from pairwise comparison of RAPDs. **Solanum demissum* (Sd), *S. tuberosum* cvs., Hanna (Ha), Lady-Olympia (Lo), Lady-Rosetta (Lr), Spunta (Sp), Diamant (Di), and Cara (Ca). The closest genetic similarity (GS) was found between the moderate resistant cultivar Spunta and the susceptible cultivar Diamant (GS = 0.75).

Table 3 Similarity coefficients among wild potato *S. demissum* and six potato cultivars varied in their resistance to *P. infestans*.

Potato genotypes*	Sd	Ha	Lo	Lr	Sp	Di	Ca
Sd	1.0000						
Ha	0.6034	1.0000					
Lo	0.4923	0.5757	1.0000				
Lr	0.4461	0.6557	0.7213	1.0000			
Sp	0.5000	0.5873	0.6774	0.6557	1.0000		
Di	0.4769	0.6349	0.6718	0.7333	0.7457	1.0000	
Ca	0.5714	0.7272	0.5468	0.5737	0.6379	0.6065	1.0000

* Sd, *S. demissum* (very resistant); Ha, *S. tuberosum* cv. Hanna (resistant); Lo, *S. tuberosum* cv. Lady-Olympia (susceptible); Lr, *S. tuberosum* cv. Lady-Rosetta (susceptible); Sp, *S. tuberosum* cv. Spunta (moderately resistant); Di, *S. tuberosum* cv. Diamant (susceptible); Ca = *S. tuberosum* cv. Cara (resistant).

Cara (GS = 0.57). Lowest genetic similarity were obtained with the susceptible cultivars Lady-Rosetta (GS = 0.44), Diamant (GS = 0.48) and Lady-Olympia (GS = 0.49).

RAPD primer K17 yielded a band with molecular weight of 936 bp found in all susceptible potato cultivars (Lady-Rosetta, Lady-Olympia and Diamant). On the other hand, band with molecular weight of 765 bp were detected in the wild potato and the resistant cultivars Hanna and Cara (Fig. 3).

4. Discussion

Few attempts have been made earlier to assess diversity between late blight resistant and susceptible potato genotypes (Pattanayak et al., 2002). In the present work, the DNAs of six potato cultivars Hanna, Cara, (resistant) Spunta, (moderate resistant) Diamant, Lady-Rosetta and Lady-Olympia (Susceptible) and a wild potato variety *S. demissum* (very resistant) were compared using 10 RAPD primers to study the genetic diversity between late blight resistant and susceptible potato cultivars. All reactions were repeated at least twice. Representative results obtained with those primers are given in Fig. 1.

The obtained results showed that, each primer generated distinct RAPD patterns differ than the others. RAPD primers generated a number of 78 bands ranging from 34 to 3690 bp. The appearance of specific amplified bands from each potato genotype was used as a measure of polymorphism, defined by amplified band of characteristic size appearing in at least one isolate.

The obtained results in Table 2 showed that, out of the 57 polymorphic bands (polymorphism 73%), 21 bands generated by random primers were unique and were present in both the late blight resistant and susceptible potato cultivars. The high levels of fragment polymorphism observed in the present study (73%) demonstrated the potential of this method in evaluating the genetic diversity. Heterozygosity and polyploidy in the potato have given rise to high levels of DNA polymorphism by RAPD markers (Pattanayak et al., 2002).

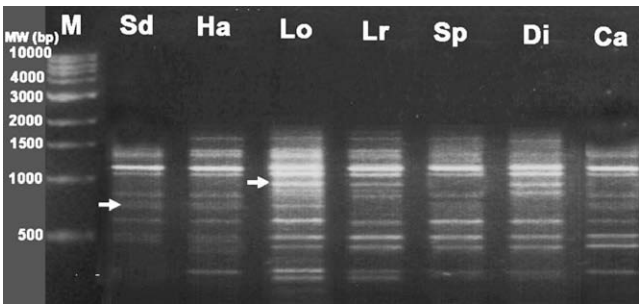

Figure 3 RAPD patterns for the tested wild potato *S. demissum* and six potato cultivars varied in their resistance to *P. infestans*, generated by 10-mer random primers (K17). **Solanum demissum* (Sd), *S. tuberosum* cvs., Hanna (Ha), Lady-Olympia (Lo), Lady-Rosetta (Lr), Spunta (Sp), Diamant (Di) and Cara (Ca). Gray arrow indicates to band with molecular weight of 936 bp. White arrow indicates to band with molecular weight of 765 bp.

Dendrogram of six potato cultivars and the wild potato variety using UPGMA analysis from pairwise comparison of RAPDs showed that, all tested plants were separated into two clusters (1 and 2). Cluster 1, included only the wild potato variety whereas cluster 2 divided into two groups (G1 and G2). Late blight resistant cultivars Hanna and Cara were grouped in G1. While, group 2 included the moderate resistant cultivar Spunta and the susceptible cultivars Diamant, Lady-Rosetta and Lady-Olympia (Fig. 2).

Also, we found that, the potato cultivars that showed highest genetic similarity (GS) to the wild potato variety were the resistant cultivars Hanna (GS = 0.60) and Cara (GS = 0.57). Lowest genetic similarity were obtained with the susceptible cultivars Lady-Rosetta (GS = 0.44), Diamant (GS = 0.48) and Lady-Olympia (GS = 0.49). These results were in agreement with those of Peidu et al. (2001) who found that, high drought resistant ramie cultivars were clustered into different groups or subgroups in the same class, which shows near relationship among them. Burnham et al. (2002) studied the genetic diversity present among soybean cultivars resistant to *P. sojae*. They found that, a close genetic relationship among resistant soybean cultivars. Also, Hong et al. (2006) revealed that, DNA fingerprints of 37 potato virus Y (PVY) resistant potato cultivars showed close genetic relationships between a numbers of potato cultivars. On the other hand, Pattanayak et al. (2002) studied the genetic diversity among resistant and susceptible potato cultivars to late blight using RAPD markers. They found that, no clear groupings based on late blight resistance and susceptibility was reflected on the dendrogram. Susceptible cultivars were found to have narrow genetic variation. In contrast, late blight resistant potato cultivars showed wider genetic variation. Page et al. (1997) identified four RAPD fragments as markers of Sclerotinia crown and stem rot (SCSR). Three are associated with resistance in red clover and one with susceptibility. Amplified fragment length polymorphism (AFLP) method was used by HyunMook et al. (2005) to identify late blight resistant markers in potato genotypes. They showed that, 136 bp fragment was observed only on highly resistant potato lines but not in susceptible ones. During the present study RAPD primer K17 yielded a band with molecular weight of 936 bp found in all susceptible potato cultivars (Lady-Rosetta, Lady-Olympia and Diamant). Also, a band with molecular weight of 765 bp was detected in the wild potato and the resistant cultivars Hanna and Cara (Fig. 3). These results suggested that, the RAPD marker technique could be beneficial for revealing the genetic variability of different genotypes of potato varied in their resistibility to late blight.

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