Virulence Patterns of *Phytophthora infestans* Isolates using R Differential Set of *Solanum demissum*: A Useful Tool to Identify Pathogen Races in Tunisia.

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**ABSTRACT**


The current study uses a representative set of 31 Tunisian *Phytophthora infestans* isolates selected according to geographic diversity to analyze the virulence diversity of *P. infestans* via plant disease bioassays. Thirty-one isolates were tested onto detached leaves of a differential set of *Solanum demissum* having 11 late blight resistance (R) genes. Of these isolates, twenty-one races were characterized. *P. infestans* isolates showed different virulence patterns depending on sampling regions. While virulence patterns were found to be diverse in Korba and Bizerte, they seemed to be less diverse in Takelsa and North-West. Moreover, in all sampling regions, virulence patterns never followed the chronological level (year) of sampling. The most effective R genes recorded in Tunisia are R5, R9 and R8 that withstood more than 90% of tested isolates. These R genes could be useful in breeding programs. Furthermore, the presence of individuals with less than four virulence factors and that the 2 virulence factor races were the most frequent races in the population could be due to the possible presence of avirulent and heterozygous isolates. This implies that *P. infestans* population in Tunisia has not undergone high mutations in Avr genes contrarily to what was reported in other countries. The diagnostic of virulence in *P. infestans* needs a useful and high throughput technique reliable and linked to the bioassay screening that could replace neutral markers (allozymes, SSR markers, mtDNA). Combining phenotypic (disease bioassay) and genotypic techniques (virulence markers) could be a very interesting tool to identify the pathogen population structure and find a rapid solution for the break-down of resistance of potato cultivars.

**Keywords**: Avr/R genes, bioassays, *Phytophthora infestans*, sampling regions, Tunisia

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*Phytophthora infestans*, the causal agent of the potato late blight, is the most destructive disease of potato worldwide.
Race-specific resistance is the well-known genetic resistance to *P. infestans* in *Solanum* species. It is characterized by interactions between dominant resistance (R) gene alleles and corresponding avirulence (Avr) alleles to trigger a rapid, localized and programmed cell death known as the hypersensitive response (HR). This pathosystem conforming to the gene-for-gene model was postulated first by Flor (6). Introgression breeding has been used to introduce disease resistance in many crop species. In potato, this has resulted in breeding lines and cultivars that are resistant to different races of the late blight pathogen (5, 15). Initially, 11 different recognition specificities were identified, all originating from *Solanum demissum* and named R1 to R11. This was the basis for a differential set of potato cultivars used worldwide to identify the virulence races occurring in *P. infestans* population (2, 4, 17, 21). Following the gene-for-gene model, pathogens evolve by mutating their Avr genes to evade plant recognition (14). Recently, many authors (9, 7, 10, 20, 23) reported that this ability leads to new races that are no longer recognized by the R proteins. *Phytophthora* Avr proteins, that have been identified so far, share a conserved motif named RXLR and belong to a highly dynamic superfamly consisting of more than 500 genes in the *P. infestans* genome (1, 7, 9, 10, 13). These discoveries lead to i) the importance of the screening of virulence behavior of strains against known R differentials and ii) the necessity of a molecular tool to underline virulence in *P. infestans* by developing marker systems based on single nucleotide polymorphism (SNP) detection in RXLR effector genes. Contrary to what is challenging in avirulence domain of *P. infestans* populations in many parts of the world, little is known about the pathogen in Tunisia. Recently, many efforts were implemented to learn more about *P. infestans* strains and better understand the population and race structure of this disastrous pathogen in Tunisia. Therefore, a pathogen population collected from different crop regions in the North of Tunisia was characterized using disease resistance bioassays in order to reveal its phenotypic diversity and virulence patterns. This could be a useful tool to follow the behavior of each race in the field and the temporal progress of late blight damage from year to year for developing a suitable disease management program.

**MATERIALS AND METHODS**

**Plant material.** The plant material used in this study consists on a Dutch set of 11 *S. demissum* carrying the major R genes and selected by Mastenbroek in 1953 (Table 1). Each line in this set carried one known R gene and the cultivar Bintje that has no known R gene was used as a positive control. The plant material (i.e. vitroplants) was cultured in the greenhouse. Leaves of each line were harvested four to five weeks after transferring the vitroplants to the greenhouse. Healthy and fresh leaves were selected from the middle part of the stem, while older and senescent ones were avoided.

**Pathogen isolates.** Thirty-one *P. infestans* isolates were selected from a Tunisian population (11) originating from potato and tomato plants collected from different regions (Table 2). Samplings were carried out from different hosts, organs, regions and seasons during 2006, 2007 and 2008. Small pieces from the biotrophic part of blighted areas (leaf, stem, tuber, and fruit), freshly collected from fields or greenhouses, were placed in Petri dishes on disinfected potato tuber...
slices and incubated at 18°C and 16 h light/8 h dark cycle during 6 to 7 days in a climate chamber. The mycelium growing on the upper face of the slice was transferred to fresh rye agar (RA) medium amended with rifampicin (24 mg/l). *P. infestans* mycelia were purified by repetitive transfers to RA amended with rifampicin and microscopic checks. Pure cultures were maintained at 18°C in the dark until further use.

Table 1. Differential set of *Solanum demissum* used in the study

<table>
<thead>
<tr>
<th>Clone</th>
<th>Known R gene</th>
<th>Identity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MaR0</td>
<td>No</td>
<td>Bintje</td>
<td>-</td>
</tr>
<tr>
<td>MaR1</td>
<td>R1</td>
<td>CEBECO 43154-5</td>
<td>Mastenbroek, 1953</td>
</tr>
<tr>
<td>MaR2</td>
<td>R2</td>
<td>CEBECO 44158-4</td>
<td>Mastenbroek, 1953</td>
</tr>
<tr>
<td>MaR3</td>
<td>R₃ₐ, R₃₉</td>
<td>CEBECO 4642-1</td>
<td>Mastenbroek, 1953</td>
</tr>
<tr>
<td>MaR4</td>
<td>R4</td>
<td>CEBECO 4431-5</td>
<td>Mastenbroek, 1953</td>
</tr>
<tr>
<td>MaR5</td>
<td>R5</td>
<td>Black 3053-18</td>
<td>Mastenbroek, 1953</td>
</tr>
<tr>
<td>MaR6</td>
<td>R6</td>
<td>Black XD2-21</td>
<td>Mastenbroek, 1953</td>
</tr>
<tr>
<td>MaR7</td>
<td>R7</td>
<td>Black 2182 ef (7)</td>
<td>Mastenbroek, 1953</td>
</tr>
<tr>
<td>MaR8</td>
<td>R8</td>
<td>Black 2424 a (5)</td>
<td>Mastenbroek, 1953</td>
</tr>
<tr>
<td>MaR9</td>
<td>R9 or R1R2R3R9</td>
<td>Black 2573 (2)</td>
<td>Mastenbroek, 1953</td>
</tr>
<tr>
<td>MaR10</td>
<td>R10</td>
<td>Black 3681 ad (1)</td>
<td>Mastenbroek, 1953</td>
</tr>
<tr>
<td>MaR11</td>
<td>R11</td>
<td>Black 5008 ab (6)</td>
<td>Mastenbroek, 1953</td>
</tr>
</tbody>
</table>

**Virulence bioassay.** *P. infestans* isolates (Table 1) were used to inoculate a differential set of *S. demissum* (15) carrying the major resistance R genes (1-11). Cultivar Bintje was used as a positive control of infection as it has no known R genes. Detached leaves, cut from each potato genotype, were inoculated with 10 µl of zoospore suspension adjusted at 10⁴ zoospores/ml prepared from fresh mycelia grown on RA flooded by 5 ml of sterile distilled water and incubated for 2 h at 4°C to stimulate the release of zoospores from sporangia. From each potato genotype, two freshly cut leaves were incubated in water-saturated florists foam with abaxial side up on moist filter paper in a tray (60 × 25 cm). For each leaf, five droplets of 10 µl of zoospore suspension (10⁴ zoospores/ml) were placed in one side of each of 5 individual leaflets. In order to visually evaluate symptoms between two isolates, the other side of the same leaf was inoculated by another isolate by the same method. Trays covered by plastic bags were placed at 18°C under a photoperiod of 16 h light/8 h dark cycle during 7 to 8 days. Disease scoring was determined based on infection efficiency (IE) and macroscopic observation. IEs were determined by dividing the number of lesions per leaf by the number of inoculation points of infection (10 per experiment). The *Solanum* genotypes were classified as resistant (R) or susceptible (S) if IE was lower or higher than 20%, respectively. Macroscopic scoring was performed by examining the leaves and rating the phenotype based on a scale of 8 from resistant to susceptible (R0, R1, R2, R3, S3, S2, S1, S0) depending on the infected area and the degree of sporulation in each point of plant-pathogen interaction. Results were confirmed in two independent experiments. Virulence
patterns of each isolate were deduced from resistance/susceptibility patterns of each *Solanum* genotype. Numbers of virulence factors for each isolate were assessed from virulence patterns in order to deduce arbitrary classes called virulent, intermediate and avirulent. The isolate was ranged at an avirulent class when it has between 0 and 2 virulence factors. It was ranged at an intermediate class if it has 3 or 4 virulence factors. The isolate was classified at a virulent class when it has 5 virulence factors or more.

Table 2. Tunisian *Phytophthora infestans* isolates used in the current study

<table>
<thead>
<tr>
<th>Location</th>
<th>No</th>
<th>Sample ID</th>
<th>Crop season</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>North-East-DA</td>
<td>1</td>
<td>TU0625</td>
<td>Season</td>
<td>Potato</td>
</tr>
<tr>
<td>North-East-K</td>
<td>11</td>
<td>TU0609, TU0619, TU0779, TU0806,</td>
<td>Early season</td>
<td>Potato</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TU0818, TU0827, TU0862, TU0756,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TU0789, TU0790, TU0816</td>
<td>Late season</td>
<td>Potato</td>
</tr>
<tr>
<td>North-East-T</td>
<td>4</td>
<td>TU0618 TU0646, TU0765</td>
<td>Late season</td>
<td>Potato</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TU0614</td>
<td>Early season</td>
<td>Tomato</td>
</tr>
<tr>
<td>North-East-S</td>
<td>1</td>
<td>TU0758</td>
<td>Late season</td>
<td>Potato</td>
</tr>
<tr>
<td>North-GM</td>
<td>7</td>
<td>TU0629, TU0630, TU0793, TU0849,</td>
<td>Late season</td>
<td>Potato</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TU0850, TU0856, TU0858</td>
<td></td>
<td></td>
</tr>
<tr>
<td>North-West-J</td>
<td>2</td>
<td>TU0645, TU0899</td>
<td>Late season</td>
<td>Potato</td>
</tr>
<tr>
<td>North-West-BS</td>
<td>2</td>
<td>TU0843, TU0846</td>
<td>Late season</td>
<td>Potato</td>
</tr>
<tr>
<td>North-West-G</td>
<td>2</td>
<td>TU0785, TU0786</td>
<td>Late season</td>
<td>Potato</td>
</tr>
<tr>
<td>North-West-MB</td>
<td>1</td>
<td>TU0784</td>
<td>Late season</td>
<td>Potato</td>
</tr>
</tbody>
</table>


RESULTS

Phenotypic diversity among geographic sites. The phenotypic diversity of *P. infestans* isolates was relatively dependent on prospected sites. In fact, the most diverse geographic sites are Korba and Bizerte and the less diverse ones are Takelsa and North-West. *P. infestans* isolates collected from Korba were ranged either in avirulent, intermediate or virulent class. They were represented by almost all races and the highly virulent isolate (TU0609) belonged to this region. In Bizerte, virulence patterns also fluctuate from the lowest (0 virulence factor) to the highest (6 virulence factors) profiles. However, in the North-West region, pathogen isolates exhibited at least intermediate virulence and the highest number of virulence factors detected was 4. Also, race 0 was detected twice in this geographic region. Furthermore, virulence patterns of most *P. infestans* isolates, belonging to the same class based on the number of virulence factors, were similar or nearly similar (TU0785, TU0843 and TU0899). In addition, all isolates collected from Takelsa (North-EastT) were arranged in avirulent and intermediate virulence classes. Elsewhere, in all sampling regions, virulence patterns of *P. infestans* isolates did not present a chronologic trend variation. Indeed, isolates collected between 2006 and 2008 were found in all three classes revealed in this study.

Virulence spectrum of *P. infestans* in Tunisia. The virulence spectrum of Tunisian *P. infestans* isolates was highly variable. Of 31 isolates tested, twenty-one...
races were characterized (Table 3) and eight categories, depending on the number of virulence factors, were deduced (Fig. 1; Table 3). Almost all isolates were virulent on one or more R differentials except three isolates that were race 0. The number of isolates in each category varied from 1 to 7. The most frequent category in the tested \textit{P. infestans} population was 2VF followed by 3VF and 4VF. Categories 0VF and 6VF were present with equal proportions in the population and each one comprised three isolates. The lowest frequency was detected for 5VF and 7VF categories. Indeed, the highly virulent pattern (7VF) was detected in only one isolate (TU0609) of the population tested.

\begin{table}
\centering
\caption{Phenotypic diversity of \textit{Phytophthora infestans} isolates among sampling regions}
\begin{tabular}{llllll}
\hline
Isolate & Host & Location & Virulence pattern & VF & Class \\
\hline
TU0846 & Potato & NorthWest BS & 0 & 0VF & A \\
TU0784 & Potato & NorthWest MB & 0 & V & \\
TU0849 & Potato & North area & 0 & I & \\
TU0630 & Potato & North area & 1 & 1VF & R \\
TU0806 & Potato & North-East K & 1 & U & \\
TU0862 & Potato & North-East K & 1 & L & \\
TU0614 & Tomato & North-East T & 1 & E & \\
TU0645 & Potato & NorthWest J & 1.2 & 2VF & N \\
TU0786 & Potato & NorthWest G & 1.7 & T & \\
TU0765 & Tomato & North-East T & 1.7 & & \\
TU0779 & Potato & North-East K & 1.4 & & \\
TU0827 & Potato & North-East K & 1.4 & & \\
TU0619 & Potato & North-East K & 4.8 & & \\
TU0818 & Potato & North-East K & 3.7 & & \\
TU0756 & Potato & North-East K & 1.6.7 & 3VF & IN \\
TU0625 & Potato & North-East DA & 1.4.6 & T & \\
TU0646 & Tomato & North-East T & 1.4.6 & E & \\
TU0816 & Potato & North-East K & 1.4.6 & R & \\
TU0858 & Potato & North area & 1.3.7 & M & \\
TU0899 & Potato & NorthWest J & 1.2.7 & E & \\
TU0785 & Potato & NorthWest G & 1.2.3.7 & 4VF & DI \\
TU0843 & Potato & NorthWest BS & 1.2.3.7 & A & \\
TU0850 & Potato & North area & 1.2.3.7 & T & \\
TU0618 & Potato & North-East T & 1.3.6.7 & E & \\
TU0758 & Tomato & North-East S & 1.4.6.7 & & \\
TU0793 & Potato & North area & 1.2.4.6.7 & 5VF & VI \\
TU0629 & Potato & North area & 1.3.6.7.11 & R & \\
TU0789 & Potato & North-East K & 1.3.4.6.10.11 & 6VF & U \\
TU0790 & Potato & North-East K & 1.3.4.7.10.11 & L & \\
TU0856 & Potato & North area & 1.2.3.7.10.11 & EN & \\
TU0609 & Potato & North-East K & 1.2.3.4.6.10.11 & 7VF & T \\
\hline
\end{tabular}
\end{table}

Useful R genes to be integrated in Tunisian crops. To investigate the importance of different R genes in Tunisia, we deduced from the virulence bioassay the proportion of each R gene presented in the R differentials that withstood the tested isolates. Data from Fig. 2 shows that all isolates tested were avirulent on the R5 differential (Black 3053-18) and R9 differential (Black 2573 (2)) followed by R8 (Black 2424 a (5)) (97%), R10 (Black 3681 ad (1)) and R11 (Black 5008 ab (6)) (87%). The R2 differential (CEBECO 44158-4)
withstood at 23 out of 31 isolates (< 75%) followed by R3 (CEBECO 4642-1) and R4 (CEBECO 4431-5) (20 out of 31) and R6 (Black XD2-21) (21 out of 31). The R1 differential (CEBECO 43154-5) withstood only to 6 out of the 31 isolates (< 20%) followed by R7 (Black 2182 ef (7)) that was resistant to 15 isolates (50%). Therefore, the most effective R
gene in the differential set used here is R5 and R9. Although R10 and R11 were withstood at almost all tested isolates, they were overcome by highly complex races in the *P. infestans* population tested. Hence, nine R differentials were resistant to more than 50% of the isolates tested and only R1 and R7 differentials were relatively overcome by most of them.

![Fig. 1. Different virulence factors categories in the *Phytophthora infestans* population tested sorted by individual number.](image1)

![Fig. 2. Percentage of avirulence on the potato differential set (R1-R11) for a representative set of Tunisian *Phytophthora infestans* isolates.](image2)

**DISCUSSION**

The phenotypic diversity within *P. infestans* seems to be relatively high within and among regions prospected. The presence of 21 races among 31 isolates collected leads to the conclusion that the whole population may have a diverse virulence spectrum. The most widely virulent isolates were collected from Korba and Bizerte areas whereas the most avirulent isolates were from Takelsa and the North-West region. The lack of Avr factors in *P. infestans* population of Korba and Bizerte and the high number of races in both areas could explain the changes of the race structure of *P. infestans* in recent years. However, in both Takelsa and North-West areas, we recorded the presence of more Avr factors. Elsewhere, high virulence diversity was also reported in many countries in the world (3, 10, 17, 18, 21).

On the other hand, some R genes from *S. demissum* (R5, R8, R9, R10, and R11) could be still effective within some regions. The R5, R9 and R8 differentials that were capable to withstand to almost all tested isolates seem to be highly useful in Tunisia. These R genes could be effective when introduced into potato cultivars (*S. tuberosum*) and deployed in Tunisia. These results differ from those reported in Toluca Valley region, Mexico, where race 5, race 8 and race 9 were detected in more than the half of tested isolates (16). Nevertheless, our results confirmed those of Guo *et al.* (10) who
concluded that R5, R8 and R9 were the most resistant differentials against a Chinese population of *P. infestans*. Furthermore, although R10 and R11 differentials were resistant to more than 80% of the total isolates, they were overcome by highly complex races in the population (1.3.4.6.10.11, 1.3.4.7.10.11, 1.2.3.7.10.11 and 1.2.3.4.6.10.11). Probably, the breakdown of the resistance of these differentials could be caused by the new generation of the pathogen (most complex races). The R2 differential was resistant to 75% of the isolates tested but can be overcome by more complex races. R3, R4 and R6 differentials confer moderate resistance to some isolates especially those collected from the North-East (Korba and Solimane) and North (Bizerte) areas. R1 and R7 differentials were infected by tested isolates and races 1 and 7 were detected in all prospected regions.

Elsewhere, the rearrangement of the population and the breakdown of several R genes were probably related to the change in the genetic structure of the pathogen recorded in the recent years. Indeed, the occurrence of the sexual reproduction and the presence of the A2 mating type of *P. infestans* in Korba and Bizerte (12) contributed at the emergency of new races more complex and currently localized in these regions as reported elsewhere (8, 16, 19, 22).

Although the high diversity and the race complexity detected in the population, the virulence spectrum of this *P. infestans* population could be still comforting compared to the findings reported in Mexico twenty years ago (16) in which more than the half of races found had more than six virulence factors. In addition, the presence of individuals with less than four virulence factors and that the 2 virulence factor races were the most frequent races in the population could be due to the possible presence of avirulent and/or heterozygous isolates.

Therefore, the diagnostic of the virulence in *P. infestans* needs a useful and a high throughput technique more reliable and complemented to the bioassay screening. Nowadays, this tool is available and is currently being optimized for two Avr effectors (Avr3a and Avr4) by real-time PCR technique via Taq-Man technology in the Plant Research International in Wageningen University, the Netherlands. Indeed, this new technique of SNPs (Single Nucleotide Polymorphisms) detection between avirulent and virulent alleles was performed for two effectors Avr3a and Avr4 for screening a Tunisian population of *P. infestans* (11) and more Avr effectors will be performed in the coming years. Altogether, with the importance of virulence screening of *P. infestans* strains, both phenotypic diversity (disease bioassay) and genotypic diversity (SNPs detection) will be the new way for genetic characterization of *P. infestans* populations that will replace neutral markers (allozymes, SSR markers, mtDNA). This could be very useful to screen and monitor the population structure and understand better the breakdown of resistance in potato cultivars by the highly complex races of the pathogen.

**RESUME**

L'étude en cours consiste à l'utilisation d'une population représentative de 31 isolats tunisiens de Phytophthora infestans choisis selon leurs diversités géographiques afin d’analyser la virulence de P. infestans par des essais biologiques in planta. Trente et un isolats ont été utilisés pour inoculer des feuilles détachées d’une série différentielle de 11 clones appartenant à Solanum demissum ayant de 1 jusqu’à 11 gènes R de résistance au mildiou. Vingt et une races ont été caractérisées à partir des 31 isolats testés. Ces isolats ont montré une diversité phénotypique variable en fonction des régions de collecte. Bien que les profils de virulence soient relativement diversifiés dans les deux régions Korba et Bizerte, ils ont été moins diversifiés dans les régions de Takelsa et du Nord-Ouest. Par ailleurs, les profils de virulence des isolats collectés à partir de différentes régions n’ont jamais suivi le niveau chronologique (année) d’échantillonnage. Ceux les plus résistants en Tunisie sont R5, R9 et R8 qui sont résistants à plus de 90% des isolats testés. Ces gènes pourraient être très utiles dans les programmes d’amélioration de la pomme de terre. En d’autres termes, la présence de races à 4 facteurs de virulence ou moins et le fait que les races avec 2 facteurs de virulence sont les plus prépondérants dans la population pourraient être expliqués par la présence probable d’individus avirulents et/ou hétérozygotes. Ceci pourrait informer sur le fait que la population tunisienne de P. infestans ne subit pas des mutations extrêmes au niveau des gènes Avr contrairement à ce qui a été rapporté dans d’autre pays dans le monde. Le diagnostic de la virulence chez P. infestans nécessite une technique fiable et à haut débit qui serait complémentaire aux essais in planta en ce, pour remplacer les marqueurs neutres (allozymes, marqueurs SSR, mtDNA). La combinaison des techniques phénoménetiques (essais in planta) et génétiques (marqueurs d’avirulence) pourrait être un outil très intéressant pour identifier la structure des populations du pathogène et trouver une solution rapide à la rupture de la résistance chez les variétés cultivées.

Mots clés: Essais in planta, gènes Avr/R, Phytophthora infestans, régions de collecte, Tunisie

ملخص

حرفياً، كثّل كثّل ومنصف هرابي وفينيان فليسيو. 2011. أنماط الدراسة لعزلات من Phytophthora infestans باستخدام مجموعة تجريبية من Solanum demissum


تتمثل الدراسة الحالية في استعمال مجموعة مكونة من 31 عزلة تونسية من الجنس Phytophthora infestans من خلال تجارب حيوية لأمراض اللبنات. تم اختيار العزلات المجموعة بإنتاج أوراق مفصولة تتنتمي لسلسلة تجريبية من نباتات Solanum demissum للحَفَة المأخوذة (الميتوكوندري). تم توصيف 21 سلالة من هذه العزلات. أظهرت هذه العزلات أنماط سلسلة R مورثة مقاومة مختلفة حسب مناطق الجوع. في حين كانت أنماط الدراسة متوفرة في منطقتي قرية وبنزرت، كانت أقلّ تنوّعا في منطقة تاكاسك والنواحي الشمالية. إضافة إلى ما قلّدناه، لم تتبع أنماط الدراسة لذا السلسلة الرمزية (أي سنة الجوع). تم تعبير مورثات المقاومة الأكثر فاعلية في تونس هي R9 و R8، والتي صمدت أمام أكثر من 90% من العزلات المختارة. يمكن أن تكون هذه المورثات مهمة جدا في برامج الترطيب. بعبارة أخرى، إن وجود أفراد بأقل من أربعة عوامل سلسلة ذات عاملة فعالة في الدراسة، يمكن أن يفسر ذلك يوجد محتمل للعزلات غير ستة أو غير متميزة للاستخدام. هذا يمكن أن يدل على أن المجتمع التونسي من P. infestans يعكس نمطًا جغرافيًا تجانسًا. يمكن أن يكون مصدرًا محتملًا لأمراض العظام. وملاذًا عادل في بلدان أخرى من العالم. إن تشخيص الدراسة لنفس حادًا على مستوى مورثات عدم الدراسة. عكس ما هو مسجل في بلدان أخرى من العالم. إن تحديد الدراسة النتائج تجريبية عالية ذات مصداقية ومرتبطة مع تجارب الغرفة الحيوية التي يمكن أن تخالف الوسائط الطبيعية (الألوئيزيات) DNA، المكررات أحادية السلسلة allozymes، SSR، المورثات أحادية السلسلة Tunisian Journal of Plant Protection Vol. 6, No. 1, 2011
Phytophthora infestans

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