Characterization of resistance genes to late blight (Phytophthora infestans) 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.

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...
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

Elevens 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 × 10⁶ 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-pha1*, *Rpi-her*, *Rpi-bib1*, and *Rpi-bib3* 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 MgCl₂, 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) (INIRON 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. acule* CGN 17674 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, or sporulation were not clear. They were recorded as highly resistant, such as 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*](http://www.wur.nl) 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, *Solanum 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 containing (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-pha1*, *Rpi-
Table 1: Sequence of primers used in this study.

<table>
<thead>
<tr>
<th>Specific primers/marker type</th>
<th>Resistance gene name</th>
<th>Chromosome No.</th>
<th>Annealing temperature (AT) °C</th>
<th>Primer name</th>
<th>Product size (bp)</th>
<th>Forward (3'-5')</th>
<th>Reverse (5'-3')</th>
<th>Solanum species</th>
<th>Ref. and accessions used for primers in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific primers</td>
<td>R3a</td>
<td>XI</td>
<td>55</td>
<td>R3a1</td>
<td>194</td>
<td>Tgtcgggttcagatgtg</td>
<td>Tgtcgggttctg</td>
<td>Solanum tuberosum</td>
<td>Accession (AY849382) (present study)</td>
</tr>
<tr>
<td>R3a</td>
<td>XI</td>
<td>55</td>
<td>R3a2</td>
<td>247</td>
<td>Gcagacagtgaacacctcg</td>
<td>Cggatcgtgcttcagga</td>
<td>Ggtcgtgctcctg</td>
<td>Solanum tuberosum</td>
<td>Accession (AY849382) (present study)</td>
</tr>
<tr>
<td>R3b</td>
<td>XI</td>
<td>55</td>
<td>R3b1</td>
<td>226</td>
<td>Ggatgcgtgcaagatgtt</td>
<td>Taggctgctcaagatag</td>
<td>Ggtcgtgcctg</td>
<td>Solanum demissum</td>
<td>Accession (JF900492) (present study)</td>
</tr>
<tr>
<td>R3b</td>
<td>XI</td>
<td>55</td>
<td>R3b2</td>
<td>244</td>
<td>Gcggaaacagtggcattta</td>
<td>Aagggaagccctctgcag</td>
<td>Ggtcgtgcctg</td>
<td>Solanum demissum</td>
<td>Accession (JF900492) (present study)</td>
</tr>
<tr>
<td>R8</td>
<td>IX</td>
<td>55</td>
<td>R8a</td>
<td>220</td>
<td>Gttgcatctctcaaggtca</td>
<td>Tctcattgcggaactc</td>
<td>Ggtcgtgcctg</td>
<td>Solanum demissum</td>
<td>Accession (KU530153) (present study)</td>
</tr>
<tr>
<td>R8</td>
<td>IX</td>
<td>55</td>
<td>R8b</td>
<td>237</td>
<td>Accttttctcagacatca</td>
<td>Ctcacagatgcgagatg</td>
<td>Ggtcgtgcctg</td>
<td>Solanum demissum</td>
<td>Accession (KU530153) (present study)</td>
</tr>
<tr>
<td>SCARs</td>
<td>R1</td>
<td>V</td>
<td>58</td>
<td>R1-517</td>
<td>517</td>
<td>Gaattggaaggtagaagatgcaaggt</td>
<td>Gtagcactacatttcgctcag</td>
<td>Solanum demissum</td>
<td>[20]</td>
</tr>
<tr>
<td>R1</td>
<td>V</td>
<td>60</td>
<td>76-2s</td>
<td>1400</td>
<td>Cacgctgcatctcatctc</td>
<td>Cacgctgcatctcag</td>
<td>Ggtcgtgcctg</td>
<td>Solanum tuberosum</td>
<td>[21]</td>
</tr>
<tr>
<td>Rpi-phi1</td>
<td>IX</td>
<td>55</td>
<td>phu6</td>
<td>298</td>
<td>Agagacacctcaatttactgct</td>
<td>Gctctgctcagacggtcagttcag</td>
<td>Ggtcgtgcctg</td>
<td>Solanum phureja</td>
<td>[22]</td>
</tr>
<tr>
<td>Rpi-ber</td>
<td>X</td>
<td>50</td>
<td>Rpi-ber1- Q133</td>
<td>395</td>
<td>Acatctgctcagatagcag</td>
<td>Acatctgctcagatagcag</td>
<td>Ggtcgtgcctg</td>
<td>Solanum berthaultii</td>
<td>[23]</td>
</tr>
<tr>
<td>Rpi-bb1</td>
<td>VIII</td>
<td>58</td>
<td>BLB1</td>
<td>821</td>
<td>Aacgctgctgtagccagt</td>
<td>Gtccaggaagggcactctg</td>
<td>Ggtcgtgcctg</td>
<td>Solanum stoloniferum</td>
<td>[24]</td>
</tr>
<tr>
<td>Rpi-bb3</td>
<td>IV</td>
<td>50</td>
<td>RGH2</td>
<td>320</td>
<td>Gcgaagacagtctgcaag</td>
<td>Tgcagatacyccatctg</td>
<td>Ggtcgtgcctg</td>
<td>Solanum bulbocastanum</td>
<td>[25]</td>
</tr>
<tr>
<td>R9a</td>
<td>IX</td>
<td>54</td>
<td>EDN61</td>
<td>450</td>
<td>Gcctggatcgtgcaacctag</td>
<td>Cttgctgctgctgctg</td>
<td>Ggtcgtgcctg</td>
<td>Solanum ediriae</td>
<td>[26]</td>
</tr>
<tr>
<td>CAPS</td>
<td>R8&amp;R9a</td>
<td>IX</td>
<td>58</td>
<td>184-81F</td>
<td>680</td>
<td>Cacgctgctgctgctg</td>
<td>Gtccaggaagggcactctg</td>
<td>Solanum demissum</td>
<td>[27]</td>
</tr>
<tr>
<td>R3a</td>
<td>XI</td>
<td>54</td>
<td>TG105</td>
<td>650</td>
<td>Tcagcctgctgtagcagatg</td>
<td>Aagggcctgctgtagcag</td>
<td>Ggtcgtgcctg</td>
<td>Solanum tuberosum</td>
<td>[28]</td>
</tr>
</tbody>
</table>

CAPS: Cleaved amplified polymorphic sequence, SCARs: Sequence-characterized amplified regions
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 potato genotypes [Figure 3 and Table 2]. Moreover, the Rpi-bfb3 gene amplified by primer combination SCAR RGH2 gave one amplicon with the expected size of 320 bp in all tested potato genotypes 

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. demissum 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-bfb3. 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-bfb1, and Rpi-bfb3 genes using different sequence-characterized amplified region primers amplified from 17 potato genotypes. Marker: 100 bp DNA ladder.
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].
In this study, 17 potato genotypes were tested for the presence or absence of eight resistance (R) genes to LB, namely R1, Rpi-pha1, 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 the Scars of the Rpi-pha1, 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 or susceptible 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


Available from: http://www.pan-europe.info/Resources/Reports/Potato_production_review.pdf


