

New data on early blight of potato and tomato caused by a complex of large-spored *Alternaria* species in Algeria

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SUMMARY

Potato and tomato occupy an important place in the Algerian agriculture. These two crops are highly threatened by abiotic and biotic stresses. The surveys carried out in different areas of production since 2010 show that after the downy mildew, early blight was very damaging particularly in areas where the climatic conditions were favourable to the development of the pathogen. From 2012 to 2015, a total of 247 isolates having morphological and cultural characteristics of *Alternaria* were obtained from 12 major growing regions of potato and tomato located in the center, east, west and south of Algeria. On the basis of the morphological characteristics of the isolates (large conidia not catenulated with long beak), 156 isolates belonging to the *A. porri* group were selected for further identification and characterization. Molecular studies based on a PCR using specific primers detected two main species *A. solani* and *A. linariae* whose isolates were obtained from samples showing the symptoms of early blight. Sequential analysis of the Calmodulin gene confirmed the existence of *A. linariae* in the potato and tomato samples. In addition, these analyzes detected among the isolates of *A. solani*, isolates belonging rather to the species *A. grandis* whose samples are also from potato and tomato. Finally, the sequential analyses of the Rpb2 gene have revealed the existence of three isolates belonging to the species *A. protenta* among the isolates initially identified as *A. solani*. Pathogenicity tests carried out by artificial inoculation showed that all isolates belonging to the four species : *A. solani*, *A. linariae*, *A. grandis* and *A. protenta* isolated from potato and tomato fields cause the typical early blight symptômes on detached leaflets and whole seedlings of the two hosts with varying levels of attack. These results confirm the ability of *A. grandis* to also attack tomatoes while it is traditionally found on potato. We show especially for the first time the presence of *A. linariae* on potato culture while this species was always described on tomato. These new data oblige us to reconsider the parasitic specialization of *Alternaria* species with large conidia on solanaceae particularly on potato and tomato.

KEYWORDS

Early blight, potato, tomato, prevalence, large-spored *Alternaria* spp, parasitic specialization

INTRODUCTION

Potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.) are the most important and useful member of the family solanaceae. They represent the first vegetable crop in terms of acreage and production in Algeria (Chehat, 2008). In 2013, Algeria occupied the second rank after Egypt in the production of potatoes in Africa. The national potato production in the last decade (2003-2013) has increased from 1 879 918 to 4 400 000 tonnes for an increase in the acreage under cultivation from 88 660 to 140 000 hectares. Similarly during the same period the yield has increased significantly from 212 to 314,3 Qx/ha (FAOSTAT, 2015). These two crops are distributed differently in some areas, and are conducted according to different cultural practices. The potato which is considered as field crop occupies significant acreage in several regions of the country from north to south and from east to west. On the contrary, tomato is cultivated in the north part of Algeria on smaller surface areas and most often under plastic house as in the Biskra region. Both crops can exist side by side and can follow each other in many coastal regions of the country.

Among the fungal diseases infecting potato and tomato crops, early blight which is a widely distributed disease can cause significant economic yield losses (Pscheidt and Stevenson 1986; Rotem 1994). Early blight epidemics are particularly severe in tropical countries during warm and wet seasons (Batista et al., 2006; Mantecon, 2007). Nevertheless, the disease is becoming more severe in all regions partly due to warmer temperatures (Kapsa, 2008). The early blight symptoms of tomato and potato most frequently observed take two forms : more or less large spots often with yellowing around them and many more spots often quite small. These symptoms have been attributed to several *Alternaria* species : *A. alternata* when the conidia are small and catenate, *A. solani* on potato and *A. linariae* (= *A. tomatophila*) on tomato when the conidia are large and solitary. With the molecular markers, several species morphologically identical with small conidia like *A. alternata*, *A. arborescens*, *A. infectoria* and *A. tenuis* have been reported on both host plants (Simmons, 2000). Likewise, several species morphologically similar with large conidia have been also reported on potato (*A. solani*, *A. grandis* and *A. protenta*) (Simmons, 2000 ; Orina et al., 2009 ; Gannibal et al., 2014 ; Landschoot et al., 2016) and on tomato (*A. linariae*, *A. solani* and *A. grandis*) (Simmons, 2000 ; Rodrigues et al., 2010). In Algeria, a complex of three species *A. solani*, *A. linariae* and *A. alternata* were reported to be the causal agents of the potato and tomato early blight (Ayad, 2014 ; Bessadat, 2014, 2016). In two recent publications, it was reported for the first time in Algeria the presence of *A. protenta* on potato (Ayad et al., 2016) and *A. grandis* on tomato (Bessadat, 2016). This indicates the complexity and the difficulty to assign a given species to a symptom and/or to the morphological characteristics of the conidia.

The objectives of the present study were to identify large-spored pathogenic *Alternaria* species on the basis of molecular, cultural and morphological characteristics and to clarify their pathogenicity on their respective potato and tomato hosts. The cultural, morphological and molecular characterization were based on 156 isolates collected during 4 years (2012-2015) on various potato and tomato fields in different bioclimatic zones of Algeria. To better highlight the different species present in Algeria, the sequences of different genomic regions such as ITS region, Calmodulin and RPB2 genes were analyzed. In addition, the parasitic specialization of *A. linariae* and *A. protenta* newly identified on potato were verified by artificial cross inoculations under conditions very similar to those in the field.

MATERIALS AND METHODS

Prospection, sampling and Isolation

Surveys were carried out in the period 2012 to 2015 and sampling was performed in 12 potato and tomato-growing regions from north (10, 16, 42) to south (03, 07, 39) and from east (21,24) to west (02, 27, 29, 44) of Algeria (Fig.1). More than 247 samples with typical early blight symptoms (dark, elongated or circular lesions with concentric rings surrounded by a yellow halo) were collected for isolation. Lesions with typical early blight symptoms were cut off from infected leaflets, disinfected and placed on potato dextrose agar medium at 22°C. The isolates obtained were purified by monospore culture on potato dextrose agar medium. The occurrence and the prevalence of the disease were evaluated by the percentage frequencies of the *Alternaria* species responsible of the early blight in these two potato and tomato crops and in the different growing regions.

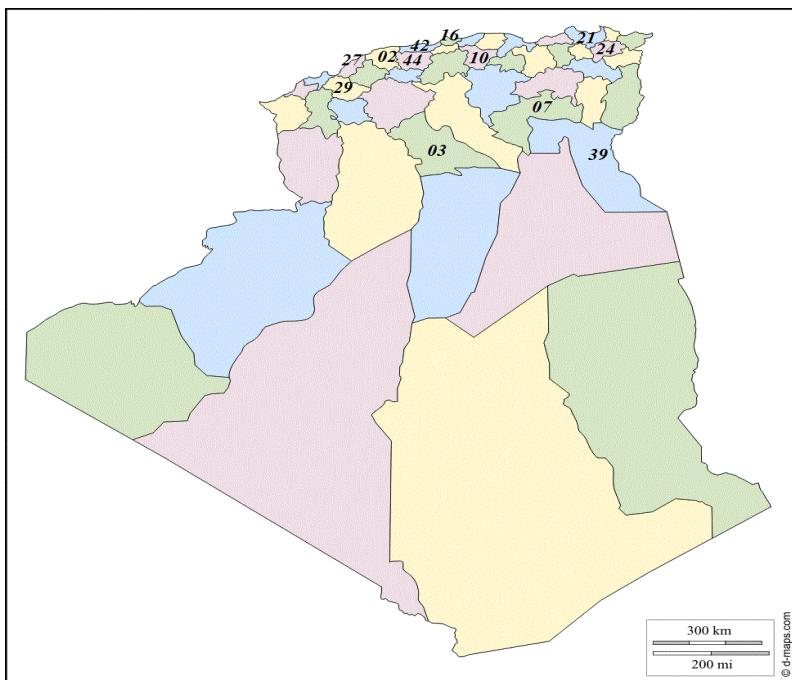


Figure 1. Geographical localization of the potato and tomato- regions prospected

*Identification of large-spored *Alternaria* species using molecular markers*

This study was carried out on the 156 isolates previously selected. Total genomic DNA was extracted according to the method described by Goodwin and Lee (1993). PCR using specific primers of *A. solani*/*A. grandis* group and *A. linniae* was performed. The primer pairs ITS1/ITS4 that gives good amplifications on the *Alternaria* species were used (White et al., 1990 ; Garde et al., 1991). All the DNA extracts of the 156 isolates were amplified. Two primer pairs OAsF7 and OAsR6 specific to both species *A. solani* and *A. grandis* for the amplification of the 164 bp of

the gene encoding Alt a1 and OatF4 and OatR2 which are specific to *A. linariae* for the amplification of the 438 pb of the gene encoding for the Calmodulin were used (Gannibal et al., 2013). These preliminary identifications were completed by PCR/RFLP using restriction enzymes *HaeII* and *RsaI* to differentiate between the two species *A. solani* and *A. grandis*. Of the 156 isolates identified by PCR and PCR/RFLP, 25 isolates were selected and their DNA was amplified at the Calmodulin loci. The PCR products were sent to GATC Biotech (Germany) for sequencing. To confirm the presence of *A. protenta* among the *A. solani* isolates, the locus Rpb2 of all *A. solani* isolates was amplified. All the sequences of the 25 isolates obtained after sequencing were analysed by the BLAST tool and compared with those in the NCBI databases. Sequence alignment was carried out with the software phylogeny.fr ("One Click").

Morphological characterization of large-spored Alternaria species

Of the 25 isolates previously identified by molecular tools, 15 isolates were morphologically characterized. The morphological characteristics used for the identification of the 15 isolates were based on the following criteria : hyphal width, body conidia length and width, beak length, number of horizontal and vertical septa. The measurements of the conidia were compared with those in the literature (Simmons 2007).

Pathogenicity of large-spored species on their respective potato and tomato hosts

To confirm the pathogenicity of the large spored *Alternaria* species, inoculations on detached leaflets and on whole plants were performed on susceptible varieties of tomato (Marmande and St Pierre) and potato (Spunta and SarpoMira). A collection of 7 *A. solani*, 3 *A. linariae*, 3 *A. grandis* and 2 *A. protenta* isolates was used. These isolates were selected for their ability to sporulate. Spore suspensions have been prepared from 15 days old cultures whose sporulation was checked with Malassez hemocytometer. The final concentrations of the suspensions were adjusted to 10^4 conidia/ml. The inoculations were made by depositing drops of 20 µl on detached leaflets and by spraying the whole plants. The disease severity was evaluated by using a visual rating scale from 1 to 9 expressing the extension of necrosis according to Duarte et al. (2013). Statistical analyzes of the results were made with Statistica 6.0 software which enable analysis of variance.

RESULTS AND DISCUSSION

Pré-identification of large-spored Alternaria species

The 247 isolates obtained were divided in two groups on the basis of the morphological characteristics : the first group (91 isolates) with abundant sporulation and small catenulate conidia typical of the section *Alternata*, the second group (156 isolates) with large, solitary, beaked conidia typical of the section *Porri*. These isolates were selected under a binocular stereomicroscope and microscope and compared with those in the literature (Simmons, 2007). The small-spored group have not been included in our study.

Molecular characterization and identification of large-spored Alternaria species

The preliminary tests carried out for the identification of the 156 isolates selected on the basis of morphological characters (large conidia with long beak) and analyzed by the universal primers specific for both groups of *Alternaria* species (*Alternaria solani* *A. grandis* OAsF7/R6 and *A. linariae* OATF4/R2) have confirmed the presence of these three species on potato crop in Algeria. These species were already reported in Algeria by Bessadat (2016) and around the

world by Gannibal et al. (2012), Rodriguez et al. (2009), Landschoot et al. (2017). Further species identification was conducted in order to differentiate between *A. solani* and *A. grandis* isolates on the one hand, and to confirm the identification of *A. linariae* on the other. PCR/RFLP by amplifying the locus Calmodulin followed by restriction enzyme digestion (*HaeII* and *RsaI*) of the PCR products has resulted in the identification of 43 *A. linariae* isolates, 92 *A. solani* isolates, 12 *A. grandis* isolates and 6 isolates which showed an abnormal restriction enzyme digestion. The sequencing of 22 isolates representing each of the three species identified previously and isolated from the two host plants collected in the 12 surveyed regions gave the phylogenetic tree shown in Figure 2. The first cluster contains the *A. linariae* isolates that are distinct from the other two clusters regrouping isolates previously identified as *A. solani*, the second cluster includes *A. solani* isolates, and the third cluster regroups the *A. grandis* isolates. Finally, the sequencing of the Rpb2 locus carried out on the second cluster which include *A. solani* isolates has identified 3 isolates as *A. protenta* two of which were isolated from potato and one was isolated from tomato (Fig.3).

Morphological characterization of large-spored Alternaria species

Of the measurements of the hyphal width, the body conidia length and width, the beak length and the number of horizontal and vertical septa of the four species (*A. solani*, *A. grandis*, *A. linariae* and *A. protenta*) previously identified by molecular tools are indicated in Table 1.

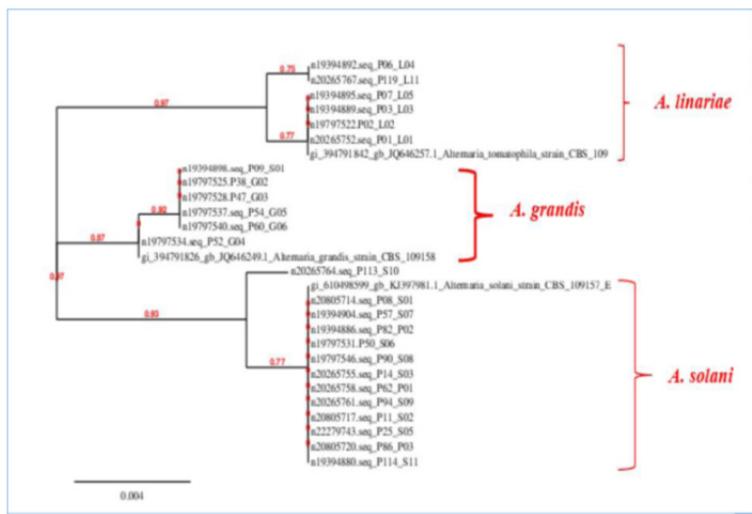


Figure 2. Cluster analysis of sequence at the Calmodulin loci

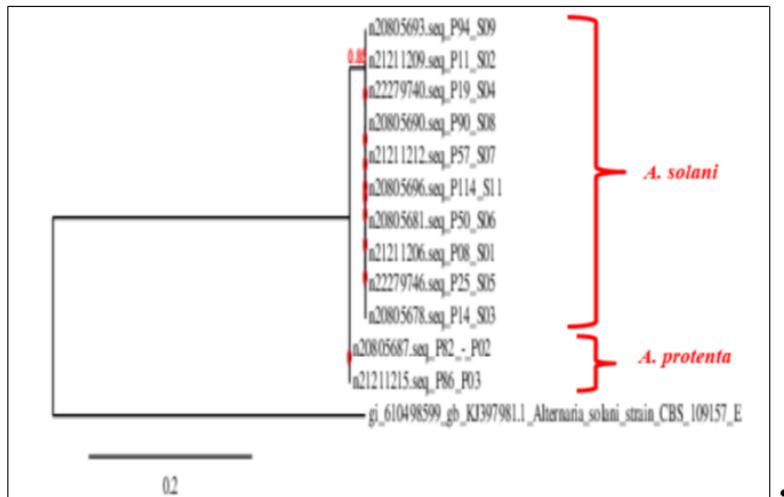


Figure 3. Cluster analysis of sequence at the *Rpb2* loci

Table 1. Morphological characteristics of 15 isolates identified as *A. solani*, *A. grandis*, *A. protenta* and *A. linariae*

Morphological characteristics	Species			
	<i>A. solani</i>	<i>A. grandis</i>	<i>A. linariae</i>	<i>A. protenta</i>
Hyphal width	4.23–7.57µm	4.40–8.74µm	3.39–8.37µm	3.40–6.67µm
Body conidia length	46.23–80.86µm	36.17–97.15µm	41.08–108.27µm	28.14–93.90µm
Body conidia width	13.47–25.54µm	10.66–21.70 µm	8.47–18.12µm	10.30–22.73µm
Beak length	30.86–114.40µm	61.28–186.57 µm	56.84–224.78µm	34.24–130.4µm
Number of horizontal septa	1–9	1–7	0–3	0–8
Number of vertical septa	1–12	2–11	1–19	1–10

The morphological characteristics of 15 isolates with large conidia showed high variability between them. In addition, there was an overlapping in the characters of the isolates in the shape and the size of the conidia. The morphological characteristics of the isolates did not clearly differentiate between the four large-spored *Alternaria* species currently reported on potato and tomato crops. Therefore, morphological characters were not sufficient for clear distinction between the four large spored *Alternaria* species. Even for some species like *A. solani*, *A. grandis* and *A. protenta*, the molecular characterization was not sufficient for their distinction; only nucleotide polymorphism at selected loci (*Rpb2*) allowed specific identification between the isolates of the two species *A. grandis* and *A. protenta*.

*Occurrence and prevalence of the large-spored *Alternaria* species in Algeria*

The survey carried out through the 12 growing regions of potato and tomato showed the presence of 4 large-spored *Alternaria* species in Algeria, i.e., *A. solani*, *A. grandis*, *A. linariae* and *A. protenta* at variable levels (Figure 4). *A. solani* is the most common species in Algeria with 63%. This species has a significant isolation frequency in the south (30.40%) and a relatively small frequency in the east (14.86%) and in the center and the west (8.78%) (Figure 5). The isolation frequencies of the four species identified in Algeria vary also with the crop. *A. solani* attacks both crops potato and tomato, but it is more prevalent on potato (50.60%) than on tomato (12.15%). These results are consistent with those obtained by Bessadat, (2014) which estimate the incidence of the early blight to 79.63%.

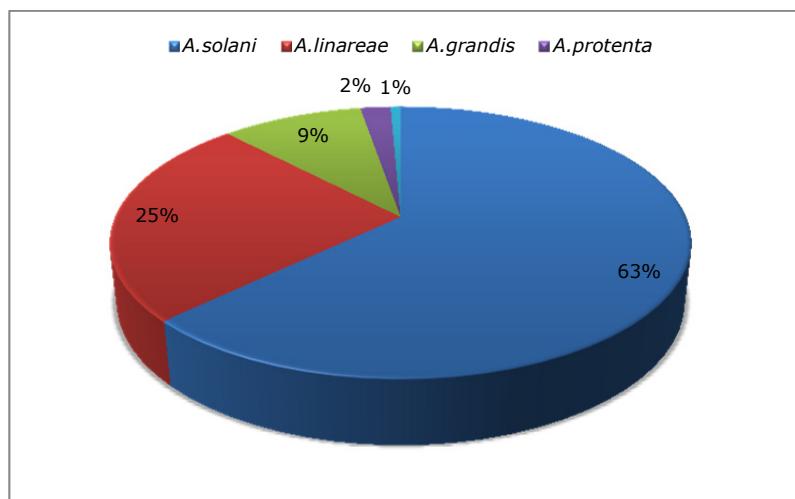


Figure 4. Distribution of *Alternaria* spp. in Algeria on potato and tomato

A. solani was considered for long time as the main species responsible for the potato and tomato early blight, however other species such as *A. grandis* et *A. linariae* have been recently reported respectively on potato and tomato (Simons, 2000 ; Rodrigues, 2009). *A. linariae* was isolated from potato for the first time in Algeria with an isolation frequency of 25% (Communication personal, 2016), whereas *A. grandis* was found on tomato (Bessadat et al., 2016). The isolation frequencies of *A. linariae* were 11.48% in the center, 8.10%, in the south, 4.72% in the west and 0.67% in the east, in addition this species is more prevalent on tomato (20.29%) than on potato (4.71%). *A. grandis* has a distribution percentage of 9% and an isolation frequency of 4.72% in the center and the west of Algeria. This species was found on potato (6.08%) and on tomato (3.37%) (Figure 6). Another species *A. protenta* was characterized through sequencing the Rpb2 gene. Two isolates from potato and one isolate from tomato were identified as *A. protenta* (Ayad, 2016). These results were confirmed by artificial cross inoculations in order to check the pathogenicity of the isolates.

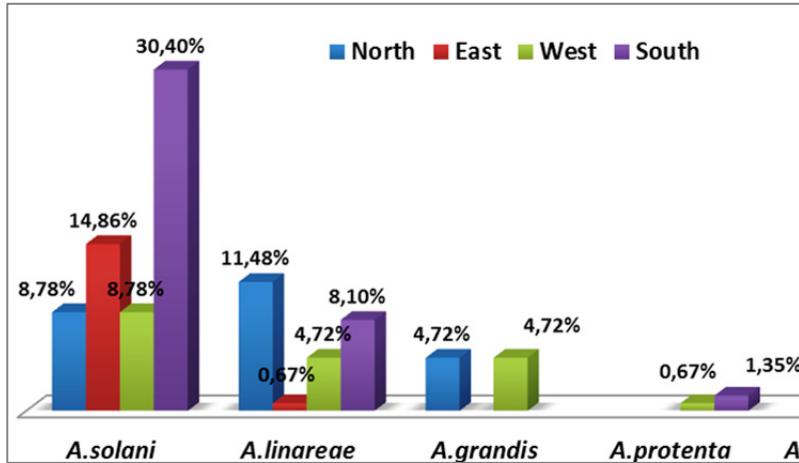


Figure 5. Distribution of *Alternaria* spp. in Algeria according to the potato and tomato growing areas

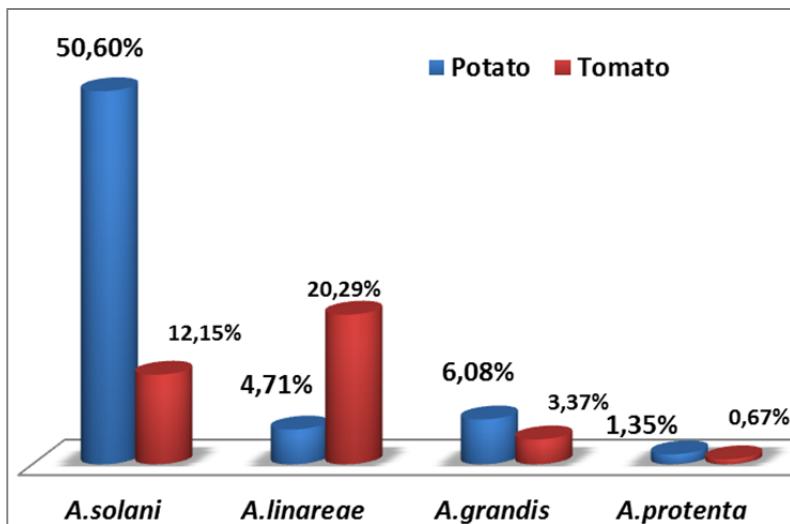


Figure 6. Distribution of *Alternaria* spp. in Algeria according to the potato and tomato crops

We therefore confirm the presence of other *Alternaria* species on potato other than *A. solani* particularly *A. grandis* and *A. protenta* which have been reported by other authors such as *A. grandis* in Europe (Landschoot et al., 2017), South America (Rodrigues et al., 2010), and Algeria (Bessadat et al., 2016). In contrast, we report for the first time in Algeria the presence in the field of *A. linareae* on potato and possibly in the world, and *A. protenta* on potato and tomato.

Aggressiveness and parasitic specialization of Alternaria species on their hosts

The high degree of aggressiveness obtained on whole seedling plants of potato and tomato, shows that the three *Alternaria* spp. (*A. solani*, *A. grandis* and *A. linariae*) isolated from potato or tomato are aggressive on both host plants (Figs. 7 and 8). These behaviors are also in agreement with those realized *in vitro* conditions by Gilbert and Webbs (2007), and Rogrigues (2009) for *A. linariae* and *A. grandis* respectively on tomato and potato (Figure 9 and Figure 10). The same results were also reported by Rodrigues et al. (2010), Gannibal et al. (2014) and Woudenberg (2014) for *A. solani* on potato and tomato. We confirmed that *A. grandis* is formerly considered as responsible of early blight of potato and also tomato in Algeria as showed by Bessadat (2016). In this study we showed that *A. linariae* is pathogenic on potato in field conditions. These results allow us to confirm the absence of parasitic specialisation in the three pathogens with respect to their two hosts: potato and tomato.

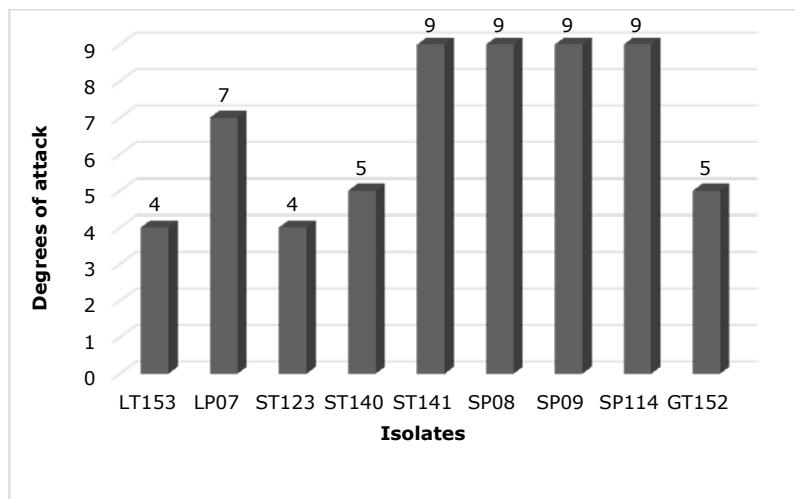


Figure 7. Degrees of attack of *Alternaria* spp. isolates on potato (S: *A. solani*; L: *A. linariae*; G: *A. grandis*; P: potato; T: tomato)

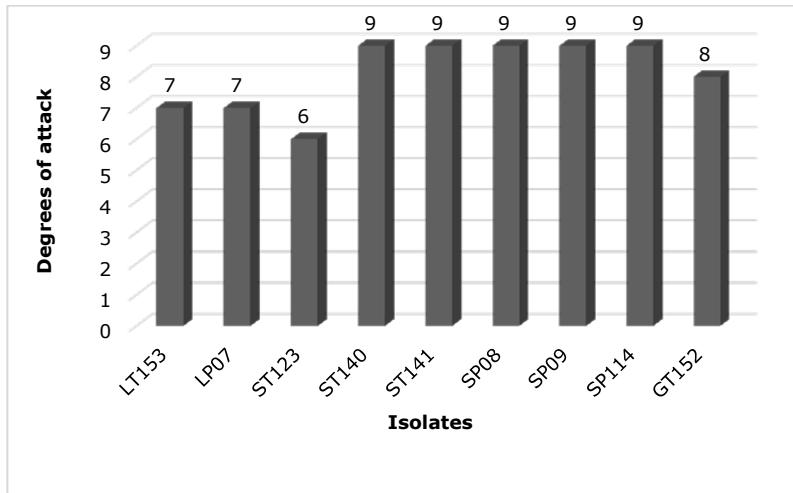


Figure 8. Degrees of attack of *Alternaria* spp. isolates on tomato (S: *A. solani*; L: *A. linariae*; G: *A. grandis*; P: potato; T: tomato)



Figure 9. Symptoms on detached leaflets and whole plants inoculated by P02 isolate (=*A. linariae* isolated from potato)



Figure 10. Symptoms on detached leaflets and whole plants inoculated by P09 isolate (=*A. grandis* isolated from potato)

CONCLUSION

The *Alternaria* large-spored species associated with potato and tomato early blight in Algeria were inventoried. *A. solani*, *A. linariae* and *A. grandis* are the common species found on potato and tomato. *A. protenta* was reported on potato and tomato for the first time in Algeria and possibly in the world. Morphological characterization remains insufficient for the distinction of *A. solani*, *A. linariae*, *A. grandis* and *A. protenta*. The identification of these four *Alternaria* species has to be confirmed by molecular markers. Beside *Alternaria alternata* species group, *A. solani* is the most common large spored species on potato; however others species with large conidia such as *A. linariae*, *A. grandis* and *A. protenta* can also infect potato in field conditions in Algeria. *A. solani* and *A. linariae* are the most common species on potato and tomato respectively in all potato and tomato growing regions of Algeria. *A. protenta* and *A. linariae* were found on potato for the first time in Algeria and have been pathogenic on this crop. Our results indicate that *A. linariae* is not specific to tomato as previously reported in the literature since it has been isolated under field conditions from potato for the first time in Algeria and possibly in the world. Also *A. grandis* is not specific to potato.

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