Journal of Applied Biology & Biotechnology Vol. 11(2), pp. 178-186, Mar-Apr, 2023
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2023.110218

Characterization of resistance genes to late blight (*Phytophthora infestants*) in potato by marker-assisted selection

Heba Amin Mahfouze*, Osama Ezzat El-Sayed, Sherin Amin Mahfouze

Department of Genetics and Cytology, Biotechnology Research Institute, National Research Centre, Dokki, Giza, Egypt.

ARTICLE INFO

Article history:
Received on: May 27, 2022
Accepted on: September 08, 2022
Available online: January 22, 2023

Key words:
Gene resources,
Disease resistance,
DNA markers,
R genes,
Solanum tuberosum L.

ABSTRACT

Multiple *R* genes referring to resistance against *Phytophthora infestants* were identified in different potato species, and the majority of these *R* genes were applied in potato breeding programs. The aim of the study was to use gene-based markers to discriminate between resistant and susceptible potato genotypes to late blight (LB). Furthermore, we correlated phenotype data obtained from the detached leaf assay with genotypic results to confirm the polymerase chain reaction (PCR)-based screening results. A total of 17 potato genotypes were tested for resistance to *P. infestans*, using the detached leaf assay. These genotypes gave different responses to resistance against LB, ranging from highly resistant to moderately susceptible. Our results showed that the data from the laboratory evaluation were run in parallel with the greenhouse experiment. Besides, these genotypes were assayed by the sequence-characterized amplified region and cleaved amplified polymorphic sequence markers for *R* genes. The results of PCR products could be used to distinguish between potato genotypes resistant and susceptible to LB. On the other hand, there is no correlation between the levels of resistance to LB and the number of *R* gene markers. The highest number of resistance gene markers was scored in the moderately resistant wild species *Solanum stoloniferum* CGN 17605 (8), followed by the highly resistant potato variety Jelly (7). In contrast, the lowest number of markers was found in the moderately resistant potato variety Deta (3). Results of the study showed that the presence of more than allele of the same gene in a single genotype give a durable resistance against different races of *P. infestans*. Therefore, potato genotypes that have multiple *R* genes could be taken into consideration in potato breeding programs for resistance against LB disease.

1. INTRODUCTION

The most serious disease of potatoes (*Solanum tuberosum* L.) is late blight (LB) disease, which is caused by *Phytophthora infestans*. It can entirely devastate a potato field [1]. LB is managed by foliar spraying using fungicides, which causes pollution of the environment. Furthermore, the appearance of novel *P. infestans* populations has led to novel strategies for the pathogen control, because the novel races will display increased virulence and resistance to fungicides. Therefore, traditional breeding programs for potatoes focused on using potato cultivars resistant to LB [2]. Conventional breeding programs take about 12 years, starting from the crossing to obtaining a novel potato variety. Besides, the selection process (involving many agronomic and quality characteristics) is laborious and needs time, so breeders must search for novel sources of LB resistance by DNA molecular markers linked to the resistance loci. This will save time, and will also help to better understand the host and pathogen interactions [3,4]. Up to now, quantitative trait loci (QTL) for LB disease resistance have been identified on *S. tuberosum* L. chromosome, which have clusters of resistance *R* loci, so *R* genes are candidates for the QTL effect [5,6]. The "candidate gene study" allows the characterization of molecular markers which can be used in potato breeding programs as marker-aided selection (MAS) [7]. Many investigations were carried out on potato genotypes resistant to *P. infestans*. Furthermore, multiple *R* loci were genotyped and identified in the potato [8]. To improve traditional methods in potato breeding programs, DNA markers closely linked to LB resistance loci may be simple to use in diploid or tetraploid potatoes. Tagging of resistance genes with DNA marker technology displays a possibility for MAS to screen disease resistance trait in the seedling stage and choose the best plants to be characterized for backcrossing. Moreover, recessive genes can be screened, which is difficult to do using traditional breeding methods [9].

The majority of the DNA markers applied formerly in mapping were either cleaved amplified polymorphic sequence (CAPS) or sequence-characterized amplified regions (SCARs) are simple to apply [10]. SCAR marker depends on variations in the primer sites, resulting in the presence or absence of an amplicon, while CAPS assays are based on polymorphism in the restriction site after polymerase chain reaction (PCR) amplification. Genetic markers representing resistance

*Corresponding Author:
Heba Amin Mahfouze,
Department of Genetics and Cytology, Biotechnology Research Institute, National Research Centre, Dokki, Giza, Egypt.
E-mail: hebaamn @ yahoo.com

allele fragments are most suitable for testing big genetic populations during breeding. The disadvantage of DNA markers is that they cannot differentiate between functionally active genes and inactive structural homologs [11]. Furthermore, with the recent progress in molecular biology of *S. tuberosum* L., the ease of use of DNA marker technology linked to resistance alleles in potato germplasm screening was reported. To make MAS an integrated part of traditional breeding, it will be important for breeders to identify the possibility molecular markers. Wherefore, to promote breeders, updates on advancements in DNA marker development for *P. infestans* resistance loci/QTLs. Genetic markers are expected to play an important role in MAS due to low costs, ease of use, linkage to target gene, and the proportion of phenotypic variance illustrated by molecular markers [10,12].

Genetic resistance to LB disease is acquisition its importance [13] due to several agents, involving the increasing demands to generate potato yields without applying fungicides [14], and alterations to legislation related to the application of chemicals [15]. Breeding of potato varieties/cultivars resistant to LB disease depends on the 11 dominant *R* loci (*R1* to *R11*), which are produced by species *Solanum demissum* and DNA markers linked to those dominant alleles were identified through MAS [16,17].

The purpose of the study was to use DNA markers to discriminate between resistant and susceptible potato genotypes to LB. Moreover, we will correlate phenotype data obtained from the detached leaf assay with genotypic results to confirm the PCR-based screening results.

2. MATERIALS AND METHODS

2.1. Plant Materials

Eleven potato varieties Annabelle, Bellini, Cara, Deta, Diamond, Herms, Jelly, Lady Rosetta, Metro, Mondial, and Spunta were collected from the brown rot project, Dokki, Giza, Egypt), as well as six wild potato species *Solanum acule* CGN 17674, *Solanum chacoense* CGN 17903, *S. demissum* CGN 17788, *S. demissum* CGN 17797, *Solanum stoloniferum* CGN 17605, and *S. tuberosum* CGN 17609 (supported by Centre for Genetic Resources, Netherland (http://www.wur.nl).

2.2. Source of *P. infestans* Isolate

The *P. infestans* isolate was obtained from the Plant Pathology Department, Agriculture Faculty, Ain Shams University [18].

2.3. LB Leaflet Tests

Five newly expanded leaflets of each potato genotype (grown in a greenhouse) were detached and used for the experiment (equivalent to five replications per genotype). Prior to inoculation, leaflets were washed and placed abaxial surface–up on filter paper in five Petri dishes (one leaflet/Petri plate). One drop (50 µl) of inoculum (3×10^3 sporangia ml^{-1}) of *P. infestans* was inoculated onto each leaflet using a micropipette. The Petri dishes were sealed with Parafilm to prevent desiccation. Then, inoculated leaflets were incubated at 16 ± 0.5°C with 12 h light cycle for the appearance of symptoms. On the 7th day after inoculation, the percent disease severity [%DS] was recorded after the disease prevailing using 1-9 Henfling scale [19].

2.4. Primer Design and Selection

In this research, nine resistance genes to LB named *R1*, *R3a*, *R3b*, *R9a*, *R8 & R9a, Rpi-phu1*, *Rpi-ber*, *Rpi-blb1*, and *Rpi-blb3* were used depending on prior publications. A total of 15 gene-specific primer pairs were screened, depending on the DNA nucleotide sequences of the nine candidate genes; nine primers were based on publications, and six primers were designed in our laboratory using the Primer3 online tool (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). The primer pairs used to amplify the nine resistance *R* loci are shown in Table 1 [21-28].

2.5. DNA Extraction and PCR Amplification

Total genomic DNA was extracted from fresh potato leaves of 17 potato genotypes, using the DNeasy plant mini-prep kit (Qiagen, CA). PCR amplification was performed in a thermal cycler (Biometra, biomedizinische Analytik GmbH) in a total volume of 25 µl containing 50 ng DNA, 10 µM of each primer, 200 mM dNTPs, 1.5 mM $MgCl_2$, and 0.5 U *Taq* DNA polymerase (GoTaq® DNA Polymerase, Promega, USA). PCR was performed under the following conditions: 94°C at 4 min and then 35 cycles of 94°C at 1 min, 50–60°C at 1 min, and 72°C at 1 min, and a final extension step at 72°C for 5 min.

All the PCR products were electrophoresed on 1% agarose gel electrophoresis in 1× TBE buffer. The genomic DNA was stained with RedSafe Nucleic Acid Staining Solution (1/20,000) (iNtRON Biotechnology, Inc. Kr) and was visualized and photographed with Gel-Documentation system (Bio-Rad Laboratories, Inc., Cali, USA). The size of each fragment was estimated with reference to a size marker of the 100 bp DNA ladder (BioRoN, Germany).

2.6. Statistical Analysis

Correlation analysis was performed using Microsoft Excel 2010 with an evaluation of Pearson's correlation coefficients.

3. RESULTS

3.1. Infection Assay in a Detached Leaf of Potato

To identify potato genotypes resistant and susceptible to *P. infestans*, 17 potato genotypes were tested against LB by detached leaf assay [Table 2 and Figure 1]. Some of the inoculated potato leaves appeared to have a few lesions. They were recorded as highly resistant, such as *S. chacoense* CGN 17903 and Jelly, when the leaves showed necrosis without sporulation, they were identified as resistant, for example, *S. acule* CGN 17674, while the leaves did not show the same reaction or sporulation were not clear. They were classified as either moderately resistant, that is, Cara, Diamond, Lady Rosetta, Metro, Mondial, Spunta, Deta, Herms, *S. demissum* CGN 17788, *S. demissum* CGN 17797, *S. stoloniferum* CGN 17605, and *S. tuberosum* CGN 17609, or moderately susceptible, such as Annabelle and Bellini [Table 2].

3.2. Detection of Resistance Genes *R3a, R3b,* and *R8* in Potato Genotypes

Six specific primer pairs were designed to detect three *R* genes, *R3a*, *R3b*, and *R8* in 17 potato genotypes as are shown in Table 1. PCR products of *R3a*, *R3b*, and *R8* resistance genes scored one specific band of (194 and 247), (226 and 244), and (220 and 237), respectively, in all the tested potato genotypes [Figure 2]. These primers have not recorded any polymorphic variations between resistant and susceptible potato genotypes. Therefore, seven SCAR and two CAPS markers were used to discriminate between resistant and susceptible potato genotypes.

3.3. Identification of *R* Genes by PCR Markers

Seven SCAR and two CAPS markers were screened to determine whether the eight candidate resistance *R* genes, *R1*, *Rpi-phu1*, *Rpi-*

Table 1: Sequence of primers used in this study.

Specific primers/marker type	Resistance gene name	Chromosome No.	Annealing temperature (AT) °C	Primer name	Productsize (bp)	Forward (3´–5´)	Reverse (5´–3´)	*Solanum* species	Ref. and accessions used for primers in the study
Specific primers	*R3a*	XI	55	R3a1	194	Tgctcggtcttcagattgtg	Ttgctggttgcttgtttctg	*Solanum tuberosum*	Accession (AY849382) (present study)
	R3a	XI	55	R3a2	247	Gccagcactaggacaactcc	Gggtaccgtctccaaactga	*Solanum tuberosum*	Accession (AY849382) (present study)
	R3b	XI	55	R3b1	226	Ggagttgccgaatgacttgt	Tagcggcgtcttcaagaaat	*Solanum demissum*	Accession (JF900492) (present study)
	R3b	XI	55	R3b2	244	Gccgaaaagagtggcattta	Aaaggaagcccttcttccag	*Solanum demissum*	Accession (JF900492) (present study)
	R8	IX	55	R8a	220	Gtgggatctcctcaaggtca	Tccttcattgcggaactacc	*Solanum demissum*	Accession (KU530153) (present study)
	R8	IX	55	R8b	237	Acccttttccaggaacatca	Ctcacaatgaatgccgaatg	*Solanum demissum*	Accession (KU530153) (present study)
SCARs	*R1*	V	58	R1-517	517	Gaatggaaagtgagcaatggcaagt	Gtagtacctatcttatttctgcaagaat	*Solanum demissum*	[20]
	R1	V	60	76-2s	1400	Cactcgtgacatatcctcacta	Caaccctggcatgccacg	*Solanum tuberosum*	[21]
	Rpi-phu1	IX	55	phu6	298	Agagaccctggatatatttcatagctct	Cgctctaggcacagggctcaatgctgat	*Solanum phureja*	[22]
	Rpi-ber	X	50	Rpi-ber1-Q133	395	Atcatctcctcaaagaatcaag	Atctccccattgacaaccaa	*Solanum berthaultii*	[23]
	Rpi-blb1	VIII	58	BLB1	821	Aacctgtatggcagtggcatg	Gtcagaaaagggcactcgtg	*Solanum stoloniferum*	[24]
	Rpi-blb3	IV	50	RGH2	320	Ggsaagaccactcttgcaag	Tggtyataatyactctgctgc	*Solanum bulbocastanum*	[25]
	R9a	IX	54	EDN61	450	Gcatcatgtctgcacctatg	Ctttgatgtggatggatggtg	*Solanum edinense*	[26]
CAPS	R8&*R9a*	IX	58	184-81F	680	Ccaccgtatgctccgccgtc	Gttccacttagccttgtcttgctca	*Solanum demissum*	[27]
	R3a	XI	54	TG105	650	Tcacatgagctgggagaaat	Aaaggcctgttgctgagag	*Solanum tuberosum*	[28]

CAPS: Cleaved amplified polymorphic sequence, SCARs: Sequence-characterized amplified regions

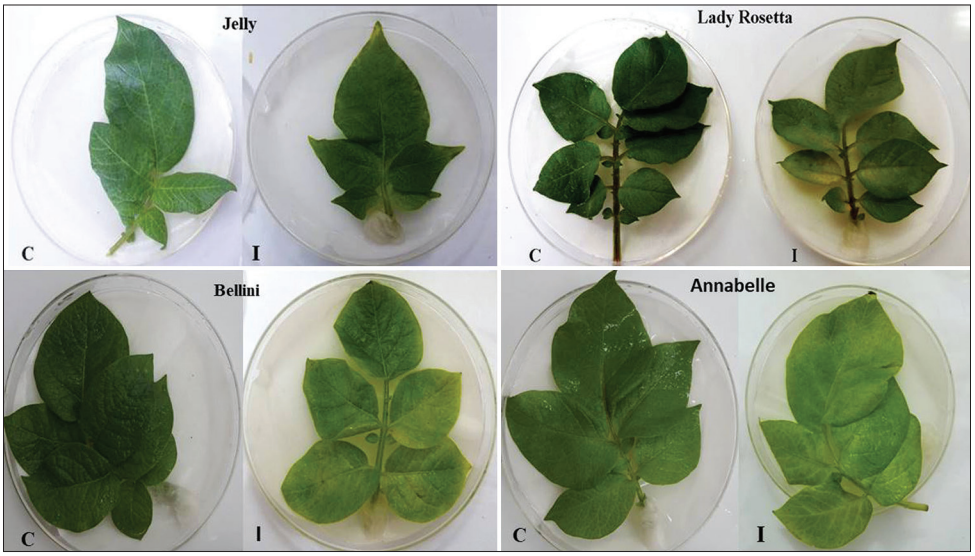

Figure 1: Assessment of detached leaf assay seven days after inoculation with *Phytophthora infestans* sporangia. Jelly cultivar a resistant phenotype that possessed genes (*R1, Rpi-phu1, Rpi-ber, Rpi-blb1, Rpi-blb3, R9a,* and *R3a*); Lady Rosetta showing moderate resistant, which possessed loci (*R1, Rpi-phu1, Rpi-ber, Rpi-blb3,* and *R8* and *R9a*); Bellini showing moderately susceptible, which possess (*R1, Rpi-phu1, Rpi-blb3, R9a,* and *R3a*), and cultivar Annabelle appeared moderately susceptible, which have genes (*Rpi-phu1, Rpi-ber, Rpi-blb1,* and *Rpi-blb3*). C: Control, I: Infected.

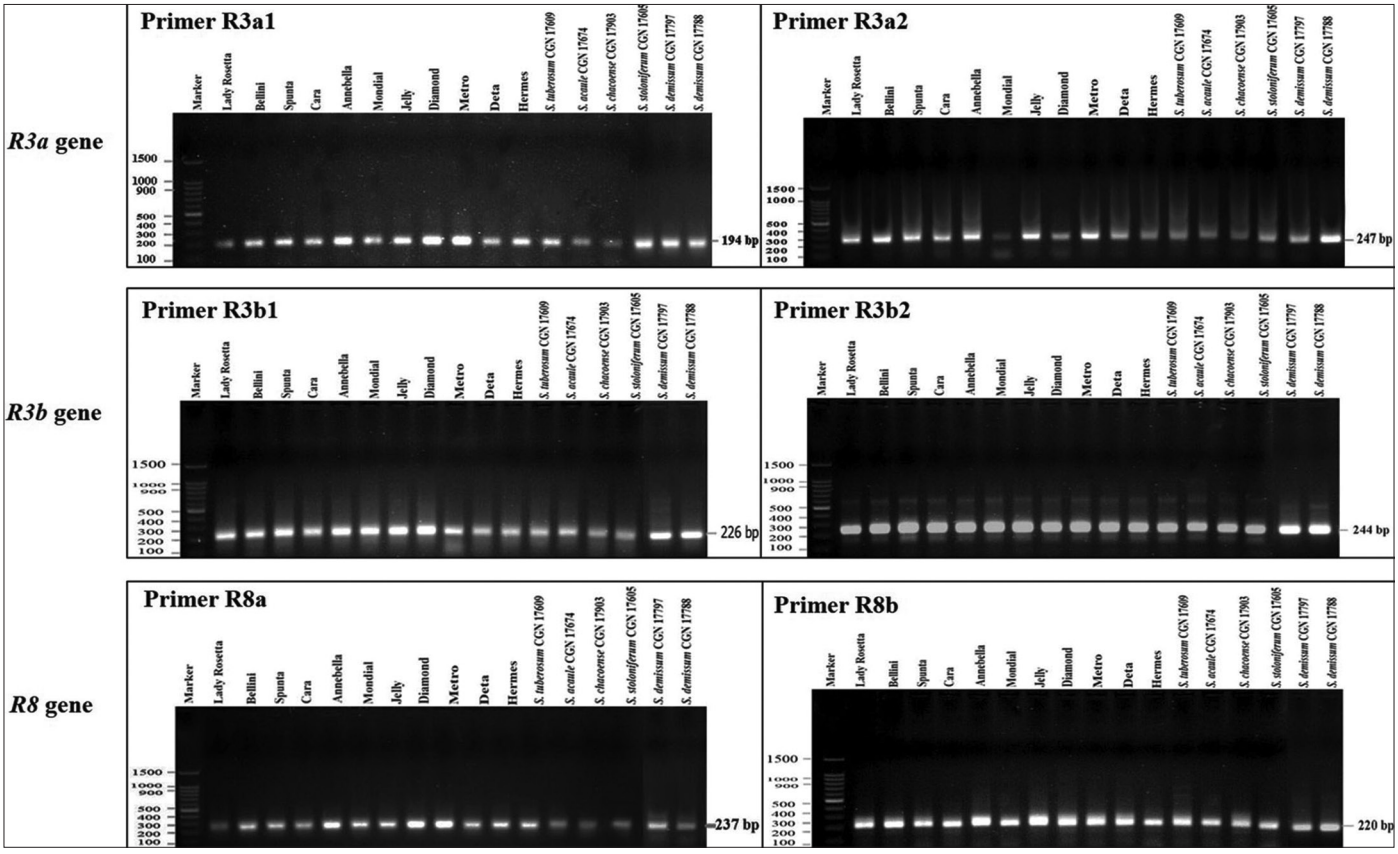

Figure 2: Polymerase chain reaction products of *R3a, R3b*, and *R8* genes using six designed primers in 17 potato genotypes. Marker: 100 bp DNA ladder.

ber, *Rpi-blb1*, *Rpi-blb3*, *R9a*, *R8* and *R9a*, and *R3a* were present or absent in the resistant and susceptible potato genotypes to LB disease [Table 2]. PCR amplicons for the *R1* gene using primer SCAR R1-517 amplified a fragment of 517 bp in 12 out of the 17 potato genotypes [Figure 3 and Table 2]. Furthermore, PCR results for the *R1* gene using SCAR 76-2s scored one band of 1400 bp in 4 out of 17 genotypes [Figure 3 and Table 2]. On the other hand, the *Rpi-phu1* gene amplified using the primer pairs SCAR phu6 gave

one fragment of 298 bp in 16 out of 17 potato genotypes [Figure 3 and Table 2]. Furthermore, the *Rpi-ber* gene amplified by SCAR Rpi-ber1-Q133 recorded a single amplified fragment of 395 bp in a total of 11 from 17 genotypes [Figure 3 and Table 2]. Moreover, the *Rpi-blb1* gene amplified by primer combination SCAR BLB1 gave one amplicon with the expected size of 821 bp in 6 of 17 potato genotypes [Figure 3 and Table 2]. Besides, PCR results of *R9a* using the primer pair EDN61 yielded an amplicon of 450 bp in 8 of the 17 genotypes of *S. tuberosum*. The presence or absence of the *Rpi-blb3* gene was analyzed using the primer set SCAR RGH2, which gave one amplified fragment of 320 bp in all tested potato genotypes. Therefore, this primer has not displayed any polymorphisms between the resistant and susceptible potato genotypes [Figure 3 and Table 2].

Amplification of the *R8* and *R9a* gene combination, using primer CAPS 184-81 gave one band of 680 bp in all studied potato genotypes [Figure 4 and Table 2]. An amplicon of 680 bp was subjected to digestion by the restriction enzyme *Rsa*I, to determine which potato genotypes are susceptible and resistant-homozygous or heterozygous for *R8* and *R9a* resistance genes. Restriction fragment, 480 bp to homozygous resistance were detected in wild species *S. acule* CGN 17674, *S. chacoense* CGN 17903, *S. stoloniferum* CGN 17605, *S. demissum* CGN 17788, and *S. demissum* CGN 17797 [Figure 4 and Table 2]. Three bands of 280, 400, and 480 bp to heterozygous resistance were found in the variety Lady Rosetta. Other potato genotypes produced restriction fragments at 280 and 400 bp, indicating susceptibility to *P. infestans*.

On the other hand, resistance gene *R3*a was amplified using primer CAPSTG105, which gave one band of 650 bp in all tested potato genotypes [Figure 4 and Table 2]. This band was digested using the *Hinf1* restriction enzyme, which gave more polymorphic fragments. Eight genotypes scored two amplicons at 150 and 500 bp only to indicate homozygous susceptible to the LB, such as Lady Rosetta, Metro, Annabelle, *S. acule* CGN 17674, *S. chacoense* CGN 17903, *S. stoloniferum* CGN 17605, *S. demissum* CGN 17788, and *S. demissum* CGN 17797 [Figure 4 and Table 2]. However, the remaining genotypes produced three bands of 150, 350, and 500 bp for heterozygous resistance [Figure 4 and Table 2], while the amplicon of 350 bp, which detects homozygous resistance. There are no genotypes for this trait [Figure 4 and Table 2].

3.4. The Relation between the Number of Markers and LB Resistance

The results of PCR amplification for all eight genes in potato genotypes resistant and susceptible, using the nine markers, are summarized in Table 2 and Figure 5. The results Spearman's correlation coefficient showed no correlation between the number of *R* gene markers and levels of resistance (r = –0.186 ns). For example, the number of *R* gene markers in the highly resistant varieties ranged from 5 to 7 markers, for example, Jelly and *S. chacoense* CGN 17903, followed by resistant varieties like *S. acule* CGN 17674 (4), and moderately resistant (3-8) such as, Diamond, Lady Rosetta, Metro, Mondial, Spunta, Cara, Deta, Herms, *S. stoloniferum* CGN 17605, *S. tuberosum* CGN 17609, *S. demissum* CGN 17788, and *S. demissum* CGN 17797. Finally, the moderately susceptible potato varieties have from 4 to 5 markers, for example, Annabelle and Bellini [Table 2 and Figure 5]. Depending on the PCR results, 17 potato genotypes were classified into eight groups. The first group: composed of 17 potato genotypes have *Rpi-blb3*. The second group: contained 16 genotypes including only the *RPi-phu1* gene. The third group: consisted of 12 genotypes involved *R1* (using marker SCAR R1-517). The fourth group: composed of 11 genotypes have *Rpi-ber*. The fifth group: included nine genotypes have *R3a* locus. The sixth groups: contained eight genotypes have *R9a*. The seventh group: involved six potato genotypes have *BLB1* and *R8* and *R9* genes. The eighth group: included four genotypes have *R1* gene (using SCAR 76-2s) [Table 2].

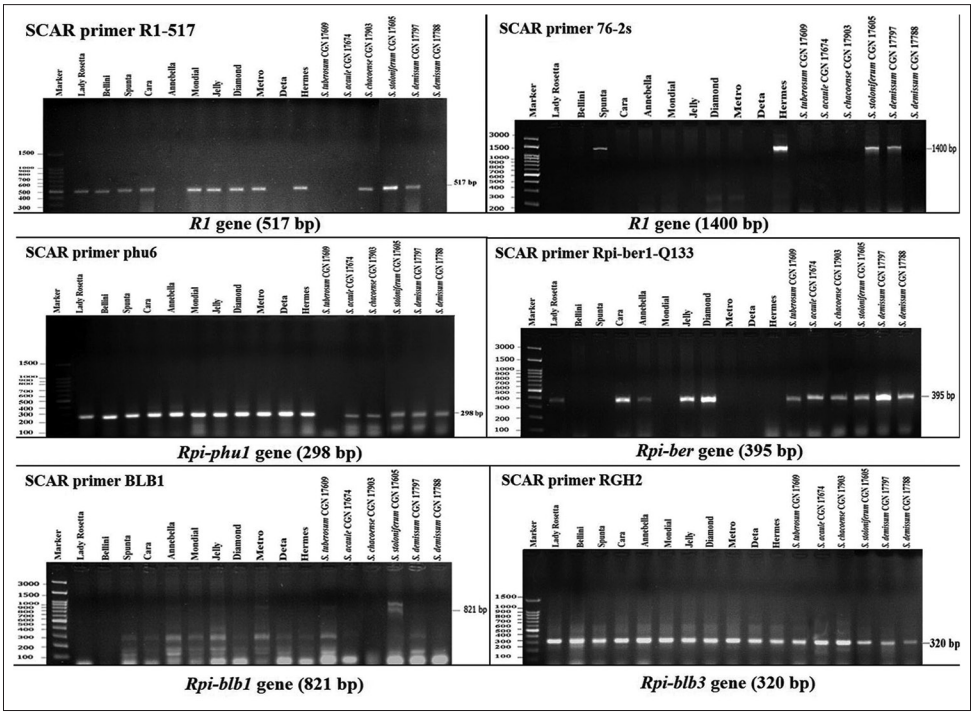

Figure 3: Polymerase chain reaction products of *R1, Rpi-phu1, Rpi-ber, Rpi-blb1,* and *Rpi-blb3* genes using different sequence-characterized amplified region primers amplified from 17 potato genotypes. Marker: 100 bp DNA ladder.

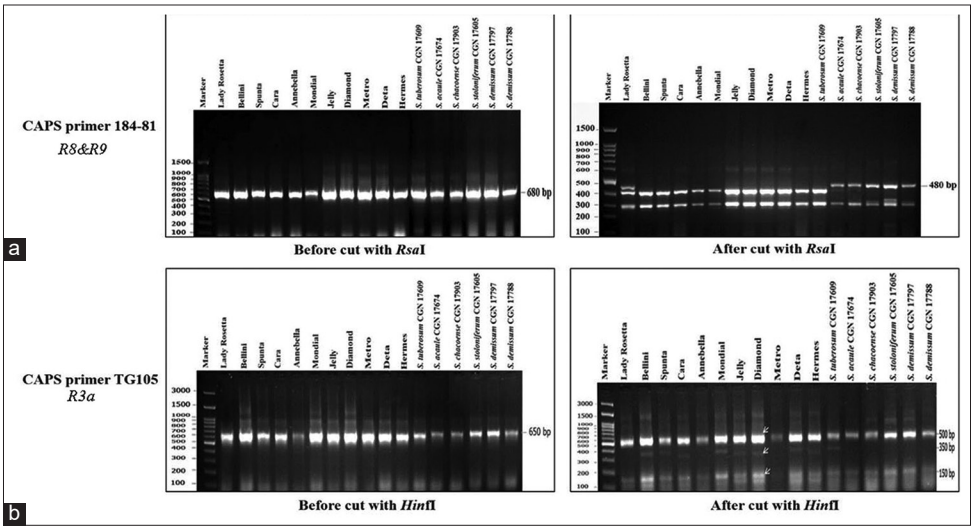

Figure 4: Detection of resistance genes *R8* and *R9a* combination and *R3a* using cleaved amplified polymorphic sequence (CAPS) primer 184-81 and CAPS primer TG105, respectively. (a) CAPS marker using primer pair 184-81 before and after cut with *RsaI*, (b) cleaved amplified polymorphic sequence marker using primer set TG105 before and after cut with *HinfI.*

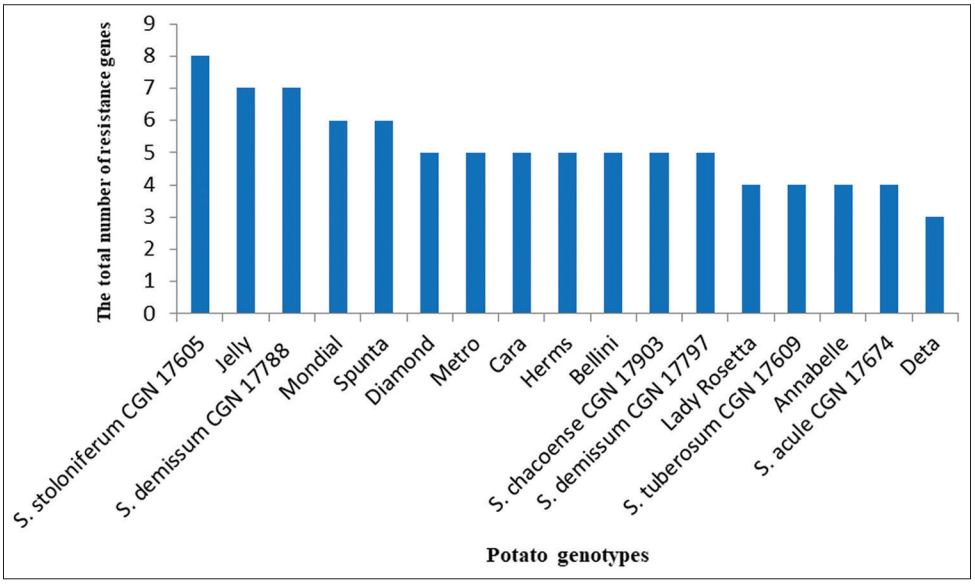

Figure 5: Illustrate the total number of late blight resistance (*R*) gene markers in 17 potato genotypes.

4. DISCUSSION

A gene-based marker is considered a powerful tool for the detection of the presence or absence of resistance (*R*) genes to LB disease in potatoes [29]. In this investigation, we evaluated 17 potato genotypes for resistance against *P. infestans* using a detached leaf assay. Results of this study showed that *S. chacoense* CGN 17903 and Jelly genotypes were highly resistant to LB (scale value; 1), while *S. acule* CGN 17674 was resistant (scale value; 2). Cara, Diamond, Lady Rosetta, Metro, Mondial, Spunta, Deta, Herms, *S. stoloniferum* CGN 17605, *S. tuberosum* CGN 17609, *S. demissum* CGN 17788, and *S. demissum* CGN 17797 were moderately resistant (scale value; 3-4), and Annabelle and Bellini were moderately susceptible (scale value; 5). In the previous study, we assessed the previous potato genotypes for LB resistance under greenhouse conditions. The results showed that the data from the laboratory trial were run in parallel with the greenhouse results, except two potato varieties, Deta and Herms, which were moderately resistant under the laboratory conditions, were moderately susceptible under the greenhouse conditions [18]. Rogozina *et al*. [30] evaluated 50 hybrids and cultivars of potatoes for resistance for LB disease in the field and laboratory for 7 years. These genotypes gave different responses to *P. infestans* infection, which varied from highly resistant to susceptible hybrids. Furthermore, the results of the laboratory evaluation agreed with the field experiments for several years. On the contrary, there are some potato genotypes, which were moderately resistant to *P. infestans* in the field experiments, were recorded as moderately susceptible under laboratory conditions. Fry [31] observed the presence of differences between results of the laboratory and field trials (1-3 points) for most potato genotypes which can be illustrated by more favorable conditions for microorganism development in laboratory experiments. Furthermore, other plant organs may be share in the resistance [32].

Table 2: SCAR and CAPS markers of *P. infestans* resistance *R* genes in 17 potato genotypes.

No.	Potato genotype	*Phytophthora infestans*		*Phytophthora infestans* resistance genes and DNA markers												The total number of *R* gene markers
				R1	*R1*	*Rpi-phu1*	*Rpi-ber*	*Rpi-blb1*	*Rpi-blb3*	*R9a*	*R8 & R9a*		*R3a*			
		Scale value	Pheno-type	SCAR*							CAPS**					
				R1-517	R1-76-2s	phu6	Rpi-ber1-Q133	BLB1	RG H2	Edn61	184-81		TG105			
1	Jelly	1	HR[a]	+	-	+	+	+	+	+	-	HoS[e]	+	HeR[g]		7
2	Lady Rosetta	3	MR[b]	+	-	+	+	-	+	-	+	HeR	-	HoS		4
3	Mondial	3	MR	+	-	+	-	+	+	+	-	HoS	+	HeR		6
4	Diamond	4	MR	+	-	+	+	-	+	-	-	HoS	+	HeR		5
5	Metro	3	MR	+	-	+	-	+	+	+	-	HoS	-	HoS		5
6	Spunta	4	MR	+	+	+	-	-	+	+	-	HoS	+	HeR		6
7	Cara	3	MR	+	-	+	+	-	+	-	-	HoS	+	HeR		5
8	Deta	3	MR	-	-	+	-	-	+	-	-	HoS	+	HeR		3
9	Herms	3	MR	+	+	+	-	-	+	-	-	HoS	+	HeR		5
10	Bellini	5	MS[c]	+	-	+	-	-	+	+	-	HoS	+	HeR		5
11	Annabelle	5	MS	-	-	+	+	+	+	-	-	HoS	-	HoS		4
12	*Solanum tuberosum* CGN 17609	3	MR	-	-	-	+	+	+	-	-	HoS	+	HeR		4
13	*Solanum acule* CGN 17674	2	R[d]	-	-	+	+	-	+	-	+	HoR[f]	-	HoS		4
14	*Solanum chacoense* CGN 17903	1	HR	+	-	+	+	-	+	-	+	HoR	-	HoS		5
15	*Solanum stoloniferum* CGN 17605	3	MR	+	+	+	+	+	+	+	+	HoR	-	HoS		8
16	*Solanum demissum* CGN 17788	3	MR	+	+	+	+	-	+	+	+	HoR	-	HoS		7
17	*Solanum demissum* CGN 17797	3	MR	-	-	+	+	-	+	+	+	HoR	-	HoS		5
A total number of bands				12	4	16	11	6	17	8	6		9			

SCAR*: Sequence characterized amplified region, CAPS**: Cleaved amplified polymorphic sequence, HR[a]: Highly resistant, MR[b]: Moderately resistant, MS[c]: Moderately susceptible, R[d]: Resistant, HoS[e]: Homozygous susceptible, HoR[f]: Homozygous resistant, HeR[g]: Heterozygous resistant, +: Presence of band, -: Absence of band

In this study, 17 potato genotypes were tested for the presence or absence of eight resistance (*R*) genes to LB, namely *R1*, *Rpi-phu1*, *Rpi-ber*, *Rpi-blb1*, *Rpi-blb3*, *R9a*, *R8 and R9a*, and *R3a*, using seven SCAR and two CAPS markers. PCR amplicons for the *R1* gene amplified using primers SCAR R1-517 and SCAR 76-2s gave clear polymorphisms between the resistant and susceptible genotypes. The results of PCR of the *Rpi-phu1*, *Rpi-ber*, *Rpi-blb1*, *Rpi-blb3*, and *R9a* genes, using SCAR phu6, SCAR Rpi-ber1-Q133**,** SCAR BLB1, SCAR RGH2, and SCAR Edn61, respectively, also showed polymorphisms between the resistant and susceptible genotypes. For the *Rpi-blb3* gene, the PCR products obtained from the primer set SCAR RGH2 have not displayed any polymorphisms between the resistant and susceptible genotypes. For (*R8* and *R9a*) and *R3a* genes, the results of CAPS markers differentiated between homozygous resistant or susceptible potato genotypes from heterozygous resistant ones. Furthermore, the digestion of fragments by a restriction enzyme revealed clear polymorphisms between susceptible and resistant potato genotypes. Therefore, these polymorphic PCR amplicons could be used as DNA markers in potato breeding programs to differentiate resistant and susceptible genotypes. These results were confirmed by Tiwari *et al*. [10] found that CAPS markers give clear polymorphisms between potato cultivars resistant and susceptible to LB after digestion by a restriction site. Cao *et al*. [33] mentioned that SCAR markers produced from randomly amplified polymorphic DNA and amplified fragment length polymorphism markers are considered important tools in the characterization of *R* genes. These markers depend on the marker sequence data, which enhances the reliability and reproducibility of PCR results [34]. Bisognin *et al*. and Pattanayak *et al*. [3,35] who mentioned that potato breeding programs can be improved with the aid of DNA markers linked to resistance loci against LB.

In the current work, it has been observed that there is no correlation between the number of markers and resistance levels to LB. In spite of, the potato variety Deta has three resistance gene markers; it was moderately resistant to LB disease. On the contrary, potato variety

Bellini contained five resistance gene markers; it was moderately susceptible to LB. These results agree with Sedlák *et al*. [36], who mentioned that there is no correlation between the presence of a marker and the degree of resistance in the plant. Mu *et al*. [37] observed the presence of a negative correlation between a number of stripe rust resistance alleles and disease scores in wheat. Rogozina *et al*. [30] indicated that some potato hybrids having only one or two markers of *Rpi* loci; they were resistant or moderately resistant to LB disease. According to Kim *et al*.; Rogozina *et al*.; and Zhu *et al*. [17,30,38] who discovered that the presence of multiple *R* resistance loci is required for a genotype to give high levels of resistance against *P. infestans*. Bouwman and Fadina *et al*. [11,39] who indicated that conventional hybridization methods using 2-3 resistance genes (stacking genes) give resistance to a broad spectrum of *P. infestans* races, compared with genetic engineering methods.

In this finding, it has been shown that the *Rpi-blb3* gene was the most common in 17 potato genotypes, followed by the *RPi-phu1* gene, while *R1* (using marker SCAR 76-2s) was the least popular in potato genotypes. On the other hand, it has been observed that some domesticated potato cultivars contain *R* genes. These cultivars were introgressed from wild species. Similar findings were reported by Beketova *et al*.; Goss *et al*. [40,41] who reported that the *R* locus was introgressed into different potato cultivars from wild species, involving *R1* to *R11*, *R3a*, *R3b*, and *R9a* from *S. demissum* [27,42,43], *Rpi-blb1*, *Rpi-blb2*, and *Rpi-blb3* from *Solanum bulbocastanum* [25,43,44], and *RPi-phu1* from *Solanum phureja* [22].

5. CONCLUSION

Gene-targeted markers were used successfully to characterize potato genotypes with resistance loci to LB disease. The selection of potato genotypes resistant against LB is the most effective strategy for the control of the pathogen. In this study, the collection of 17 potato genotypes was estimated in the laboratory using the detached leaf assay. Besides, these genotypes were assayed by the seven SCAR and two CAPS markers for *R* genes. According to the study's findings, there is no correlation between LB resistance levels and the number of *R* gene markers. For instance, moderately resistant *S. stoloniferum* CGN 17605 has the highest number of markers (8), followed by highly resistant cultivar Jelly (7). On the contrary, the lowest number of resistance gene markers was scored in the moderately resistant potato genotype Deta (3). Therefore, these genotypes have more than one resistance gene that could be taken into consideration in the potato breeding programs for resistance against LB disease.

6. AUTHORS' CONTRIBUTIONS

Dr. HAM performed SCAR and CAPS markers, and analysis data, Prof. Dr. SAM wrote the manuscript, and Prof. Dr. OEE edited the manuscript.

7. FUNDING

This work was funded by National Research Centre (Project ref. 12020111), Dokki, Giza, Egypt.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All data generated or analyzed during this paper already exist in this paper.

11. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

12. AUTHORS' INFORMATION

Genetics and Cytology Department, Biotechnology Research Institute, National Research Centre (NRC), Dokki, 12622, Egypt.

REFERENCES

1. Fry WE, Birch PR, Judelson HS, Grünwald NJ, Danies G, Everts KL. Five reasons to consider *Phytophthora infestans* a reemerging pathogen. Phytopathology 2015;105:966-81.
2. Gebhardt C, Valkonen JP. Organization of genes controlling disease resistance in the potato genome. Ann Rev Phytopathol 2001;39:79-102.
3. Pattanayak D, Chakrabarti SK, Naik PS. Genetic diversity of late blight resistant and susceptible Indian potato cultivars revealed by RAPD markers. Euphytica 2002;128:183-9.
4. Bradshaw JE. Potato breeding at the Scottish plant breeding station and the Scottish Crop Research Institute: 1920-2008. Potato Res 2009;52:141-72.
5. Danan S, Chauvin JE, Caromel B, Moal JD, Pellé R, Lefebvre V. Major-effect QTLs for stem and foliage resistance to late blight in the wild potato relatives *Solanum sparsipilum* and *S. spegazzinii* are mapped to chromosome X. Theor Appl Genet 2009;119:705-19.
6. Rojas DK, Sedano JC, Ballvora A, León J, Vásquez TM. Novel organ-specific genetic factors for quantitative resistance to late blight in potato. PLoS One 2019;14:e0213818.
7. Chen SH, Borza T, Byun B, Coffin R, Coffin J, Peters R, *et al*. DNA markers for selection of late blight resistant potato breeding lines. Am J Plant Sci 2017;8:1197-209.
8. Hein I, Gilroy EM, Armstrong MR, Birch PRJ. The zigzag-zig in oomycete-plant interactions. Mol Plant Pathol 2009;10:547-62.
9. Tanksley SD, Young ND, Paterson AH, Bonierbale MW. RFLP mapping in plant breeding: New tools for old science. Nat Biotechnol 1989;7:257-64.
10. Tiwari JK, Siddappa S, Singh BP, Kaushik SK, Chakrabarti SK, Bhardwaj V, *et al*. Molecular markers for late blight resistance breeding of potato: an update. Plant Breed. 2013;132:237-45.
11. Fadina OA, Beketova MP, Sokolova EA, Kuznetsova MA, Smetanina TI, Rogozina EV, *et al*. Anticipatory breeding: Molecular markers as a tool in developing donors of potato (*Solanum tuberosum* L.) late blight resistance from complex interspecific hybrids. Agric Biol. 2017;52:84-94.
12. Gebhardt C, Ballvora A, Walkemeier B, Oberhagemann P, Schüler K. Assessing genetic potential in germplasm collections of crop plants by marker-trait association: A case study for potatoes with quantitative variation of resistance to late blight and maturity type. Mol Breeding. 2004;13:93-102.
13. Świeżyński KM, Zimnoch E. Breeding potato cultivars with tubers resistant to *Phytophthora infestans*. Eur Potato J 2001;44:97-117.
14. Tresnik S. State of the art of integrated crop management and organic systems in europe with particular reference to pest management potato production. Pesticide Action Network (PAN) Europe; 2007.

Available from: http://www.pan-europe.info/Resources/Reports/Potato_production_review.pdf
15. Twining S, Clarke J, Cook S, Ellis S, Gladders P, Ritchie F, *et al*. Research Report: Pesticide availability for potatoes following revision of directive impact assessments and identification of research priorities; 2009. Available from: http://www.potato.org.uk
16. Barone A. Molecular marker-assisted selection for potato breeding. Am J Potato Res 2004;81:111-7.
17. Kim HJ, Lee HR, Jo KR, Mortazavian SM, Huigen DJ, Evenhuis B, *et al*. Broad spectrum late blight resistance in potato differential set plants *MaR8 and MaR9* is conferred by multiple stacked *R* genes. Theor Appl Genet 2012;124:923-35.
18. Mahfouze HA, Ahmed HZ, El-Sayed OE. Gene expression of pathogenesis-related proteins and isozymes in potato varieties resistant and susceptible to late blight disease. Int J Agric Biol 2021;26:490-8.
19. Henfling JW. Late blight of potato: *Phytophthora infestans*. Technical Information Bulletin. Lima, Peru: International Potato Center; 1979. p. 13.
20. Beketova MP, Sokolova EA, Rogozina EV, Kuznetsova MA, Khavkin EE. Two orthologs of late blight resistance gene *R1* in wild and cultivated potato. Russ J Plant Physiol 2017;64:718-27.
21. Ballvora A, Ercolano MR, Weiss J, Meksem K, Bormann CA. The *R1* gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine Zipper/NBS/LRR class of plant resistance genes. Plant J 2002;30:361-71.
22. Śliwka J, Swiatek M, Tomczynska I, Stefanczyk E, Chmielarz M, Zimnoch-Guzowska E. Influence of genetic background and plant age on expression of the potato late blight resistance gene *Rpi-plu1* during incompatible interactions with *Phytophthora infestans.* Plant Pathol 2013;62:1072-80.
23. Tan MY, Hutten RC, Visser RG, Eck HJ. The effect of pyramiding *Phytophthora infestans* resistance genes *RPi-mcd1* and *RPi-ber* in potato. Theor Appl Genet 2010;121:117-25.
24. Wang M, van der Vossen EA, Vosman B. Allele mining in *Solanum*: Conserved homologues of *Rpi-blb1* are identified in *Solanum stoloniferum*. Theor Appl Genet 2008;116:933-43.
25. Park TH, Gros J, Sikkema A,Vleeshouwers VG, Muskens M, van der Vossen EA. The late blight resistance locus *Rpi-bib3* from *Solanum bulbocastanum* belongs to a major late blight *R* gene cluster on chromosome 4 of potato. Mol Plant Microb Interact 2005;18:722-29.
26. Verzaux E. Resistance and susceptibility to late blight in *Solanum*: gene mapping, cloning and stacking. Wageningen: PhD Thesis, Wageningen University; 2010.
27. Jo KR, Visser RG, Jacobsen E, Vossen JH. Characterization of the late blight resistance in potato differential *MaR9* reveals a qualitative resistance gene, *R9a*, residing in a cluster of *Tm-22* homologs on chromosome IX. Theor Appl Genet 2015;128:931-41.
28. Huang S, Vleeshouwers V, Werij JS, Hutten R, van Eck H, Visser R, *et al*. The *R3* resistance to Phytophthora *P. infestans* in potato is conferred by two closely linked *R* genes with distinct specificities. Mol Plant Microb Interact 2004;17:428-35.
29. Arens P, Mansilla C, Deinum D, Cavellini L, Moretti A, Rolland S, *et al*. Development and evaluation of robust molecular markers linked to disease resistance in tomato for distinctness, uniformity and stability testing. Theor Appl Genet 2010;120:655-64.
30. Rogozina EV, Beketova MP, Muratova OA, Kuznetsova MA, Khavkin EE. Stacking resistance genes in multiparental interspecific potato hybrids to anticipate late blight outbreaks. Agronomy 2021;11:115.
31. Fry WE. *Phytophthora infestans*: New tools (and old ones) lead to new understanding and precision management. Annu Rev Phytopathol 2016;54:529-47.
32. Orlowska E, Liorente B, Cvitanich C. Plant integrity: An important factor in plant-pathogen interactions. Plant Signal Behav 2013;8:e22513.
33. Cao W, Hughes GR, Ma H, Dong Z. Identification of molecular markers for resistance to *Septoria nodorum* blotch in durum wheat. Theor Appl Genet 2001;102:551-4.
34. Lee SP, Kim KJ, Jung HS, Kim HY, Cho HM, Park YE, *et al*. Development of AFLP derived SCAR marker linked to disease resistance to late blight (*Phytophthora infestans*) in potato. Korean J Breed 2005;37:79-85.
35. Bisognin DA, Douches DS. Genetic diversity in diploid and tetraploid late blight resistant potato germplasm. Hortic Sci 2002;37:178-83.
36. Sedlák P, Vejl P, Melounová M, Křenek P, Domkářová J, Zoufalá J. Characterization of resistance genes resources against late blight available for Czech potato breeding by means of selected DNA markers. Plant Soil Environ 2005;51:82-6.
37. Mu J, Liu L, Liu Y, Wang M, See DR, Han D, *et al*. Genome-wide association study and gene specific markers identified 51 genes or QTL for resistance to stripe rust in U.S. winter wheat cultivars and breeding lines. Front Plant Sci 2020;11:998.
38. Zhu S, Li Y, Vossen JH, Visser RG, Jacobsen E. Functional stacking of three resistance genes against *Phytophthora infestans* in potato. Transgenic Res 2012;21:89-99.
39. Bouwman J. Durable late blight resistance in potato (*Solanum tuberosum*): *R* gene mapping and stacking. Wageningen: PBR-80436 MSc Thesis, Plant Science, Wageningen University and Research Centre; 2014.
40. Beketova MP, Drobyazina PE, Khavkin EE. The *R1* gene for late blight resistance in early and late maturing potato cultivars. Russ J Plant Physiol 2006;53:384-9.
41. Goss EM, Tabima, JF, Cooke DE, Restrepo S, Fry WE, Forbes GA, *et al*. The Irish potato famine pathogen *Phytophthora infestans* originated in Central Mexico rather than the Andes. Proc Natl Acad Sci 2014;111:8791-6.
42. Huang SW, van der Vossen EA, Kuang H, Vleeshouwers VG, Zhang N, Borm TJ. Comparative genomics enabled the isolation of the *R3a* late blight resistance gene in potato. Plant J 2005;42:251-61.
43. Lokossou AA, Park TH, van Arkel G, Arens M, Ruyter-Spira C, Morales J. Exploiting knowledge of *R/Avr* genes to rapidly genotype a new LZ-NBSLRR family of late blight resistance genes from potato linkage group IV. Mol Plant Microb Interact 2009;22:630-41.
44. Van der Vossen EA, Gros J, Sikkema A, Muskens M, Wouters D, Wolters P. The *Rpi-blb2* gene from *Solanum bulbocastanum* is a *Mi-1* gene homolog conferring broad-spectrum late blight resistance in potato. Plant J 2005;44:208-22.

How to cite this article:
Mahfouze HA, El-Sayed OE, Mahfouze SA. Characterization of resistance genes to late blight (Phytophthora infestants) in potato by marker-assisted selection. J App Biol Biotech. J App Biol Biotech 2023;11(2):178-186.
DOI: 10.7324/JABB.2023.110218