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Characterization of the Potato Late Blight Pathogen *Phytophthora infestans* in Tunisia

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ABSTRACT

This mini-review focus on summarizing different phenotypic and genotypic analysis recorded on *Phytophthora infestans* population collected from the Northern area of Tunisia. Here, we demonstrated that mating type distribution plays a primordial role in genetic structure of the pathogen between sampling regions. Also, a probably strong correlation between mating type and metalaxyl resistance could be mentioned. Next to that, we found that the aggressiveness and the virulence patterns were highly effective parameters to outline phenotypic diversity in Tunisian population that showed specific characteristics comparing with Algeria and others countries in the world. Consistently, genotypic diversity based on SSR markers seems to be a very interesting key in the genetic understanding of the pathogen. In fact, we found that *P. infestans* populations in Tunisia were divided to two major phylogenic groups: a clonal lineage group shared between all sampling regions and a diverse group detected in potato population and specific to two sub-regions. Thus, we could conclude that genotypic diversity confirmed phenotypic diversity and both analysis leaded us to conclude that population structure of *P. infestans* in Tunisia is very specific either in North African area or all over the world.

Keywords: genotypic diversity, phenotypic diversity, *Phytophthora infestans*, structure, Tunisia

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INTRODUCTION

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is one of the most destructive diseases of cultivated potato and tomato worldwide (Goodwin *et al*. 1998; Cooke *et al*. 2011). Originating from South America, this pathogen was introduced firstly into Europe in the 1840s via infected seed potatoes (Fry *et al.* 1993) and leaded to the great potato famine in Ireland in 1845-1850 (Bourke 1964). In 1941, *P. infestans* was introduced to Africa on imported potato seeds from the United Kingdom in order to feed allied troops during World War II (Cox and Large 1960). Since that, late blight appeared as a continuous and sustainable problem in potato and tomato fields. In Tunisia, damages fluctuated from year to year depending on weather conditions and could attend high levels at rainy years. Where potato is cultivated during three seasons over a period of ten months in the year, late blight epidemics can result in complete crop loss. On tomato, this pathogen can damage either greenhouses crop or field crop in the most producing regions. The Mediterranean climate is highly conducive to the late blight epidemics from November to May in potato crops and throughout the year in tomato crops. Similar to what is happening in Europe (Haverkort 2008), USA (Miller *et al.* 1997; Reis *et al*. 2005) and Asia (Koh *et al.* 1994; Guo *et al.* 2009), potato late blight epidemics in Tunisia have become more difficult to control thanks to i) the probable changes occurring in *P. infestans* population structure all over the world (Goodwin et *al.* 1998; Runno-Paurson *et al.* 2009; Corbière *et al.* 2010), ii) the lack of preventive application and the excessive use of phenylamide fungicides (Harbaoui *et al.* 2010), iii) the absence of crop rotation and the incorrect irrigation systems adopted in fields and iv) the widespread use of the susceptible cultivar 'Spunta' in more than 90% of potato acreage in Tunisia. However, information on the pathogen population structure is a prerequisite for understanding the epidemiology of the disease and for selecting durable disease resistance sources for crop breeding. Therefore, we intended to further characterize Tunisian *P. infestans* isolates (165 individuals) collected from potato and tomato during 2006 to 2008. Three crop regions in Tunisia were considered in this survey. Within these areas, Bizerte is considered as the wide Northern region that potato is cultivated

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in humid conditions. The North-Eastern area (Nabeul) is considered as the cradle of potato crop and the wide area of tomato early season crop. The North-Western area is also an important site in potato culture covered by mixed weathers oscillate between sub-humid to semi-arid climate. In this overview, we will summarize the different genetic and genotypic analysis recorded on a *P. infestans* population collected from potato and tomato crop regions at North of Tunisia during the period between 2006 and 2008.

GENETIC STRUCTURE OF *P. INFESTANS* IN TUNISIA

Sexual cycle contribution in genetic structure of the *P. infestans* populations in Tunisia

The mating type was first identified to 165 *P. infestans* isolates using CAPS (Cleaved Amplified Polymorphism Sequence) technique. The *in vitro* assay technique was used later in order to confirm molecular results. Of this total number of isolates, 141 isolates (85%) were determined as A1 mating type and 24 isolates (15%) were identified as A2 mating type. Known as the index of the genetic changes of *P. infestans* population's structure (Goodwin and Drenth 1997; Goodwin 1998; Beninal *et al.* 2009), the A2 mating type was identified in two crop regions: the North and the North-East areas but not in the North-West. Although the presence of both mating types that raises the possibility of sexual reproduction and the generation of oospores, we suggest that it could be rare or absent in some sub-regions from where no A2 isolates were detected. Furthermore, all tomato isolates (29) analyzed in this study were A1 mating type. The most likely explanation may be that genetic changes in potato were not the same in tomato due to some kind of host preference. These findings were previously investigated and showed that isolates collected from tomato had preference for tomato above potato (Harbaoui and Hamada 2008).

Occurrence of metalaxyl resistance and probable correlation with A2 mating type

Metalaxyl resistance was performed on a representative population of 65 *P. infestans* isolates via *in vitro* assays using pea agar medium amended by metalaxyl fungicide. The classification of isolates was performed based on the percentage of mycelium growth at 100 ppm. Consequently, 58% of tested isolates were classified as metalaxyl resistant and the reminder (42%) is metalaxyl sensitive. In this tested set, we found that 40 isolates were collected from protected areas either from experimental fields or from farms and 25 isolates were collected from unprotected crops. The highest number (70%) of resistant isolates was determined in Bizerte area and the highest percentage (60%) of sensitive isolates was detected in North-West area. Moreover, while potato population showed high percentage (67%) of resistant isolates, tomato population presented high level (69%) of sensitive isolates. In addition, this study indicated that all A2 mating type isolates were metalaxyl resistant. This could peel a probable correlation between the mating type and the metalaxyl resistance (Harbaoui *et al.* 2010) as demonstrated firstly by Goodwin in 1998. Consequently, metalaxyl resistance considered as a parameter to diagnose and repair different pathotypes from different regions plays an important role in the understanding of genetic structure of *P. infestans* populations.

Virulence/Avirulence and aggressiveness on potato: Two major parameters to consider for repairing *P. infestans* populations structure

Virulence diversity was investigated on 31 isolates selected from a Tunisian population of *P. infestans* sampled from potato and tomato plants collected from different crop regions. Samplings were carried out from different hosts, organs, regions and seasons during 2006, 2007 and 2008. This study was performed via bioassays technique using a differential set of 11 *Solanum demissum* carrying each one a known *R* gene. Consequently, virulence patterns of tested isolates showed high diversity and 21 races were deduced (Harbaoui *et al.* 2011). In all sampling regions, virulence patterns of *P. infestans* isolates did not present a chronologic trend variation. 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 tested isolates. Therefore, the most effective *R* genes in the differential set are R5, R9 and R8. Although R10 and R11 were withstood at almost all tested isolates, they were overcome by highly complex races. Hence, nine R differentials were resistant to more than 50% of the tested isolates and only R1 and R7 differentials were relatively overcome by most of them (Harbaoui *et al.* 2011). Overall, virulence diversity outlines the behaviour of the pathogen in the field and lead to more understanding of biologic structure of the pathogen.

Elsewhere, aggressiveness was performed with bioassay technique on 36 isolates that inoculated a susceptible cultivar 'Bintje'. Highly aggressive isolates predominated during years and represented 56% from tested isolates. Moderately and weakly aggressive isolates represented equal proportions of 22%. Highly aggressive isolates predominated within all Tunisian sub-populations, and their fraction increased from 40, 58 to 64% in 2006, 2007 and 2008, respectively. In addition, aggressiveness profiles were variable between potato and tomato isolates as the former presented more aggressive profiles on 'Bintje' cultivar than the latter (unpublished data). This could lead to the assumption that *P. infestans* population in Tunisia undergoes mutations in potato crops but not in tomato. Indeed, these changes could be due to the climate conditions conducive to the selection pressure depending on hosts.

GENOTYPIC DIVERSITY OF *P. INFESTANS* POPULATION IN TUNISIA

Mitochondrial haplotypes showed low diversity

Haplotyping were computed based on RFLP-PCR technique using primers 2 and 4 adopted by Griffith and Shaw (1998). Consequently, about 98% of the isolates (n=161) had mitochondrial haplotype Ia, whereas only 2% (n=4) had mitochondrial haplotype IIa. These four isolates were collected from the same tomato greenhouse from Takelsa, the largest tomato sampling sub-region in the North-East area. This conclusion could inform that although mitochondrial genome could not be highly informative about the genetic diversity of *P. infestans*, haplotyping analysis showed that probably potato and tomato didn't host the same population of the pathogen (unpublished data).

Microsatellites showed high genetic diversity and inform well on *P. infestans* population structure

SSR markers are classified as ideal markers for rigorous genetic analysis since they are robust, flexible, reproducible, and offer the greatest potential to study the genetic diversity of *P. infestans* (Knapova and Gisi 2002; Cooke and Lees 2004, Lees *et al.* 2006; Guo *et al.* 2009). In order to study the genetic structure of *P. infestans* population in Tunisia, 12 SSR markers were computed on 126 from 165 isolates using a multiplex PCR technique and analysed by automatic scoring using GeneMapper4.0 software (Applied Biosystems, The Netherlands). Accordingly, the 12 markers seemed to be highly informative and showed high gene diversity values depending on any classification (sampling regions, mating type, host and season). Also, they yielded 49 alleles, within which many private alleles were detected in most regions, mating type, host or season sub-populations (unpublished data). Furthermore, analysing the multilocus genotypes showed that genotypic diversity is highly variable

between considered sub-populations. However, genetic differentiation between sub-populations based on sampling regions, mating types, hosts and seasons showed low values (13%). In addition, the gene flux index computed on different sub-populations was relatively high and generally attended 2 migrants between combined populations. These two last analyses indicated the evidence of migration due to the relative quite broad geographic distances between sampling regions in Tunisia. Elsewhere, phylogenic analysis computed on 130 isolates showed a clear clustering and two phylogenic groups were detected. The first group was deduced as a clonal lineage shared by all sampling regions and contains only A1 mating type isolates and Ia haplotype. Also, all tomato isolates that had Ia haplotype were clustered in this group. The second cluster was described as a highly diverse group which comprises A1 and A2 isolates. In this second group, we identified more mtDNA haplotypes (Ia and IIa). Only IIa haplotype tomato isolates were found in the diverse group. Consistently, almost all potato isolates collected from Korba (North-East) and several isolates from Bizerte (North) were clustered in that highly diverse group. Probably the old generation (clonal lineage group) still evolved by asexual recombination and slightly undergoes mutations in Takelsa and North-West sub-regions. But, the new generation (diverse group) that presented more complex genetic structure is resulted from the occurrence of sexual cycle in both two sub-regions: Korba and Bizerte. This deduction was confirmed by analysing the clonal fraction explained by Zhan *et al.* (2003) as the proportion of genotypes derived from asexual recombination. This parameter showed high level of clonality in both first regions and low level of clonality in both last regions (unpublished data).

SPECIFICITY OF GENETIC STRUCTURE OF *P. INFESTANS* POPULATION IN TUNISIA

P. infestans population in Tunisia seems to be very characteristic comparing either with European, American and Asian populations or with neighbour countries such as Morocco (Sedegui *et al.* 2000; Hammi 2003) and Algeria (Corbière *et al.* 2010). Indeed, mating type distribution which is in favour to A1 seems to be an important index of genetic changes in *P. infestans* structure. The fact that all A2 isolates tested for metalaxyl were resistant and most of them (33 from 36) were highly aggressive on sensitive cultivar 'Bintje' could lead to the assumption that genetic changes in *P. infestans* populations in Tunisia as well as in Europe (Goodwin *et al.* 1998; Lebreton *et al.* 1998; Sliwka *et al.* 2006; Runno-Paurson *et al.* 2009), Asia (Guo *et al.* 2009); and Africa (Corbière *et al.* 2010) is due to the appearance of new genotypes resulted from sexual recombination. This assumption was strongly confirmed by genetic diversity using SSR markers. In fact, all A2 isolates was phylogenetically clustered in one highly diverse group and no one belonged to the clonal population (unpublished data). In addition, the co-existence of A1 and A2 was proved in Bizerte (North) and Korba (North-East) but not in Takelsa (tomato region) and North-West areas. These data suggest that *P. infestans* sub-populations carry out different genetic structures in each sub-region depending on selection pressure. Weather conditions and hosts played an important role to pomp specific strains in each region. Especially, all isolates from Korba were clustered in the genetic diverse group except tomato isolates that were clustered in the clonal group. Also, we found that all tomato isolates from Takelsa belonged to clonal population except the only one potato isolate that belonged to the diverse group. Elsewhere, virulence diversity analysis demonstrated that the most effective R genes to be deployed would be R5, R8 and R9, for which virulent isolates were found. Also the differentials R10 and R11 were effective against most tested isolates, and virulence was only detected in a few, highly complex isolates. In addition, the differentials R2, R3, R4, and R6 were resistant, but the differentials R1 and R7 were infected by more than 50% of tested isolates. These results could be informative for breeding programs that can focus introgressing highly effective R genes such as R5, R8 and R9. Also substitution of the highly susceptible potato cv. 'Spunta', which is cultivated at more than 90% from total potato acreage in Tunisia, by other candidate cultivars that contain identified effective R genes, could reduce the disease on potato. Altogether, despite the presence of complex pathotypes in two interesting potato crop regions, the population detected in the other regions and in tomato crops still not highly complex. Compared with the situation in Europe and the New World, or even in close countries such as Algeria, the genetic changes in Tunisia are still comforting thanks to the absence of new strains such as Blue 13 strains which were recently detected in Europe (Cooke *et al*. 2010; Sophien Kamoun, pers. comm.) and probably identified in Algeria (Corbière *et al*. 2010). Although the genetic diversity and the race complexity detected in Tunisian population, the race structures (virulence diversity) are still less complex than in Mexico 20 years ago (Rivera-Pena 1990). Therefore, as Tunisian isolates are still less complex pathotypes than Algerian and Moroccan, we suggest following more strict monitoring programs either chemicals spray or irrigation means in order to avoid dispersion or appearance of more resistant genotypes in regions undergoing genetic changes. In addition, we should avoid internal seeds exchange between provinces in order to limit the spread of A2 strains in new regions. Finally, this survey could be highly useful in the future because it will aid management decision on late blight control in Tunisia considered as an important North African area.

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