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Phenotypic and genotypic characteristics of Algerian isolates of *Phytophthora infestans*

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SUMMARY

Late blight is one of the most important biotic constraints to potato production in Algeria. Characterization of Algerian *P. infestans* isolates collected on potato and tomato during 2007 to 2009 was performed for mating type, metalaxyl sensitivity, virulence on a set of potato *R*-gene differentials, *in vitro* mycelial growth on agar medium at five temperatures, aggressiveness on leaflets of four potato cultivars and genotypic diversity with 11 microsatellite loci. All isolates from potato but two were A2 mating type, and most of them overcame all 11 *R*-specific genes from the international differential host set; all A2 isolates were metalaxyl resistant. The two isolates collected from tomato were A1 and metalaxyl sensitive. The presence of both mating types in *P infestans* in Algeria raises the possibility of the formation of oospores. Mycelial growth was fastest at 19°C for all the isolates, but A1 isolates from potato and tomato grew significantly faster than A2 isolates at 27°C. No aggressiveness differences were noticed between A1 and A2 isolates, but the relative rankings of the isolates changed according to the cultivar and to the component (lesion area, sporangia production) measured. Cvs. Bintje and Spunta (the dominant cv. in Algeria) were highly susceptible. By contrast, the sporulation of the isolates was relatively low on the moderately susceptible cvs Désirée and Atlas. SSR markers revealed a large genotypic diversity and distinct genetic groups, according to mating types and host plants. Phenotypic and genotypic traits suggest that the A2 Algerian population of *P. infestans*, collected from potato, could be closely related to Western European populations.

KEYWORDS

Phytophthora infestans, *Solanum tuberosum*, *Lycopersicon esculentum*, mating type, aggressiveness, virulence, metalaxyl, mycelial growth, temperature, microsatellite markers

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INTRODUCTION

Late blight is one of the main biotic constraints to potato production in Algeria. A nation-wide crop failure due to late blight was observed in 2007, and regional outbreaks are annually noticed on both potato and tomato crops in Western and Central Algeria. In these major potato production areas, the climate is favourable for *Phytophthora infestans* and severe late blight epidemics occur during spring and autumn, as two main potato crops are grown during a calendar year. Furthermore, in some areas, potato and tomato are grown in close proximity to each other, and volunteers and refuse piles are often observed in or close to the fields. These factors lead to the potential for late blight and aerial inoculum at any time of the year. The severity of blight epidemics is further fuelled by a number of additional causes, such as incorrect spray programs (no preventive application and excessive use of phenylamine fungicides), overhead irrigation, absence of crop rotation, and the widespread use of susceptible cultivars, such as Spunta (the dominant cv. in Algeria). New *P. infestans* populations, and/or presence of oospores in soil, making possible early attacks and the survival of the pathogen outside its host, over several years, may also worsen the severity of late blight epidemics. Algeria annually imports 100 000 tons of potato seeds, primarily from the Netherlands (58%), France (16%) and Denmark (13%), and part of these are multiplied locally in the spring for the autumn potato crop. Latently infected seed tubers are thus an important potential source of primary inoculum for late blight epidemics, and European *P. infestans* isolates might have been introduced in Algeria through such seed tubers. Except a study on a small number of Algerian isolates (Beninal *et al*., 2009), very little is known about the population characteristics of *P. infestans* in Algeria. 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 Algerian *P. infestans* isolates collected on potato and tomato during 2007 to 2009, and compared them with some French isolates sampled on potato at the same period. We studied phenotypic and genotypic traits to determine : (i) the mating type ratio in the studied population ; (ii) the population level of metalaxyl sensitivity ; (iii) which *R*-genes are these Algerian isolates able to overcome; (iv) the effect of temperature on *in vitro* mycelial growth on agar medium ; (v) if these isolates differ from each other with respect to aggressiveness on four potato cultivars ; (vi) their genotypic diversity with microsatellite markers.

MATERIALS AND METHODS

Phytophthora infestans isolates

A total of 36 isolates was sampled in 2007, 2008 and 2009 from naturally infected potato and tomato crops in Western and Central wilayas (Algerian regions) between Algiers and Tlemcen (distant from about 450 km) (Table 1). Some French isolates, collected in 2008, were added for comparison with Algerian populations. Infected leaves or stems were collected from independent plants from different cultivars. Single-lesion isolates were obtained by placing 1 cm^2 pieces of infected tissue on tuber slices of a susceptible potato cultivar. Pure axenic cultures were then obtained by transferring small pieces of mycelium growing on the upper side of the potato slice on pea agar medium, and subsequently maintained in darkness by serial transfers on pea agar medium.

Mating type determination

The mating-type of each isolate was determined by individually pairing them on pea agar with known A1 and A2 testers. After 10-14 days incubation in darkness at 15°C, the presence or absence of oospores was recorded under a microscope.

Effect of temperature on *in vitro* mycelial growth

Radial growth of mycelium on agar medium was used to assess behavior of the isolates at several temperatures. Mycelial growth was compared at five constant temperatures (11, 15, 19, 21 and 27°C) for nine Algerian isolates (2 from tomato and 7 from potato) and two French isolates (1 A1, 08-P13-02 and 1 A2, 08-P13-08). Agar plugs (8 mm diameter) were cut from the edge of 2-week old cultures and placed in the center of Petri dishes (90 mm diameter), containing pea agar medium. All Petri dishes were prepared on one day with the same medium. There were three replicate dishes per isolate and temperature. Cultures were placed together in a plastic box and incubated in darkness at each temperature. After seven days, the size of each colony was measured along two perpendicular directions. After the diameter of the mycelium plug was substracted, the two measurements were averaged. The experiment was carried on for one further week at the two extreme temperatures (11°C and 27°C), as cultures had not reached the edge of dishes after 7 days, and the colony diameters were measured again as described above. Data were subjected to analysis of variance (ANOVA) using SAS statistical software. Whenever significant effects were detected, means were compared using the Student-Newman and Keuls test.

Pathogenicity tests

Potato plant material

Plants from potato genotypes were grown from seed tubers in pots filled with 1:1:1 sand-peat-compost mixture, in a glasshouse regulated at 15-20°C. They were watered with a nutrient solution (Hakaphos; NPK 15/10/15) once a week. Leaflets were collected for experiments on 6-8 week-old plants.

Virulence patterns were determined using Black's differential set of potato clones, each having one of the *R1-R11* pathotype-specific resistance genes, and Bintje as susceptible cultivar. This set was originally provided by the Scottish Agricultural Science Agency (SASA, Edinburgh, UK) and seed tubers were multiplied by INRA (UMR APBV, Ploudaniel, France). Recent work has shown that the *R3* differential (CEBECO-4642-1) actually contains two closely linked genes, *R3a* and *R3b* (Huang *et al*., 2004). Virulence to each of these could not be assessed separately, due to the lack of available differential hosts with only one of these two genes. Therefore, virulence to *R3* was regarded as a single factor.

Aggressiveness was quantified on four potato cultivars: Bintje (highly susceptible to late blight in Europe and not cultivated in Maghreb), Spunta (susceptible and dominant in Algeria), Désirée and Atlas (moderately susceptible with partial non-specific resistance, and grown in Algeria).

Inoculum preparation

Each isolate was multiplied separately on detached cv. Bintje leaflets. Leaflets were stored abaxial side up on the lids of inverted Petri dishes containing 1% water agar. They were infected by deposing a 20µL drop of suspension of *P. infestans* sporangia collected by flooding a 3-week-old culture with 5-6 mL sterile distilled water and gently scraping the colony surface to remove sporangia. Prior to inoculation, sporangial suspensions were chilled at 4 °C for at least two hours to promote zoospore release. Dishes containing the inoculated leaflets were deposited in clear plastic boxes, in an illuminated incubator. After seven days of incubation in humid chambers under controlled conditions (15°C/18°C night/day temperatures, 16h daylight), the sporangia produced on infected leaflets were collected in sterile water ; the resulting suspensions were adjusted to 5×10^4 sporangia mL^{-1}, chilled at 4°C for two hours, and used for pathogenicity experiments.

Metalaxyl resistance test

The sensitivity to metalaxyl of 28 Algerian isolates and 5 French isolates was assessed in a floating leaf disk bioassay as previously described (Beninal *et al*., 2009). Sensitivity was tested with metalaxyl

(Ridomil 25 WP, Novartis experimental compound) at concentrations of 10 and 100 mg L^{-1}. Isolates sporulating on the disks floating on water containing 100 mg L^{-1} metalaxyl were rated as resistant, those on 10 mg L^{-1} were rated as intermediate and those that sporulated only on water were rated as sensitive.

Virulence phenotype determination

A total of 19 Algerian isolates collected in 2007 and 2008 (2 from tomato, 17 from potato) were tested and compared to 7 French isolates (4 A1 and 3 A2). Each leaflet was placed abaxial face up on a moist filter paper in a clear plastic dish and inoculated by depositing a 20 µL drop of the sporangial suspension on each side of the midrib. Two leaflets per isolate and differential host were inoculated. After incubation as described above, each inoculation site was scored for the presence or absence of a sporulating lesion and interaction was considered compatible if sporangiophores were visible.

Aggressiveness quantification

Aggressiveness was performed with 13 isolates : 2 Algerian isolates from tomato, 7 Algerian isolates from potato and 4 French potato isolates tested for comparison (2 A1 named 08-P15-02 and 08-P43-01, and 2 A2 named 08-P13-11 and 08-PON01-01). Experiment was conducted on four potato cultivars, Bintje, Spunta, Désirée and Atlas, chosen according to their level of susceptibility to late blight and grown in Algeria, except cv. Bintje, used as reference. Six leaflets were inoculated for each isolate-cultivar combination. Each leaflet was placed abaxial face up on the lids of inverted Petri dishes containing 10 g L^{-1} water agar (two leaflets per dish), and inoculated by depositing a 20 µL drop of sporangial suspension (about 1 000 sporangia) at the leaflet center. Infected leaflets were incubated for six days as described before. Lesion area (LA, in cm^2) was measured with a image analyser and the Histolab software (Microvision Instruments, Evry, France). Each leaflet was washed in 10 mL saline buffer (Isoton II), and sporangia were counted with a Beckman Coulter Z2 counter (Villepinte, France) to determine sporangia production per lesion (SP). The spore capacity (SC) was then calculated as the mean number of sporangia produced per cm^2 of lesion. Data were subjected to analyses of variance using the general linear models (GLM) procedure of the SAS statistical software. Whenever significant effects were detected, means were compared using the Student-Newman and Keuls test.

DNA extraction and microsatellite amplification

Eighteen Algerian isolates (2 from tomato and 16 from potato) were grown separately in pea broth, previously autoclaved for 20 min at 120°C. After three weeks of incubation at 15°C, mycelium was washed three times in sterile water, and lyophilized. DNA was extracted using the Blood and Tissue 96 kit (Qiagen) according to the manufacturer's instructions, and stored at -20°C. Alleles at 11 polymorphic microsatellite loci - Pi4B, Pi4G and PiG11 developed by Knapova and Gisi (2002); and Pi02, Pi89, Pi04, Pi16, Pi33, Pi56, Pi63 and Pi70 developed by Lees *et al.* (2006) - were amplified in Polymerase Chain Reactions (PCR) performed in a 12,5 µL volume containing between 20 and 200 ng of DNA of *P. infestans*, 2.5 µL of 10X PCR Buffer (Promega), 0.3 mM of each dNTP, 2 mM of $MgCl_2$ and 0,5 U of Taq DNA polymerase (GoTaq, Promega). Concentrations of forward and reverse primers followed Eucablight protocols for SSR analysis of *P. infestans* (SCRI, Scottish Crop Research Institute, UK). In order to detect simultaneously the alleles at several loci, primers were labeled with fluorescent dyes and pooled into four panels : 1) Pi02, Pi89 and Pi4B, 2) PiG11, Pi04, Pi70; Pi56 and Pi63, 3) Pi16 and Pi33, and 4) Pi4G. PCRs were performed under the following conditions: the PCR started with a cycle of 5 min at 94°C, followed by 30 cycles of 20 s at 94°C, 25 s at 58°C, 30 s at 72°C, and finished with an elongation cycle of 5 min at 72°C. PCR products were added to deionized formamide loading buffer, and samples were loaded into an ABI Prism DNA sequencer run according to manufacturer's instructions (Applied Biosystems). DNA fragments were

automatically sized with the GeneMapper™ 3.5 software. Allele sizes were calibrated to the allele sizes of reference isolates and with SSR allele information sheet, kindly provided by D.E.L. Cooke (SCRI).

RESULTS

The A2 mating type is prevalent on potato, in Algeria

Of the 36 isolates collected between 2007 and 2009, 34 were from potato and 2 from tomato. All isolates from potato but two (GH-2007 collected in 2007 and AG7 in 2009) were of the A2 mating type. The two isolates sampled from tomato (ITC and AD) in 2008 were of the A1 mating-type (Table 1).

All A2 isolates from potato are resistant to metalaxyl

All 25 Algerian A2 isolates tested proved to be metalaxyl resistant, while the two A1 isolates from tomato were metalaxyl sensitive (Table 1). The A1 Algerian isolate from potato (GH-2007) had intermediate sensitivity, whereas the 4 French A1 isolates from potato were sensitive.

The virulence phenotypes are highly complex

The virulence spectrum in the Algerian isolates was highly complex, except for the A1 isolate from potato, GH-2007 (Table 1). The majority of the A2 isolates from Algeria overcame all 11 *R*-specific genes of the differential set, as did those from France sampled at the same period. Four A2 isolates, collected in 2007, were not virulent to *R9*, but they overcame all the other specific resistance genes. This pathotype was not found in 2008. The two A1 isolates from tomato also had complex profiles, but they were different from each other: ITC was avirulent to *R2*, while AD was avirulent to *R9*, as some A2 isolates from potato. The A1 isolate GH-2007 from potato showed the least complex pathotype, with 7 virulence factors; this pathotype (1.3.4.7.8.10.11) was very common in French A1 isolates (Corbière *et al*., 2010).

Table 1. *Origin, mating-type, metalaxyl sensitivity and virulence profiles of P. infestans Algerian isolates collected from 2007 to 2009 and of French isolates.*

Isolate name	Date of isolation	Location (wilaya)	Cultivar	Mating type (MT)	Metalaxyl sensitivity *	Virulence profile on 11 R specific genes (from R1 to R11)
Algerian	***isolates from***	***potato***				
Z0	5/05/2007	Ain Defla	Spunta	A2	R	virulent to 11 genes
Z1	5/05/2007	Ain Defla	Martina	A2	R	**avirulent to *R9***
Z3	5/05/2007	Mostaganem	Spunta	A2	R	virulent to 11 genes
Z5	5/05/2007	Mostaganem	Spunta	A2	R	virulent to 11 genes
Z12	10/05/2007	Oran	-	A2	R	**avirulent to *R9***
Z13	10/05/2007	Tiaret	Atlas	A2	R	**avirulent to *R9***
Z18	10/05/2007	Tiaret	Atlas	A2	R	virulent to 11 genes
Z21	10/05/2007	Chlef	Désirée	A2	R	**avirulent to *R9***
GH- 2007	10/11/2007	Boutlelis (Oran)	Spunta	**A1**	**I**	**1 3 4 7 8 10 11**
G33-2008	10/03/2008	Boutlelis (Oran)	Spunta	A2	R	virulent to 11 genes
SABL	10/03/2008	Mazagran (Mostaganem)	Safrane	A2	R	virulent to 11 genes
ABD	23/03/2008	Mostaganem	Spunta	A2	R	virulent to 11 genes
AT	02/03/2008	Chentouf (Ain Temouchent)	Spunta	A2	R	virulent to 11 genes

Isolate name	Date of isolation	Location (wilaya)	Cultivar	Mating type (MT)	Metalaxyl sensitivity *	Virulence profile on 11 R specific genes (from R1 to R11)
TLE	15/03/2008	Honaine (Tlemcen)	Spunta	A2	R	virulent to 11 genes
SBA	19/01/2008	Tabia (Sidi Bel Abbès)	Spunta	A2	R	virulent to 11 genes
G28	2008	-	-	A2	-	-
Z22	24/01/2008	Algiers (CNCC)	Atlas	A2	R	-
Z30	13/05/2008	Zerralda (Algiers)	Atlas	A2	R	-
Z31	13/05/2008	Boumerdes	Timate	A2	R	-
Z32	15/05/2008	Staoueli (Algiers)	-	A2	R	virulent to 11 genes
Z33	15/05/2008	Tipaza	Kondor	A2	R	virulent to 11 genes
ST5	17/07/2008	Ain Defla	Spunta	A2	R	-
ST6	17/07/2008	Ain Defla	Spunta	A2	R	-
ST7	17/07/2008	Khemis Meliana (Ain Defla)	-	A2	R	-
ST8	17/07/2008	Khemis Meliana	-	A2	R	-
ST9	25/07/2008	Algiers	-	A2	R	-
ST10	25/07/2008	Algiers	-	A2	R	-
AG1	2009	Algiers (INA)	Spunta	A2	-	-
AG2	05/05/2009	Ain Defla	Spunta	A2	-	-
AG3	20/05/2009	Mostaganem	Spunta	A2	-	-
AG4	20/05/2009	Mostaganem	Atlas	A2	-	-
AG5	20/05/2009	Mostaganem	Amila	A2	-	-
AG6	20/05/2009	Mostaganem	Désirée	A2	-	-
AG7	2009	Oued Smar (Algiers)	Fabula	**A1**	-	-
Algerian isolates from tomato						
ITC	24/02/2008	Hassi Bounif (Oran)	Zahra	**A1**	**S**	**avirulent to *R2***
AD	11/03/2008	El Abadia (Ain Defla)	Actana	**A1**	**S**	**avirulent to *R9***
French isolates from potato						
08-P13-02	23/06/2008	Ploudaniel (29)	Bintje	**A1**	**S**	**1 3 4 7 10 11**
08-P13-08	23/06/2008	Ploudaniel (29)	Bintje	A2	-	-
08-P13-05	23/06/2008	Ploudaniel (29)	Bintje	**A1**	**S**	**1 3 4 5 6 7 8 10 11**
08-P13-01	23/06/2008	Ploudaniel (29)	Bintje	A2	R	virulent to 11 genes
08-P15-02	23/06/2008	Ploudaniel (29)	Bintje	**A1**	**S**	**1 3 4 5 6 7 10 11**
08-P43-01	23/07/2008	Plougar (29)	Atlas	**A1**	S	**1 3 4 6 7 8 10 11**
08-P13-11	23/06/2008	Ploudaniel (29)	Bintje	A2	-	virulent to 11 genes
08PON01-01	25/06/2008	Pluméliau (56)	Atlas	A2	-	virulent to 11 genes

** S : sensitive ; I : intermediate ; R : resistant.*

- : not tested

Algerian and French isolates do not differ for *in vitro* mycelial growth

Radial growth of mycelium was fastest at 19°C for all 11 isolates tested, and was significantly slower at 11°C and 27°C Figure 1 (part A, A2 isolates and part B, A1 isolates). Variance analysis showed significant differences among temperatures (F = 521,92, P<0.0001), isolates (F = 46,91, P<0.0001) and interactions between temperatures and isolates (F = 8,75, P<0.0001). The isolates could be classified into three distinct groups according to their mean growth. The first group was composed of 2 A1 isolates from potato, the second one of 7 A2 isolates and 1 A1 isolate from tomato (AD), and the third group of 1 A1 isolate from tomato (ITC). Growth of A2 isolates from Algeria and France was not different.

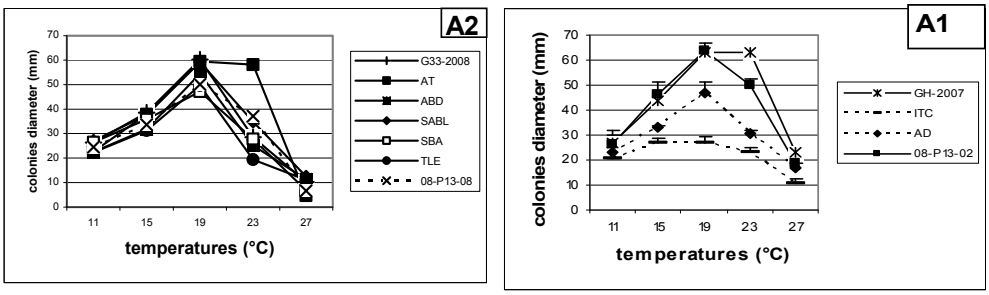

Figure 1. *In vitro mycelial growth of 11 P. infestans isolates cultivated on pea agar medium and incubated in darkness during 7 days, at 5 constant temperatures : 11, 15, 19, 23 and 27°C. Each number is the mean value of three replicate culture plates per treatment.* **A** *: colonie diameter of A2 mating type isolates (6 Algerian isolates and 1 French isolate, 08-P13-08) ;* **B** *: colonie diameter of A1 mating type isolates (in dotted lines : 2 isolates from tomato and in fulled lines : 2 isolates from potato).*

The experiment was carried on for a second week at 11°C and 27°C, as colonies have not reached edge of dishes, and radial growth of mycelium of the 11 isolates for 14 days is presented in Figure 2 (part A, 11°C and part B, 27°C). Variance analysis revealed significant differences among temperatures (F = 480,92, P<0.0001), mating types (F = 37,97, P<0.0001) and interactions between temperatures and mating-types (F = 18,56, P<0.0001). Growth of the isolates was twice as fast at 11°C than at 27°C. At 27°C, A1 isolates grew significantly faster than A2 isolates; the A1 isolate ITC from tomato had the highest colony diameter at that temperature. After 14 days of incubation at 27°C, there was no difference between A1 isolates according to their hosts of origin, potato or tomato.

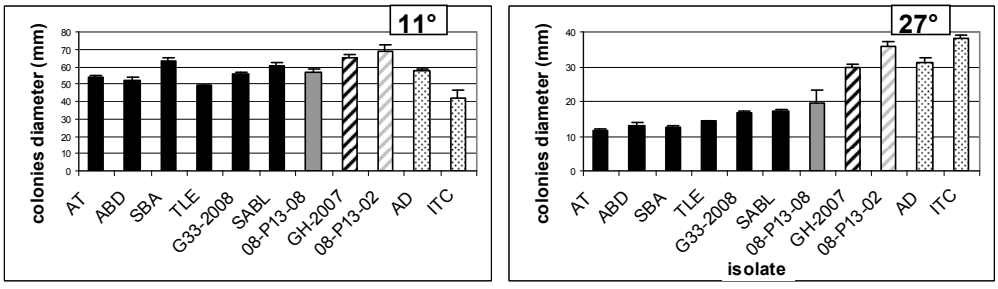

Figure 2. *In vitro mycelial growth of 11 P. infestans isolates cultivated on pea agar medium and incubated in darkness during 14 days, at 2 constant temperatures,* **A** *: 11°C and* **B** *: 27°C. Dotted columns : A1 Algerian isolates from tomato ; shaded columns : A1 isolates from potato ; fulled columns : A2 isolates from potato (in black : Algerian isolates ; in grey : French isolate). Each number is the mean value of three replicate culture plates per treatment.*

No large differences are noticed between aggressiveness on potato of A1 and A2 isolates

Infection experiments showed highly significant effects of cultivars and isolates when the three aggressiveness components were analysed for 13 isolates on 4 potato cultivars. Lesion areas (LA) ranged from 6 cm^2 for isolate ITC on cv. Désirée to more than 11 cm^2 for isolates SABL and 08-PON01-01 on cv. Bintje. Spores production per lesion (SP) ranged from 48000 sporangia for isolate GH-2007 on cv. Désirée to more than 350 000 sporangia for isolates AD and 08-PON01-01 on cv. Bintje (Figure 3). Sporulation capacity (SC) was also highly variable, ranging from 6 500 sp/cm^2 for isolate GH-2007 on cv. Désirée to more than 36 000 sp/cm^2 for isolates AD and AT on cv. Bintje. Isolate ITC showed the lowest LA on all cultivars, except on cv. Spunta; it also had the smallest radial growth of mycelium, at 15°C and 19°C after 7 days of incubation on pea agar medium.

ANOVA performed for LA data showed highly significant differences for cultivars (F = 22,45, P<0.0001) and isolates (F = 17,06, P<0.0001), but a non significant C x I interaction (F = 1,34, P = 0,1). Isolates were fully aggressive to cv. Bintje for LA. Mean LA values on cv. Spunta were not significantly different to those on cvs Désirée and Atlas. ANOVA of SP values also revealed significant effects for cultivars (F = 75,94, P<0.0001), isolates (F = 3,04, P = 0.0005) and cultivar x isolate interaction (F = 2,06, P = 0,0006), while variance analysis of SC data indicated high significance for cultivars (F = 42,67, P<0.0001), isolates (F = 4,08, P<0.0001) and C x I differential interaction (F = 2,21, P = 0.0002). Among the four potato cultivars, Desirée and Atlas presented the lowest SP and SC values. On these two cultivars, isolates produced on average two times less sporangia than on cv. Bintje (SP mean values of $12,6.10^4$ and $13,7.10^4$ sporangia versus $27,6.10^4$ sporangia, respectively). However, the relative rankings of isolates changed according to the cultivar (Figure 3) and to the component. Nevertheless, and quite interestingly, all Algerian isolates were highly aggressive on cv. Bintje and were adapted to this cultivar, although it is not grown in Algeria. There was no significant difference between isolates according to their mating type or to their country of origin.

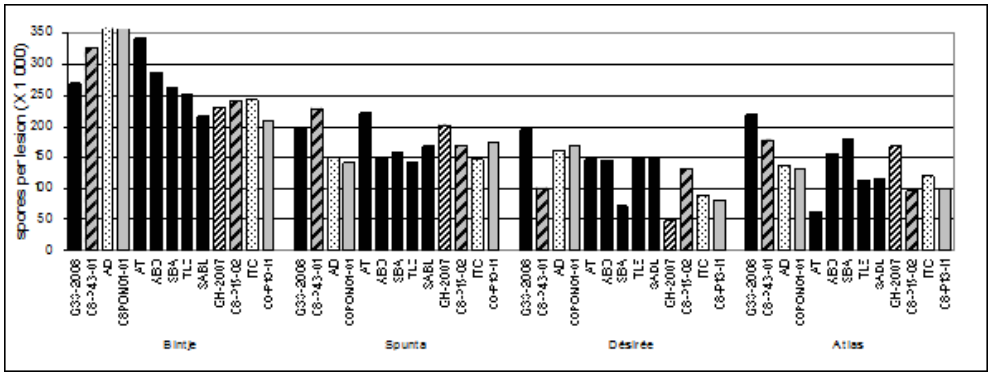

Figure 3. *Spore production (number of sporangia per lesion) of 13 P. infestans isolates on 4 potato cultivars : Bintje, Spunta, Désirée and Atlas. Data were obtained in a detached-leaflet assay, after 6 days of incubation at 15°C/18°C night/day temperatures, 16h daylight. Dotted columns : 2 A1 Algerian isolates from tomato ; shaded columns : 3 A1 isolates from potato (1 from Algeria and 2 from France) ; fulled columns : A2 isolates from potato (in black : 6 Algerian isolates ; in grey : 2 French isolate). Each number is the mean value of six replicate leaflets per treatment.*

SSR markers reveal different *P. infestans* populations on potato and tomato

The genotypes of 18 Algerian isolates (2 from tomato and 16 from potato) were explored using microsatellite markers. A total of 32 alleles were detected over the 11 microsatellite loci, with two to five alleles per locus (Table 2).

Table 2. *Single sequence repeat multilocus genotypes detected with 10 microsatellite markers, in 18 Algerian isolates of P. infestans from potato and tomato.*

			Alleles detected with 10 SSR markers									
Isolate name	MT	Plant *	Pi02	Pi89	Pi4B	G11	Pi04	Pi70	Pi56	Pi63	Pi16	Pi33
AD	**A1**	**T**	**162/164**	179/**181**	**213/217**	**162/162**	**160/168**	192/**195**	174/**174**	**148/**157	176/178	203/203
ITC	**A1**	**T**	**162/164**	179/**181**	**213/217**	**162/162**	**160/168**	192/**195**	174/**174**	**148/**157	176/178	203/203
GH 2007	**A1**	P	**152/**162	179/179	**217/217**	**156/156**	166/170	192/**195**	174/176	-	176/178	**200/200**
Z33	A2	P	**162/**162	179/**181**	**217/217**	**140/140**	166/170	192/192	**176/**176	151/157	176/178	203/**206**
G28	A2	P	160/162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	**200/200**
G33	A2	P	160/162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	**200/200**
AT	A2	P	160/162	179/179	205/**205**	154/160	166/170	192/192	174/176	151/157	176/178	203/203
Z13	A2	P	**162/**162	179/179	205/**205**	154/160	166/170	192/192	174/176	151/157	**178/**178	203/203
Z18	A2	P	-	179/179	-	154/160	166/170	192/192	174/176	151/157	**178/**178	203/203
Z12	A2	P	**162/**162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	**178/**178	203/203
Z32	A2	P	**162/**162	179/179	-	**160/**160	166/170	192/192	174/176	151/157	**178/**178	203/203
Z21	A2	P	-	179/179	-	**160/**160	166/170	192/192	174/176	-	**178/**178	203/203
TLE	A2	P	**162/**162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	203/203
Z3	A2	P	**162/**162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	203/203
SABL	A2	P	160/162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	203/203
Z0	A2	P	160/162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	203/203
Z1	A2	P	160/162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	203/203
ABD	A2	P	160/162	179/179	205/213	154/160	166/170	192/192	174/176	151/157	176/178	203/203

*Plant * : T = tomato ; P = potato ; - : no data*

Of 11 SSR markers tested, all primers showed polymorphism, except Pi4G marker (two alleles were revealed, 159 and 161 bp and data were missing for 5 isolates). The 18 *P. infestans* isolates genotyped split into 10 unique multi-locus genotypes (MLGs), five of which being represented by a single isolate (GH2007, AT, Z12, Z13, Z33) and one of them detected in four A2 isolates (Z0, Z1, SABL, ABD). Five of these 10 MLGs were not present in French populations collected in 2006 to 2008 on potato (Corbière *et al*., 2010). That concerned the three A1 isolates sampled on potato (GH-2007) and on tomato (AD and ITC), and four A2 isolates (Z12, Z21, Z32 and Z33) collected on potato in 2007 and 2008 in different wilayas. The remaining 5 MLGs, present within French populations, all corresponded to A2 isolates. Although the number of isolates was limited, the 8 MLGs corresponding to A2 isolates showed a limited genotypic diversity (polymorphism at 1-3 of the 11 loci), except that corresponding to isolate Z33. Among A1 isolates, two genotypic groups were distinguished according to their host of origin. The two isolates AD and ITC from tomato belonged to the same MLG, which differed markedly from that of isolate GH-2007 from potato. Moreover, the MLG of isolates AD and ITC presented alleles relatively rare in Europe, *e.g.* allele 164 at locus Pi02, and alleles 160 and 168 at locus Pi04 (SSR allele information sheet, D.E.L. Cooke, SCRI, UK).

DISCUSSION AND CONCLUSIONS

Monitoring of *P. infestans* in the Western and Central coastal regions of Algeria showed that the A2 mating type is present at a high frequency in potato crops in Algeria, although some A1 isolates were also detected. In contrast, only A1 mating-type isolates were isolated from the few tomato samples that could be analysed. The frequency of A2 isolates is similar to that found in Western European populations on potato where, since 2005, a dramatic shift in mating type was noted (Cooke *et al*., 2010 ; Corbière *et al*., 2010). In Maghreb, the presence of the A2 mating type was also reported in

Moroccan populations (Hammi, 2003; Andrivon *et al*., 2007), and at a lower frequency, in Tunisian population (Hamada and Harbaoui, 2010). In Algeria, further work needs to be performed to obtain a more comprehensive sampling, and thus better evaluate the respective frequencies of the two mating types. However, the presence of both A1 and A2 isolates within *P infestans* populations in Algeria raises the possibility of sexual reproduction and the generation of oospores.

All the Algerian A2 isolates tested were metalaxyl resistant. The continued use of foliar application of metalaxyl on potatoes in Algeria, in spite of resistance, may have contributed to the predominance of the metalaxyl-resistant isolates on this crop, by a high selection pressure. Nevertheless, within Western European and Maghrebian populations, a strong association between metalaxyl resistance and A2 mating type has also been noticed (Hammi, 2003; Cooke *et al*., 2010; Corbière *et al*., 2010; Hamada and Harbaoui, 2010; Kildea *et al*., 2010). The A1 isolate recovered from potato in Algeria (GH-2007) was identified with intermediate sensitivity. According to Klarfeld *et al*. (2009), isolates with intermediate resistance to metalaxyl could suggest an oospore origin. Isolate GH-2007 with intermediate sensitivity and a unique MLG could thus be a recombinant isolate. Continuous blight monitoring in potato and tomato production regions must therefore be carried on to identify any trend towards sexual reproduction. On tomato, the two A1 isolates exhibited in contrast metalaxyl sensitivity. The absence of metalaxyl resistance in tomato isolates has also been found in the Netherlands, South Africa and Morocco where high levels of resistance have been found on potato (Mc Leod *et al*., 2001; Hammi, 2003). This result might be explained by host preference of *P. infestans* isolates, although all isolates are pathogenic on potato.

The interaction between climate changes and thermal adaptation of *P. infestans* may have profound effects for the future of potato production in Algeria. Our results on mycelia growth at different temperatures are consistent with early work which found that growth on medium was the most rapid at 20-21°C, with a minimum temperature of 2-3°C and an upper temperature limit close to 30°C (Crosier, 1934 *in* Harrison, 1992). They thus do not support the idea that *P. infestans* isolates from Algeria and France show a different pattern of temperature adaptation than earlier populations, or from current French populations of the pathogen. However, the behavior of the isolates from potato was slighly different according to their mating type; mycelial growth of A1 isolates was higher than those of A2 isolates, especially at 27°C, after two incubation weeks. This observation suggests that A1 isolates could be more tolerant to high temperatures than A2 isolates, but this needs confirmation. These data should however be supplemented by additional results from *in-planta* experiments, since Harrisson (1992) demonstrated that temperature and polygenic resistance to blight interact to determine hyphal growth in leaflets.

Investigating pathotype composition provides information that is especially important in breeding for crop resistance. This study confirms the presence of highly complex pathotypes of A2 *P. infestans* isolates in Algeria, as reported previously (Beninal *et al*., 2009). These results are also consistent with data from French populations, where 75% of the A2 isolates, collected in 2007 and 2008, had these two virulence profiles (Corbière *et al*., 2010). Our data showed that the virulence spectrum of the Algerian A1 isolate from potato differs from those of A2 isolates, and corresponds to a pathotype which is prevalent in many European countries (Lehtinen *et al*., 2008; Hannukkala *et al*., 2009; Chmielarz *et al*., 2010; Corbière *et al*., 2010). By contrast, on tomato, A1 isolates presented highly complex profiles with 10 virulence factors. It is unclear at this point whether *P. infestans* isolates from tomato have host-specific virulence profiles, and it should be considered in future research. Based on our results, pathotypes in Algeria are not associated with potato cultivars or with regions. The isolates that were virulent on all 11 *R* differentials were collected from different cultivars (Spunta, Atlas and Kondor), in several wilayas between Algiers and Tlemcen. Since most cultivars grown

in Algeria (or sampled in this study) have no or few *R* genes, it is likely that random mutation plays an important role in the diversification of pathotypes. Moreover, *P. infestans* seems highly mobile through airborne sporangia or infected tubers and could migrate on hundreds of kilometers (Montarry *et al*., 2010).

An increase in aggressiveness has often been postulated to be linked to the appearance of the A2 mating type isolates in *P. infestans*. Here, no significant differences between A1 and A2 isolates, except for isolate ITC from tomato, were noticed. This result is consistent with a previous report on French populations, where A1 isolates were slightly more aggressive than A2 isolates on cv. Bintje (Corbière *et al*., 2009). Some of the isolates differed strikingly in aggressiveness on the four cultivars tested, so pathogenic fitness on one potato cultivar was not related to pathogenic fitness on the other potato cultivars. Therefore, using several cultivars with different levels of pathotype-nonspecific resistance is desirable, as it increases the value of the results (Lehtinen *et al*., 2009). In the same way, the present work confirms, as reported by several authors, different levels of pathogenic fitness across isolates. It is therefore important to evaluate resistance levels of potato genotypes with different isolates of *P. infestans*.

Our results showed a large variation in sporangial production on the four potato cultivars. Spore production is an important component when assessing pathogen aggressiveness, because, in a polycyclic disease such as late blight, secondary cycles and new lesion formation are determined to a large extent by the amount of spores produced. This component has also important implications for late blight management regarding the use of decision support systems (DSS). According to this aggressiveness component, the classical behavior of the cultivars was confirmed. Cv. Bintje was the most susceptible to Algerian isolates; cv. Spunta, dominant in Algeria, was also fully susceptible, while cvs Désirée and Atlas exhibited moderate susceptibility. In Moroccan *P. infestans* population, isolates also produced significantly fewer sporangia on cv. Désirée compared with cv. Spunta (Hammi, 2003). We did not notice local adaptation of Algerian isolates to cv. Désirée in comparison with cv. Bintje, although cv. Désirée is grown for a number of years in Algeria and cv. Bintje is not cultivated. Algerian and French isolates did not show significant aggressiveness differences. Then, as with others factors analysed in the work, *P. infestans* populations of these two countries seem presented large similarities. On the other hand, because of the small sample size, it was not possible to study regional aggressiveness differences in *P. infestans* populations within wilayas separated by hundreds of kilometres, or within types of potato production (cultivated on spring and autumn or grown from locally produced and imported seeds).

In our experimental conditions, AD isolate from tomato was highly pathogenic on potato and this result did not indicate evidence for host adaptation. However it is practically impossible to know how many generations this isolate has spent on the host it was isolated from. This tomato isolate might have been on potato in former generations and still possess high fitness on potato. It would thus be premature to conclude before more studies are conducted to evaluate host preference and quantitative aggressiveness of isolates collected on potato and tomato, on both hosts, and with larger sample sizes. Indeed, Hammi (2003) showed differencies between potato and tomato isolates when he tested the ability of Moroccan isolates to infect leaves of both hosts. According to this author, isolates from potato equally attacked both potato and tomato ; in contrast, isolates from tomato were highly pathogenic on tomato cv. Daniela , but less aggressive on potato cvs Spunta, Désirée, Nicola and Kondor.

Algerian *P. infestans* isolates, collected from potato, proved to be highly aggressive, complex pathotypes. This emphasizes the need to incorporate diverse sources of resistance into breeding programs and to focus on non-specific resistance. Indeed, specific *R* genes, *e. g. R9*, is now overcome although it has never been introduced into commercial cultivars. The plasticity of *P. infestans* genome will reduce the efficacy of breeding resistance based simply on the accumulation of *R*-genes.

In contrast, cv. Sarpo Mira has proved to possess high partial blight-resistance, with no apparent changes in resistance level in recent cultivar trials (Lees *et al*., 2009 ; Chmielarz *et al*., 2010 ; Galfout *et al*., 2010).

Finally, molecular markers were used to assess genomic variations, which are not affected by host or environmental factors that influence the expression of phenotypes. SSR markers revealed three distinct genetic groups according to mating types and host plants : one is composed with two A1 isolates from tomato; a second one with one A1 isolate from potato and a third one with A2 isolates from potato. The predominant A2 genotype, characterized in our study with four isolates, had a multilocus genotype close to the genotype identified by D.E.L. Cooke as genotype 13 or "Blue 13", but D13 marker missed in our analysis. The other 10 A2 isolates, except Z33, deviated from the predominant pattern with variation at one, two or three loci. Such minor changes could be attributed to mutation or mitotic recombination within clonal lineages. The origin of A2 isolate Z33 has to be explored to know whether it could be a recombinant isolate, as it is suspected for isolate GH-2007. In Great Britain, Ireland and France, the clonal genotype 13 is now dominant within A2 isolates (Cooke *et al*., 2010; Corbière *et al*., 2010; Kildea *et al*., 2010). It is possible that this clonal A2 MLG will have higher pathogenic fitness than other genotypes and then spread in potato crops of many regions. In cold winter regions, as in Nordic European countries, the *P. infestans* populations have been shown to be genetically diverse and it is suggested that this variation is maintained by sexual reproduction, as both mating types were present in 29-56% of the fields (Widmark *et al*., 2007; Lehtinen *et al*., 2009). Due to an effective clonal propagation and spread, a highly pathogenic *P. infestans* genotype would have the ability to respond quickly to selective pressure, and successful isolates can, over short periods, become dominant in the pathogen population. However, in Algeria as in France, none of the factors (metalaxyl resistance, effect of temperature on mycelial growth, virulence, aggressiveness) could apparently explain the invasion of potato crops by A2 mating type isolates of *P. infestans*.
Although only a limited number of tomato crops were sampled, the SSR analysis suggests that the population of *P. infestans* on tomato is genotypically distinct from that on potato. Reports on the host specificity of *P. infestans* populations on tomato and potato vary among regions. In some locations, *P. infestans* populations that infected both tomato and potato could not be distinguished by neutral genetic markers (Chen *et al*., 2008). In contrast, the same genetic markers have shown that distinct genotypes are associated with different hosts. The current study revealed that the two isolates collected from tomato are genotypically identical with the markers used, but that they are really different in their phenotypic characters. Moreover, despite the clonal structure of A2 *P. infestans* isolates, there was also a lack of association between genotypic and phenotypic traits of the isolates. This result is not unexpected because molecular neutral markers are not necessary linked to phenotypic markers. Further research is warranted to investigate the genetic diversity of Algerian isolates with a wider range of isolates and SSR markers, as new sets are now available (Guo *et al*., 2009, Cooke *et al*., 2010). A comparative study of Algerian *P. infestans* population structure with European populations, *e.g.* from the Netherlands, France and Denmark from where large amounts of seed tubers are imported, could help to understand origin and diversity of Algerian isolates.

In conclusion, this study provides important data to understand the population diversity and pathogenicity fitness of *P. infestans* in Algeria. Such data might be helpful in supplying information to breeders for Algerian markets, extension specialists and farmers to make rational decisions regarding late blight control.

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