Characterization of *Phytophthora infestans* populations in northwestern Algeria during 2008-2014

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ABSTRACT

A total of 161 *Phytophthora infestans* isolates, collected from infected potato and tomato plants during 2008-2014, were characterized based on mating type, metalaxyl sensitivity and polymorphism at 12 simple sequence repeat (SSR) loci, in order to investigate the population of P. infestans in the north-west of Algeria, an emerging potato production region. The majority of isolates were of A2 mating type (112 isolates). A high percentage (89 %) of resistance to metalaxyl among isolates was detected. The metalaxyl resistant phenotype was present in both mating types with a higher percentage in A2 mating type isolates. SSR based genotypic analysis of P. infestans population showed a low diversity. Genotype 13_A2 was the predominant in the population with a frequency of 67 % followed by 2_A1 (21 %) and 23_A1 (5 %). Genotype 23_A1 was detected only in tomato and potato isolates collected in 2013 and 2014.

Keywords: Late blight, Mating type, Metalaxyl, Potato, SSR markers, Tomato

Introduction

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is considered to be the most important potato disease in the world. Under favourable conditions it can destroy the whole potato haulm and cause considerable yield loss. *P. infestans* is responsible of $ 6.7 billion annually in potato, and crop losses up to 100 % in tomato (Chowdappa et al. 2015). In the 19th century this pathogen caused the famous Great Famine in Ireland resulted in the death of approximately one million and the emigration of a million more people (Fry & Godwin 1997). Since this period, migration probably within exported seedtubers, allowed the late blight pathogen to spread throughout the world (Fry *et al.* 1993). As a consequence of the latest of these movements, populations of *P. infestans* have changed in several North European countries during the 1980s. Several studies on characterization of *P. infestans* showed that the 'old' European populations of the pathogen were rapidly being replaced by 'new' populations, genetically different and probably originated from central Mexico (Fry *etal.* 1993). Across many potato growing regions, populations of *P. infestans* have been dominated by clonal lineages that are define dusing a combination of genotypic and phenotypic characters (Cooke & Lees 2004). A successful A2 mating type, metalaxyl resistant lineage with increased aggressiveness and virulence that originated in Europe was named genotype 13_A2 (Cooke *et al.* 2012; Lees *etal.*2012). It was first reported in the Netherlands and Germany in2004,Polandin2006,2008and2009(Chmielarz*etal.*2014)and reached 80 % of the population in Great Britain in 2008 (Cooke *et al.* 2012). The 13_A2 lineage was responsible for severe late blight outbreaks on potato and tomato in southern India and has replaced the prior population represented by the US-1 and other genotypes (Chowdappa *et al.* 2015).

In Algeria, late blight is a very common disease on potato, but it was also reported on tomato in some areas of the country and it is controlled through extensive use of chemical fungicides, especially metalaxyl, as in many other regions in the world. In north-western Algeria, an emerging potato production region, this disease reached epidemic proportions in 2007-2008, probably due to the favourable weather conditions. Consequently, heavy yield losses were recorded despite the widespread use of late blight fungicides and potato production has decreased by 30 % in this period.

In Algeria, potato and tomato are the most important vegetable crops, grown on 156176 and 22 646 ha, with an annual production of 4.67 and 1 million tones, respectively (FAOSTAT 2014). Potato is planted in three seasons, from Aug. to Jun. The late season, from Jan. to Jun. is the main cropping

period in all production regions, but early and very early crops are typical practices in coastal regions. The majority of seed potato tubers are imported from European countries, especially the Netherlands, France, Denmark and Scotland. Conversely, tomato is grown all year round in greenhouses and open fields. In some areas, potato and tomato are grown in adjacent fields increasing the risk of the spread of late blight from one crop to another. In addition, some cultural practices, such as absence of crop rotation, sprinkler irrigation, and the widespread use of susceptible cultivars may also promote early attacks. The practice of leaving infected potato tubers in fields after harvest could also be a potential source of primary inoculum for late blight epidemics. A study of some *P. infestans* isolates collected from the northwestern Algeria during 2008 revealed the appearance of metalaxyl resistance and the presence of the two mating types, A1 and A2, with a high prevalence of A2 (Corbiere *et al.* 2010). Thus, monitoring pathogen insensitivity, especially to systemic fungicides, is an important aspect of disease management (Gisi & Cohen 1996). The development of *P. infestans* isolates' resistance to metalaxyl was reported also in many regions of the world (Deahl *et al.* 1993; Gisi & Cohen 1996; Hammi *et al.* 2002; Cooke*etal.*2010;Hamada&Harbaoui2010;Chmielarz*etal.*2014).

Phytophthora infestans is a heterothallic, hemi-biotrophic oomycete with two mating types (A1 and A2). The presence of both mating types enables sexual reproduction, which generates genotypic diversity in the pathogen population leading to increased adaptability. Another effect of sexual reproduction is the production of oospores, which are highly tolerant to adverse environmental conditions and can survive in soil between growing seasons and over several years (Turkensteen*etal.*2000).

Before 2008 there were no data on Algerian *P. infestans* population characteristics. The first report of the presence of the A1 and A2 mating types in Algeria (Corbiere *et al.* 2010) indicates that all A2 *P. infestans* isolates collected during 2007e2008 have highly complex virulence patterns. Despite the importance of late blight disease on potato and tomato cultivation in Algeria, very little is known about the characteristics of the *P. infestans* population in this country.

The aim of this study was to characterize *P. infestans* populations in order to understand the repeated epidemics recorded in the north-western Algeria region since 2008 and thus develop new efficient late blight control strategies. Phenotypic and genotypic traits were analyzed to determine: (i) the mating type distribution in the Algerian population; (ii) the population level of metalaxyl sensitivity; (iii) their genotypic diversity using microsatellite markers.

Materials and methods

Sampling and isolations

Samples were collected during 2008-2014 from potato crops grown in the field and tomatoes grown in the field and greenhouses, located in different sites in north-western Algeria (Fig 1). Two most important potato production areas from this region, Mostaganem (coastal region) and Ain Defla (interior region) were chosen to compare the populations of *Phytophthora infestans* for mating type ratio, level of metalaxyl resistance and genotypes frequency.

One to four fields were sampled per site at the onset of the epidemic and at 15e20 d thereafter. Five to ten samples with a single lesion were randomly collected from each field. Samples were packed individually in paper bags and maintained at 4 C until isolation.

Phytophthora infestans was isolated from potato and tomato leaves, stems, fruits and tubers showing typical late blight symptoms by placing small pieces of infected tissue under potato tuber slices of susceptible cultivar Spunta. After four to five days of incubation at 18 C in darkness, small tufts of mycelium growing above the potato slice were transferred to Petri dishes containing pea agar medium (protocol from Corbiere & Andrivon 2003) amended with rifamycin (30 mg L^{1}) and ampicillin (200 mg L^{1}). Pure cultures were obtained by repetitive transfers on pea agar medium.

Mating type

The mating type of the isolates was determined by pairing them with reference isolates of A1 and A2 mating type (P13-06 and P27-05, respectively) on pea agar medium. Mycelial plug from an active growing colony of the test isolate was placed on the opposite side (2 cm apart) of the plug of either the A1 or A2 tester isolate. After 11e15 d of incubation at 15 C in the dark, plates were examined microscopically for the presence of the oospores in thehyphal interactionarea between the isolates paired (Cooke *et al*. 2003). Isolates that produced oospores with the A1 tester but not with the A2 isolate were designated as A2. Isolates that formed oospores when paired with the A2 tester isolate but not with the A1 isolate were designated as A1.

Metalaxyl resistance test

The sensitivity of 92 *Phytophthora infestans* isolates was assessed *in vivo* using potato leaf discs (Hermansen *et al.* 2000). Six leaf discs of the susceptible cultivar Spunta were floated abaxial side up in Petri plates each containing distilled water amended with metalaxyl (Ridomil 25 WP, Novartis experimental compound) at different concentrations (0, 10and 100 mg L^{1}). Leaf discs for each plate

were inoculated with a 20 mL droplet of the sporangial suspension of each isolate prepared as previously described by Andrivon *et al.* (2007).

After 7 d of incubation at 18e20 C, the sensitivity to metalaxyl was determined by the isolates' ability to grow and sporulate on the leaf discs. Isolates sporulating on the discs floating on water containing 100 mg L^1 metalaxyl were rated as resistant (R), those on 10 mg L^1 were rated as intermediate (I) and those that sporulated only on water were considered sensitive (S).

DNA extraction

For DNA extraction, 117 isolates were grown individually in pea broth. After 20 d of incubation at 15 C, mycelia of isolates were rinsed with sterile distilled water, harvested by filtration and stored at 20 C. Genomic DNA was extracted from 10 mg of dry mycelium using the Power Plant Pro DNA Isolation Kit (MO BIO Laboratories, USA). Quality and quantity of extracted DNA samples were evaluated using a DNA Quant-it assay kit (Molecular Probes, Carlsbad, CA, USA) and by electrophoresis in 1 % agarose gels containing SYBR Safe (Invitrogen, Life Technologies, USA) DNA gel stain.

Simple sequence repeat marker analysis

Polymorphic simple sequence repeat (SSR) regions of Algerian *Phytophthora infestans* isolates were amplified using primers for loci Pi02, Pi4B, G11, Pi04, Pi63, Pi70, D13, SSR2, SSR4, SSR6,SSR8, and SSR11. The Type-itMicrosatellite PCR kit(QIAGEN) was used following previously published methods (Li *et al.* 2013). Samples were analyzed using an ABI 3730 capillary DNA sequencer according to the manufacturer's instructions (Applied Biosystems). The peak size was determined against a GeneScan 500 LIZ standard and alleles were scored using GeneMapper Software v. 3.7.

Data analysis

The genetic diversity of representative selection of Algerian *Phytophthora infestans* isolates chosen after clone correction was analyzed according to the major allele frequency, number of alleles per locus and gene diversity. Based on SSR data, these basic statistics were determined by Matlab programming. Gene diversity (H) was calculated for each locus according to the following formula (Anderson *et al.* 1993) and implemented under Matlab as m-function named (*pic.m*):

$$H = 1 - \sum_{i=1}^{k} p_i^2$$

where k is the total number of alleles detected per SSR marker and p_i is the sum of the i-th allelic frequency of each microsatellite locus for the genotypes.

An H value equal to 0 means no diversity at that locus and 1 means highest possible diversity.

Results

Mating type

A total of 161 isolates of *Phytophthora infestans* were obtained from 2008 to 2014 (Table 1). Most of them were collected in 2013 and 2014 years and 45 isolates were collected during the 2008e2012 period (Supplementary information, Table S1).

Both A1 and A2 mating type isolates were detected among Algerian isolates of *P. infestans* population collected from north-western Algeria over the period of 2008-2014. Of the 161 isolates tested, 112 were A2 mating type (70 %), 49 were A1 mating type (30 %) (Fig 2A). The A1:A2 frequency ratios during the sampling years were 0.4:0.6 in 2008e2012, 0.15:0.85 in 2013 and 0.4:0.6 in 2014. Interestingly, all isolates from tomato were A1 mating type except one (BT16) being an A2 mating type isolate collected from Mostaganem in 2013. Conversely, the A2 mating type prevailed among isolates from potato in all years (Fig 2B). Finally, both A1 and A2 mating types were sometimes found in the same field in both the Mostaganem and Ain Defla regions. A higher proportion of A2 mating type was found in isolates collected from Ain Defla with 0.1:0.87 (A1:A2) frequency ratio. In contrast, the approximate 0.45:0.54 (A1:A2) ratio of the two mating type was found in isolates collected from Mostaganem region
(Fig 2C).

Metalaxyl resistance

Among the 92 isolates tested for their response to metalaxyl, 89 % (n ¼ 82) were classified as resistant, 7 % (n ¼ 6) as sensitive and 4 % (n ¼ 4) as intermediate (Fig 3A). The metalaxyl resistant phenotype was the most common in all years (Fig 3B). During the period 2008e2012, 73 % (n ¼ 19) of isolates tested were resistant, the remaining isolates were both sensitive (19 %, n ¼ 5) and intermediate (8 %, n ¼ 2). In 2013 and 2014, similar proportions of resistant isolates were found, 96 % (n ¼ 24) and 95 % (n ¼ 39), respectively. Isolates of both A1 and A2 mating types were found to be metalaxyl resistant but a greater proportion of the A2 mating type isolates were resistant (Fig 3C). Finally, a higher proportions of resistance to metalaxyl among isolates collected from Mostaganem and Ain Defla were found (93 %, n ¼ 42 and 86 %, n ¼ 32, respectively) (Fig 3D).

SSRs analysis

A total of 57 alleles were detected over the 12 SSR loci, ranging from 2 (at loci Pi70 and SSR2) to 12 (at locus G11) (Table 2). Alleles 173 from locus SSR2 and 244 from locus SSR6 were detected in all tested *Phytophthora infestans* isolates. Some alleles were detected only once among all tested isolates (frequency of 0.015). The H value ranged between 0.162 (locus SSR2) and 0.823 (locus G11) (Table 2).

The SSR fingerprints from the 117 isolates of *P. infestans* subject to SSR analysis were compared to profiles from other lineages (e.g. Cooke *et al.* 2012; Li *et al.* 2012) and categorized into four major types. The most frequent of these was 13_A2 (n ¼ 78) with other types 2_A1 and 23_A1 genotypes detected 25 and 6 times, respectively (Fig 4A). The genotype and expected mating type corresponded in every case. Finally, eight 'Misc' (abbreviation of miscellaneous) isolates that did not group within any known lineage were detected (Fig 4A) and were of mating types, A1 (n ¼ 6) and A2 (n ¼ 2). Genotype 2_A1 was the predominant (10 isolates) in *P. infestans* isolates collected from 2008 to 2012 (Fig 4B), followed by 'Misc' and 13_A2 genotypes (five and four isolates, respectively). Conversely, genotype 13_A2 was the predominant one in the population sampled in 2013 and 2014 (48 and 26 isolates, respectively). Finally, the genotype 23_A1 was found once in an isolate (BT9) collected on tomato from Mostaganem in 2013 and 5 times on potato from Mostaganem in 2014 (Fig 4B). The population of *P. infestans* in this region showed a higher genotypic diversity than the population in Ain Defla and other regions from the north-west of Algeria (Figs 4C and 5).

Minor variation within the fingerprints of isolates of the 13_A2 clonal lineage were identified and defined as subclonal lineages, 13_A2_1, 13_A2_2, etc (Fig 6A). This variation was primarily at loci G11, D13 and SSR4. The 13_A2_2 subclonal lineage was the predominant, represented by 50 isolates followed by 13_A2_22 (8 isolates) and 13_A2_1 (6 isolates). In addition, other subclonal genotypes (13_A2_50, 13_A2_61, 13_A2_68, 13_A2_86 and 13_A2_101) were found but with low frequencies (Fig 6A). All of the eight subclonal lineages detected were found in Mostaganem region with the dominance of 13_A2_2 which was also the predominant in Ain Defla. In this last region, three subclonal genotypes, 13_A2_68, 13_A2_86 and 13_A2_101 were absent (Fig 6B).

Discussion

In this study, a total of 161 Algerian *Phytophthora infestans* isolates collected over the period of 2008-2014 were characterized both for phenotypic traits, such as mating type and metalaxyl sensitivity and genotypically, using SSR markers. The obtained results revealed important aspects of *P. infestans* population in northwestern Algeria over this period. An analysis of mating types indicated a shift from the A1 to the A2 mating type of Algerian *P. infestans* isolates. Fungicide resistance testing indicated a high proportion of metalaxyl resistance over the years 2008-2014. Genotypic analysis with SSR markers revealed the spread of a dominant 13_A2 clone, a lower frequency of two other clones and occasional infection by previously undescribed genotypes.

Potato and tomato production is important in Algeria but despite the damage caused by late blight there have been few studies on the pathogen. Previous reports on relatively few isolates (Beninal *et al.* 2009; Corbiere *et al.* 2010) documented the appearance of the A2 mating type as early as 2007. More detailed analysis of isolates collected from 2013 and 2014 crops in this study confirms the dominance of the A2 type in two important Algerian production areas. The importation of large quantities of infected potato tubers every year from European countries such as the Netherlands (accounting for more than 50 % of the total), Denmark, France and the UK where recent increases in the frequency of the A2 mating type have been reported (Montarry *et al.* 2008; Cooke *et al.* 2012; Li *et al.* 2012; Mariette *et al.* 2016) is a probable cause of this increase. Similar results were reported in Morocco (Sedegui *et al.* 2000; Hammi *et al.* 2002). In contrast, a lower frequency of A2 mating type was reported in many countries, such as Tunisia (Hamada & Harbaoui 2010; Harbaoui*et al.* 2013), Italy (Savazzini & Galletti 2015) and China (Tian *et al.* 2015). The data on the 15 tomato isolates in this study showed that all except one were A1 mating type. This distinction between isolates of both hosts needs to be confirmed with a large number of tomato isolates. Many reports demonstrate that distinct clonal lineages of *P. infestans* are associated with potato and tomato within the same region (Legard et *al*. 1995; Oyarzun *et al.* 1998; Suassuna *et al.* 2004) but are in contrast with the monitoring results from India reported by Chowdappa *et al.* (2015), who found that the A2 population of genotype 13_A2 was responsible for severe late blight outbreaks on potato and tomato in South India. The high frequency of A2 isolates on potato crop in most of sampling sites might be related to the emergence of 'new' more aggressive A2 isolates, introduced from Europe through imported potato tubers as has been reported previously. It was found that the frequencies of A1 and A2 isolates vary greatly between Ain Defla and Mostaganem, two emerging potato production regions in north-western Algeria. The high frequency of A2 isolates in Ain Defla may be interpreted as an adaptation of these isolates to

potato crops present in the majority of the collection sites but which are far from tomato production areas. It is important to note that all tomato production sites surveyed in Ain Defla were free of late blight. In contrast, potato and tomato are grown in adjacent fields in the Mostaganem region. The closeness of the two mating type frequencies in this region may be explained by the spread and exchange of isolates between both crops. The coexistence of both mating types in most of the sampling sites means that sexual reproduction and the production of oospores (Turkensteen *et al.* 2000) may occur in this region. These sexual structures greatly contribute to the overwintering survival and to the nature of the population structure of *P. infestans* (Andersson *et al.* 1998; Lehtinen & Hannukkala 2004; Fry 2008).

In this study, a high level of metalaxyl resistance in the Algerian *P. infestans* population in every year (73 % from 2008 to 2012, 96 % in 2013 and 95 % in 2014) and in most sampling sites was observed (Fig 3). Similar results were reported in many studies worldwide (Gisi & Cohen 1996; Goodwin *et al.* 1998; Jmour & Hamada 2006; Harbaoui *et al.* 2013; Savazzini & Galletti2015).In contrastlow levelsof resistancehave been reported in Norway, Sweden, Finland, Denmark (Lehtinen *et al.* 2008), Poland (Chmielarz *et al.* 2014) and Latvia (Aav *et al.* 2015). In this study resistance to metalaxyl was observed in both regions, Ain Defla and Mostaganem, and with a higher proportionintheA2matingtypeisolates.Thisrelationshipbetween metalaxyl resistance and A2 mating type was detected amongst 25 isolates collected in 2007 and 2008 in northwestern Algeria (Corbiere *et al.* 2010). Similar results were also reported in many regions of the world (Miller *et al.* 1997; Goodwin *et al.* 1998; Hammi *et al.* 2002; Cooke *et al.* 2003; Fontem *et al.* 2005). The higher metalaxyl resistance observed inA2matingtypemightbealsoassociatedwiththeoccurrence of 'new' population of A2 isolates which are more tolerant to this fungicide as found in many regions in Europe (Cooke *et al.* 2012). In addition, the extensive use of metalaxyl in Algeria, especially since the severe late blight epidemics recorded during 2007e2008 (Corbiere *et al.* 2010), has probably been another factor in the rapid increase of the resistant population. These results suggest that the use of metalaxyl to control late blight may not be a suitable management strategy in Algeria.

The genetic structure deduced from the microsatellite genotyping revealed a low genetic diversity among *P. infestans* population in north-western Algeria. The 13_A2 clonal lineage was the predominant genotype, especially on potato crops, and it was found with several subclonal variants in multiple locations. The genetic diversity data are consistent with the introduction and spread of 13_A2 into Algeria by means of infected potato tuber seed. *P. infestans* MLG 13_A2 known as 'Blue 13' was first detected in the Netherlands and Germany in 2004 and is known to be resistant to

metalaxyl (Cooke *et al.* 2012). Over the last decade the dominance of 13_A2 strain was reported in several European countries (Cooke *et al.* 2010; Kildea *et al.* 2010; Mariette *et al.* 2016) and it has spread into Asia (Chowdappa *et al.* 2015). According to some authors (Lees *et al.* 2009; White & Shaw 2009; Cooke *et al.* 2012), this dominance is attributed to this genotype's increasedvirulence and aggressiveness (Cooke *et al.* 2012; Chmielarz *et al.* 2014). Isolates of 13_A2 were reported to be the most aggressive on five potato cultivars and were better suited to cooler (13 C) than warmer (18 C) conditions (Cooke *et al.* 2012). However, in southern India, where the average temperature varied from 20.8 C to 22.5 C, the European 13_A2 lineage was also highly suited to local temperatures and was highly aggressive on potato and tomato (Chowdappa *et al.* 2013; Chowdappa *et al.* 2015). This is consistent with its adaptation to conditions in Algerian crops where the average temperature varied from 12 to 22 C during the main cropping season. The dominance of 13_A2 isolates in Algeria may be also associated with its adaptation to Spunta, dominantcultivar preferredby farmersfor its short cycle and productivity. The study of few *P. infestans* isolates collected from the northwest of Algeria in 2008 showed significant aggressiveness of these isolates on cultivar Spunta (Corbiere *et al.* 2010). In common with other studies (Cooke *et al.* 2012; Li *et al.* 2012), subclonal variation was observed in the 13_A2 population in Algeria. The 13_A2_2 subtype was most commonly recovered from 2008 to 2014 with other sub-types of this also found locally.

Isolates of the 2_A1 lineage were also recovered on both tomato and potato hosts in this survey. This lineage has been present in Europe since at least 1982 (Cooke *et al.* 2012) also probably imported into Algeria with seed potato tubers in the past. The decline of 2_A1 isolates over the period of this survey may be a sign of its displacement by the aggressive and metalaxyl resistant 13_A2 clonal lineage. The third genotype detected among Algerian *P. infestans* population was 23_A1.This lineagewas present on both hosts but only in Mostaganem region and it was detected for the first time in 2013 on tomato and then in 2014 on potato crops. The 23_A1 strain was dominant on tomato, but uncommon on potato during 2012 in Great Britain (Stroud *et al.* 2016). In contrast, it was present with a higher frequency on potato among *P. infestans* population in Egypt (El-Ganainy *et al.* 2013). The three clonal lineages identified in this current study were not recovered in the Tunisian population which comprised strains never reported in European countries (Harbaoui *et al.* 2014) or southern India (Chowdappa *et al.* 2015).

In this study, 8 'Misc' isolates were detected on both hosts from different sites. Six of them were of the A1 mating and two belonging to the A2 mating type. These Misc MLGs are interpreted as being evidence of the germination of sexually generated oospores in some regions of Europe (Sjoholm€ *et*

al. 2013). As Misc isolates are collected from areas where both mating types are present, it is possible that these genotypes have generated through the sexual recombination of A1 and A2 mating types. Furthermore, in some prospected potato fields where furrow irrigation system is used, the first symptoms of late blight were observed on lower stems of plants, which probably caused by oospore-derived infection. However, as it is not confirmed whether oospores are formed in Algerian crops it is possible that these represent locally spread ephemeral seed-borne sources of inoculum that have not spread to be recognized as clonal lineages.

Finally, a high genotypic diversity level was observed in potato population collected from the Mostaganem region compared with Ain Defla region. More than three MLGs were sometimes detected in the same field in some potato production areas of Mostaganem region. This may be due to a larger population on a more diverse range of hosts in Mostaganem or the rare occurrence of the sexual reproduction in this region where both mating types were present in the majority of the collection sites. According to Goodwin *et al.* (1995), sexual recombination is currently contributing to the increase of the genetic composition of the pathogen. In fact, a high genetic diversity, reported in Nordic and Estonian *P. infestans* populations was attributed to the implication of sexual reproduction (Sjöholm *et al.* 2013; Runno-Paurson *et al.* 2016).

The high level of genotypic diversity observed in Mostaganem region might be also related to its specific weather conditions which allow the cultivation of potato all year round, so several generations of the pathogen can occur, and this could implicate the appearance of new genotypes. In contrast, potato population in Ain Defla region was composed mainly of the Blue 13 strain. It seems that this clonal lineage is welladapted to the warm environmental conditions which characterize this region. The study realized by Shakya *et al.* (2015) of the effects of some climatic factors, such as temperature on *P. infestans*, showed response variability between different clonal lineages.

Conclusion

In conclusion, the results presentedin this study revealed that *Phytophthora infestans* population in northwestern Algeria is mainly composed of the A2 mating type isolates associated with the clonal lineage 13_A2 and A1 isolates of 2_A1 and 23_A1. The high level of metalaxyl resistance in *P. infestans* population suggests that the use of metalaxyl formulations should be carefully planned in late blight management in
Algeria.

This study is a preliminary contribution to the worldwide effort to characterize *P. infestans* and it provides some information on the pathogen populations in strategic regions of Algeria. Further investigations are required to establish a complete structure of the entire population of this pathogen, especially on tomato and thus complete the map of all the production areas. Moreover, by monitoring the distribution and evolution of *P. infestans*, it is possible to investigate and understand the evolution and epidemiology of late blight in Algeria, and thus improve the management strategies of this disease.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.funbio.2017.01.004.

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Table 1 - Mating type, metalaxyl resistance and genotypes of *Phytophthora infestans* isolates in the north-west of Algeria from 2008 to 2014.

Sampling year	Number of isolates								
	A1	A2	Metalaxyl			Clonal lineage			
			Sensitive	Intermediate	Resistant	13_A2	2_A1	23_A1	Misc
2008-2012	19	27	5	2	19	4	10	0	8
2013	10	53	1	0	24	48	3	1	3
2014	20	32	0	2	39	26	12	5	0
Total	49	112	6	4	82	78	25	6	11

Table 2 - Allele frequencies and Gene diversity of SSR loci**.**

SSR Locus	Fragments	Alleles detected and allele frequencies (in parentheses)	Gene diversity (H)
Pi02	5	258 (0.103) 266 (0.310) 268 (0.517) 270 (0.051) 272 (0.017)	0.561
Pi4B	3	205 (0.285) 213 (0.393) 217 (0.321)	0.660
G11	12	142 (0.092) 152 (0.015) 154 (0.277) 156 (0.107) 158 (0.015) 160 (0.246) 162 (0.046) 164 (0.031) 168 (0.015) 206 (0.123) 208 (0.015) 210 (0.015)	0.823
Pi04	3	166 (0.423) 168 (0.077) 170 (0.500)	0.565
Pi63	3	270 (0.163) 273 (0.327) 279 (0.509)	0.607
Pi70	2	192 (0.839) 195 (0.161)	0.269
D13	9	136 (0.523) 138 (0.031) 140 (0.169) 144 (0.015) 152 (0.015) 154 (0.200) 158 (0.015) 206 (0.015) 210 (0.015)	0.655
SSR11	3	331 (0.074) 341 (0.796) 355 (0.129)	0.343
SSR2	2	173 (0.0911) 175 (0.089)	0.162
SSR4	9	284 (0.278) 288 (0.115) 290 (0.131) 292 (0.098) 294 (0.262) 296 (0.049) 298 (0.016) 300 (0.033) 302 (0.016)	0.809
SSR6	3	240 (0.259) 242 (0.167) 244 (0.574)	0.574
SSR8	3	260 (0.426) 264 (0.111) 266 (0.463)	0.592
Total	**57**		

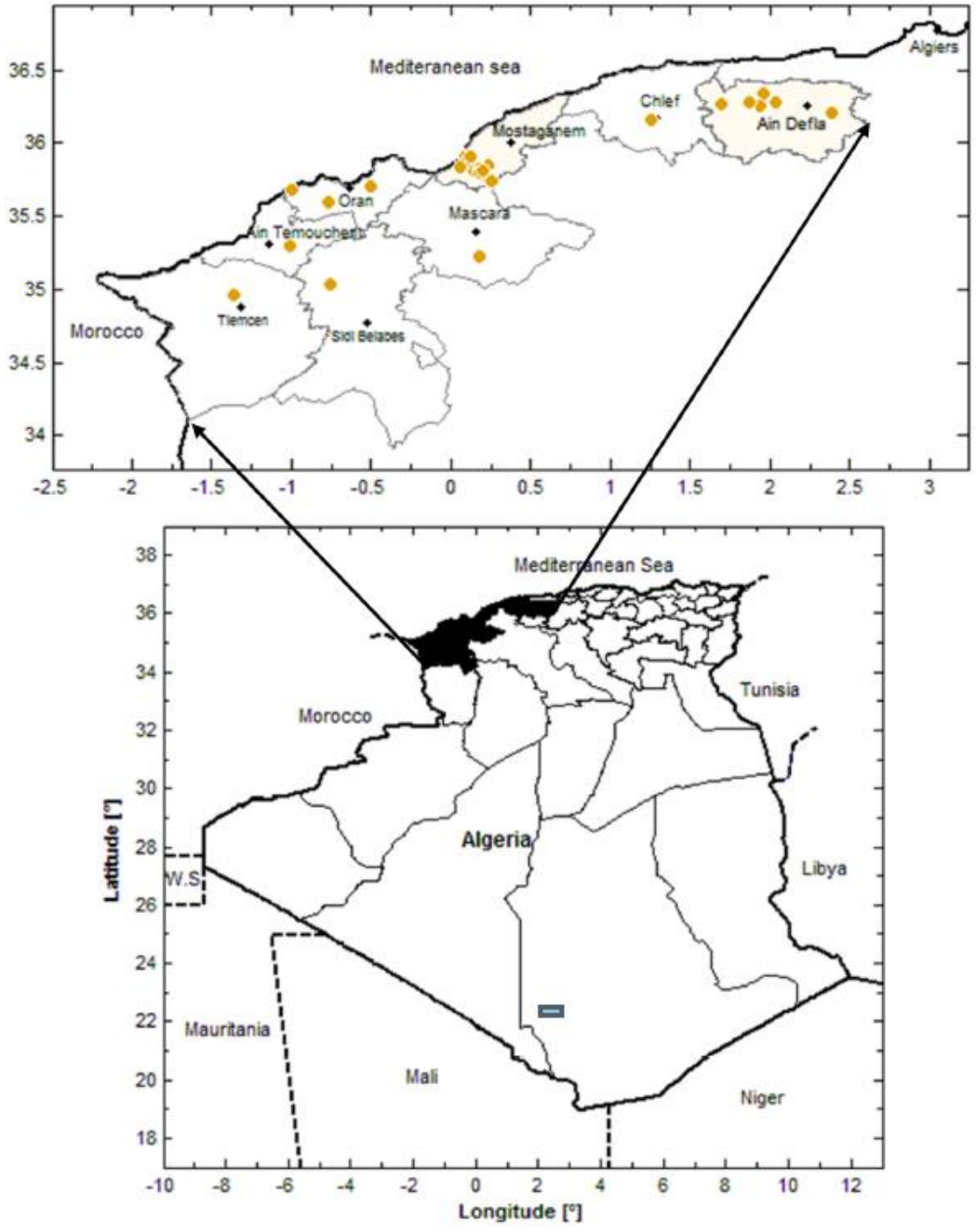

Fig 1 - The Survey map indicating locations where tested isolates were taken during 2008-2014 in northwest of Algeria. The position (longitude and latitude) of each sampling site was determined using GPS (Global Positioning System).

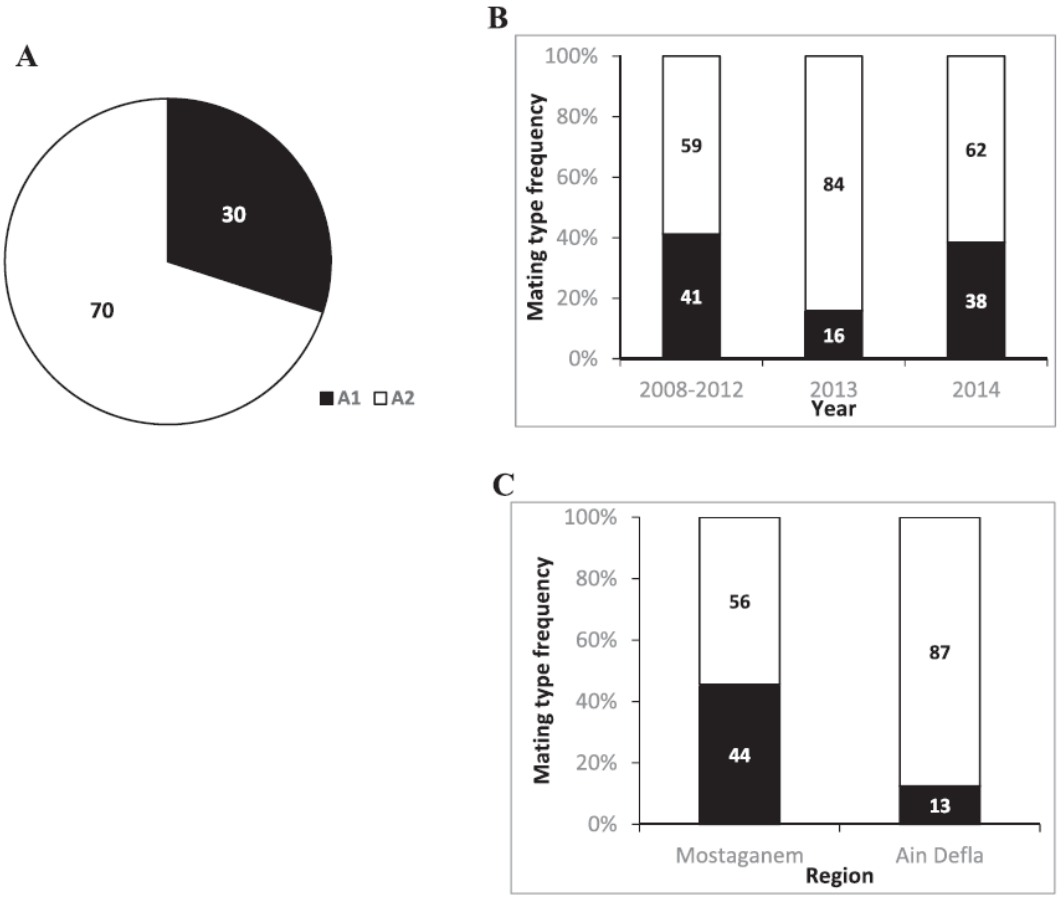

Fig 2 - Frequency of mating types (A1, A2) among *Phytophthora infestans* isolates collected from the northwestern Algeria during 2008-2014 (n = 161). (A) For all isolates. (B) Depending on the sampling year. (C) With respect to the region of sampling (Mostaganem and Ain Defla).

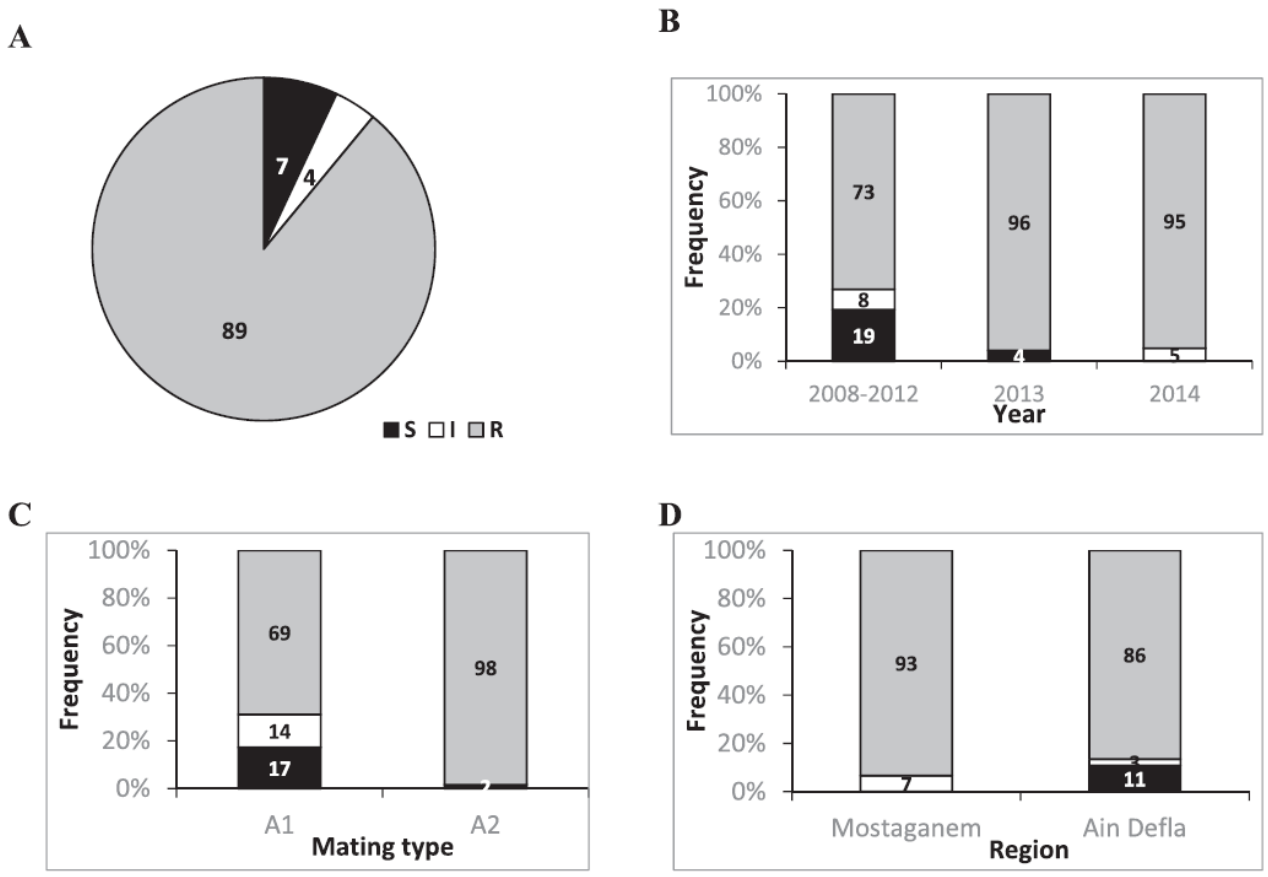

Fig 3 - Metalaxyl resistance among *Phytophthora infestans* isolates collected from the northwestern Algeria during 2008-2014. (A) For all tested isolates (n = 92). (B) With respect to the sampling year. (C) With respect to mating type. (D) With respect to two regions (Mostaganem and Ain Defla). S: sensitive, I: intermediate, R: resistant.

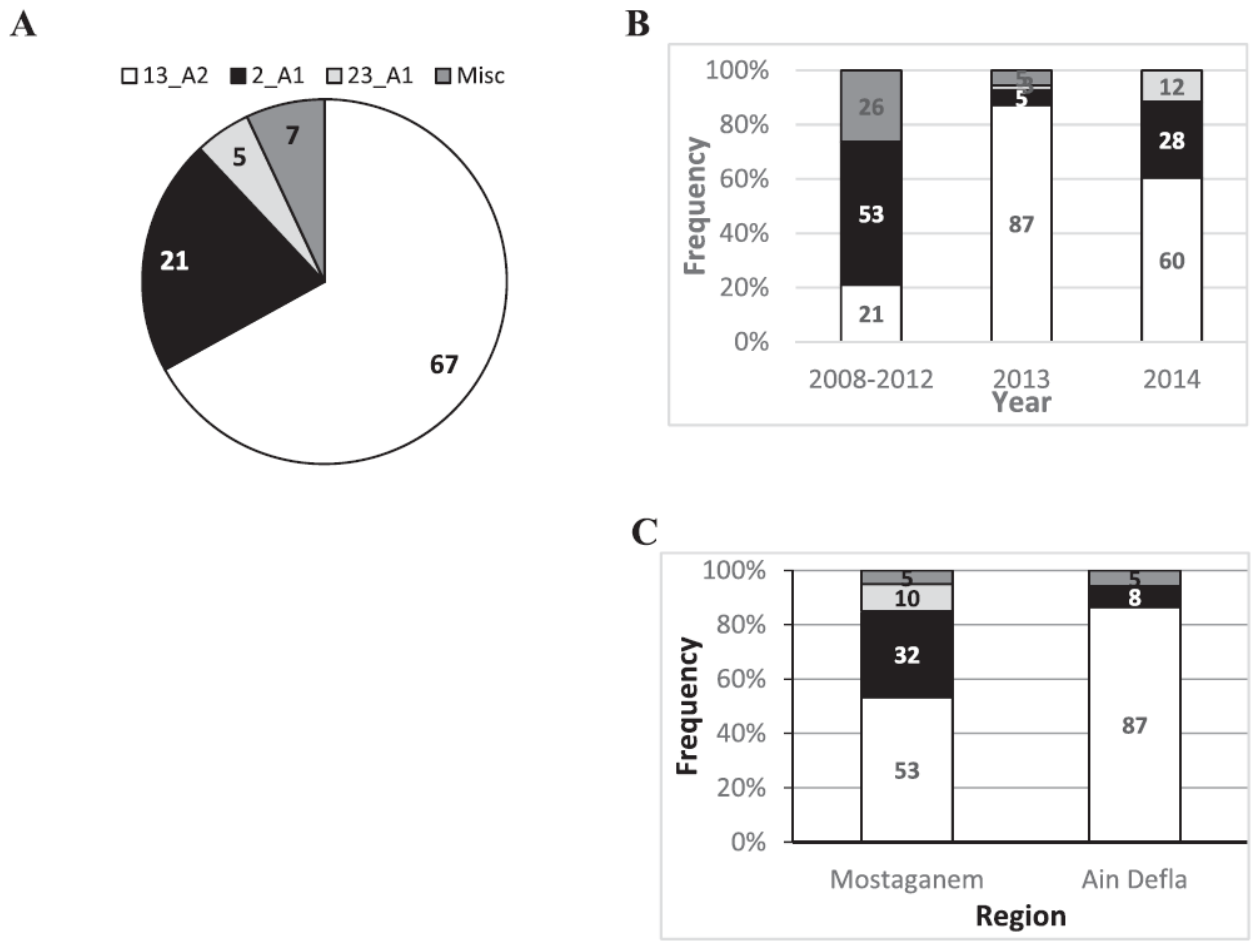

Fig 4 - Genotype frequency of Phytophthora infestans isolates collected from the northwestern Algeria during 2008-2014. (A) For all isolates tested (n = 117). (B) With respect to sampling year. (C) With respect to two regions (Mostaganem and Ain Defla).

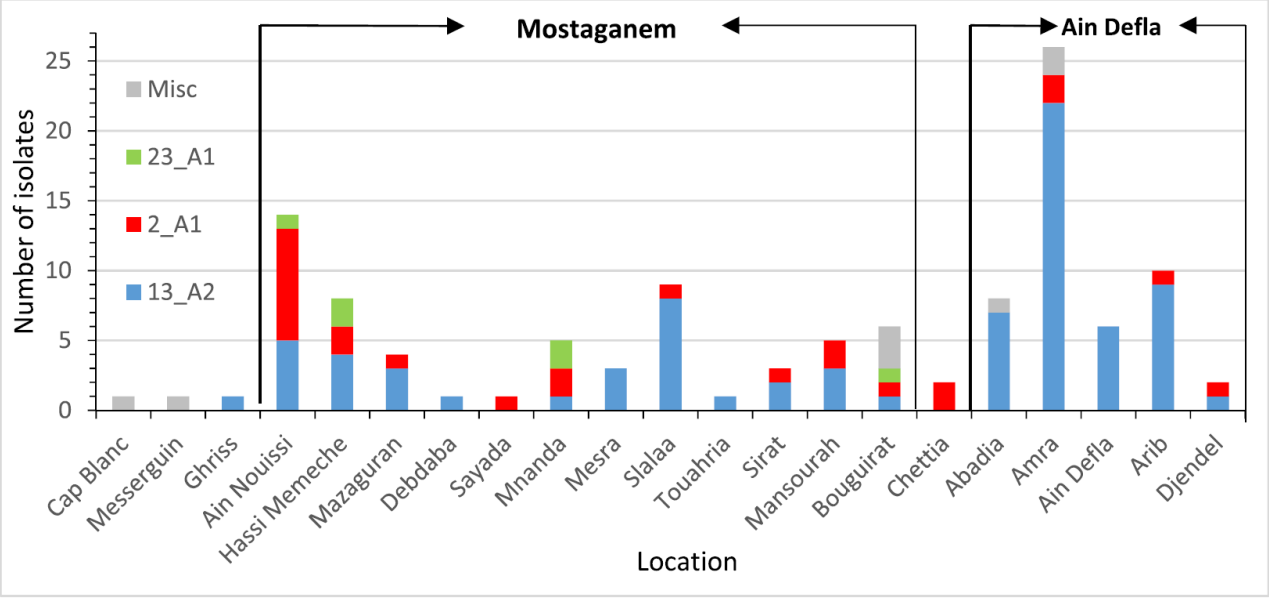

Fig 5 - Frequency of *Phytophthora infestans* genotypes in the northwest of Algeria during 2008-2014 with respect to sampling sites (from West to East).

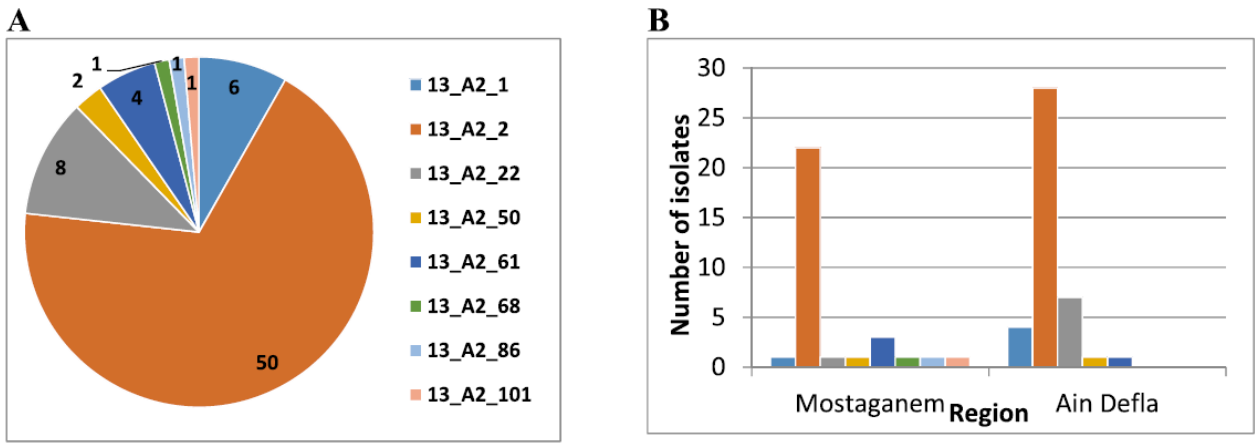

Fig 6 - Sub-groups frequency analysis of the genetic '13_A2' lineage of *Phytophthora infestans* isolates collected from northwestern Algeria during the period of 2008-2014. (A) For all isolates identified as '13_A2' group (n = 73). (B) Depending on the region of sampling (Mostaganem and Ain Defla).