



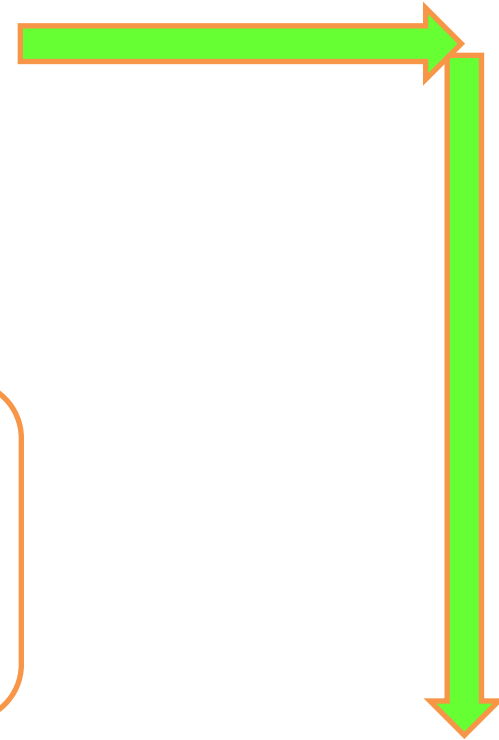
New data on early blight of potato caused by a complex of large-spored *Alternaria* species in Algeria



Djida AYAD, Bruno HAMON, Abdelaziz KEDAD, Philippe SIMONEAU & Zouaoui BOUZNAD

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Introduction: Potato production



World

fourth world rank
after wheat, rice
and maize

Algeria

considered as food on the
same basis as cereals and
pulses

Acreage
136,735 ha
(2014)

Production
46,735,155 Qx
(2014)

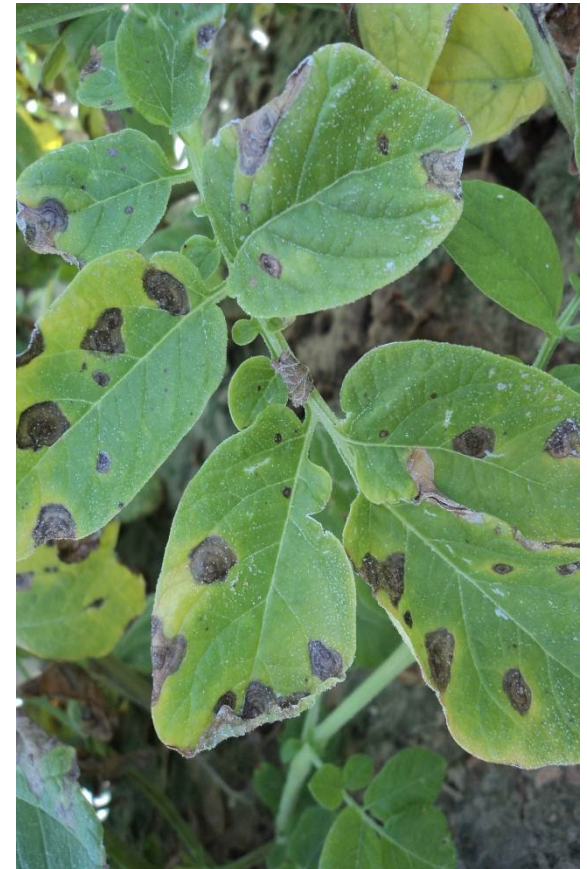
Yield
341.79 Qx/ha
(2014)

Prominent place in
the algerian
agricultural
economy

Symptoms of foliar Early Blight



large-spored *Alternaria* species with long beack



Causal agents of early blight on solanaceae (potato)

Frequently mentioned
in the past

Group of small-spored
species: *A. alternata*

Group of large-spored
species: *A. solani*

Recently considered by
molecular tools

A. alternata
A. tenuissima
A. arborescens
A. infectoria

A. solani
A. grandis
A. protenta

Aim of our research

- ❖ Identification of of large-spored *Alternaria species*
- ❖ Occurrence and prevalence of early blight caused by Large-spored *Alternaria species* in Algeria
- ❖ Aggressiveness and parasitic specialisation of *Alternaria species* on their natural hosts

Methodology used in our research

1

1. Prospection, Isolation and selection of isolates

2

2. Molecular characterization of isolates

3

3. Morphological characterization of isolates

4

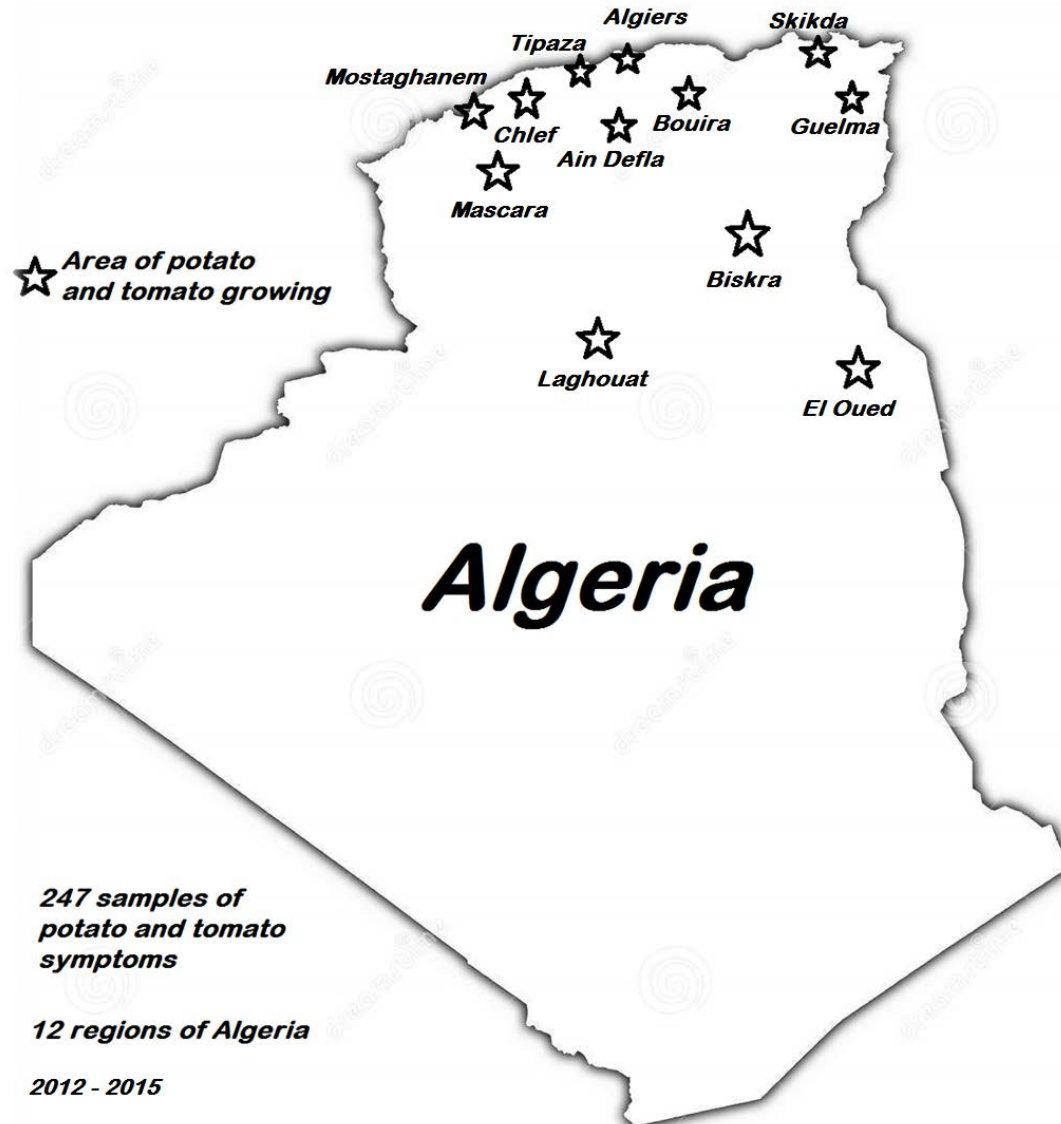
4. Parasitic specialization of species

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1. Prospection and morphological characterization of isolates

Growing area of potato prospected

**S
A
M
P
L
I
N
G**



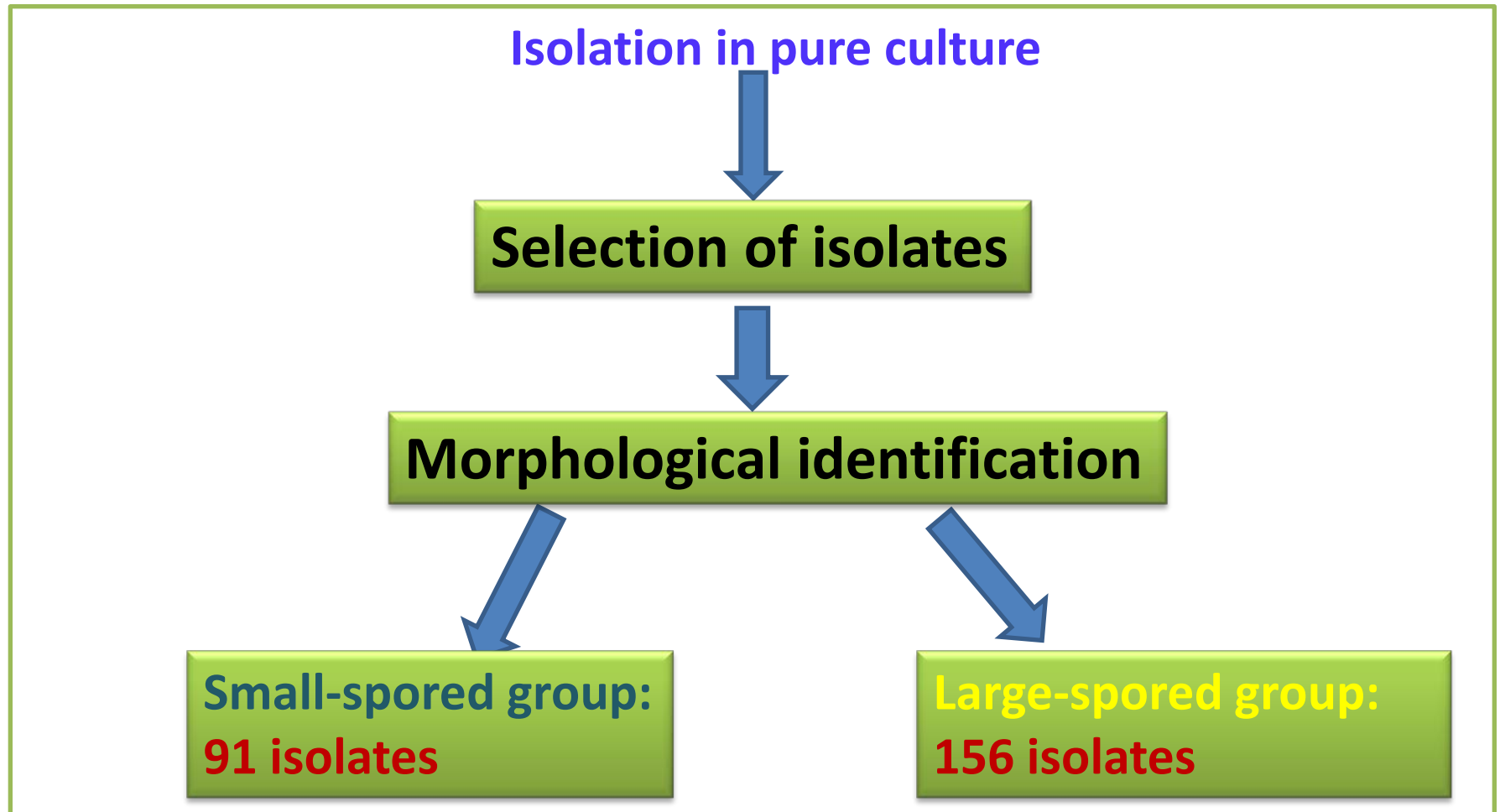
Collecting blighted samples



Samples with typical symptoms were used for isolation

Isolation and selection of isolates

This first step was performed on the basis of morphological characters: large solitary conidia with a beak more or less long



Morphological aspect of conidia



- a: large conidia = groupe *A. solani* ;
- b: small conidia = groupe *A. alternata*

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**Characterization and identification
of large-spored *Alternaria species*
by molecular markers**

The identification of large-spored *Alternaria* species by molecular markers is carried out in three steps

1. Discrimination between large-spored *Alternaria* species using specific primers of *Alternaria solani*/*A. grandis* and *A. linariae*

2. Discrimination between *A. solani*, *A. grandis* and *A. linariae* using polymorphism at the Calmodulin locus

3. Discrimination between large-spored *Alternaria* species using sequence analysis

Discrimination between the two species groups (*A. solani*/*A. grandis* and *A. linariae*)

PCR – SPECIFIC PRIMERS

OAsF7
and
OAsR6



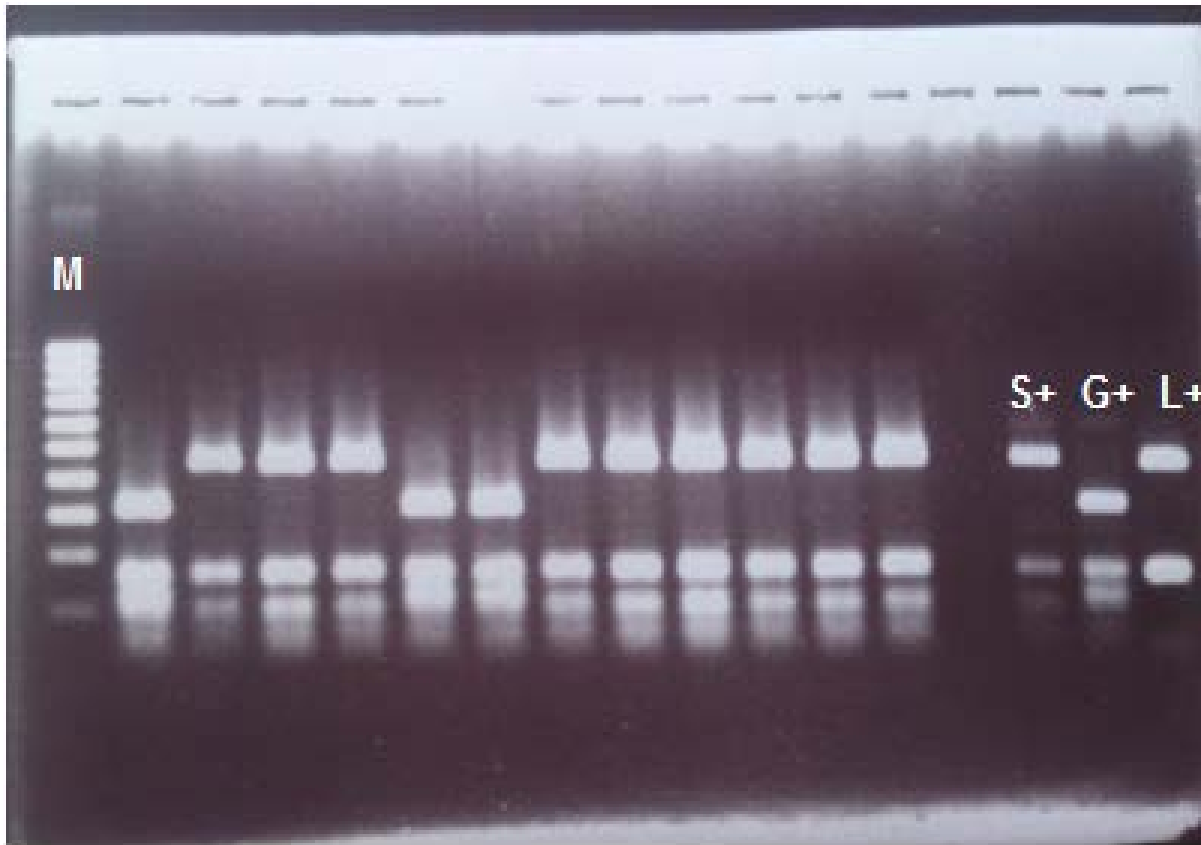
OatF4
and
OatR2

Amplification of a 164 bp fragment of
the gene encoding Alt a1
to separate *A.solani* and *A.linariae*

Amplification of a 438 bp fragment of
the gene coding for calmodulin
to confirm *A. linariae*

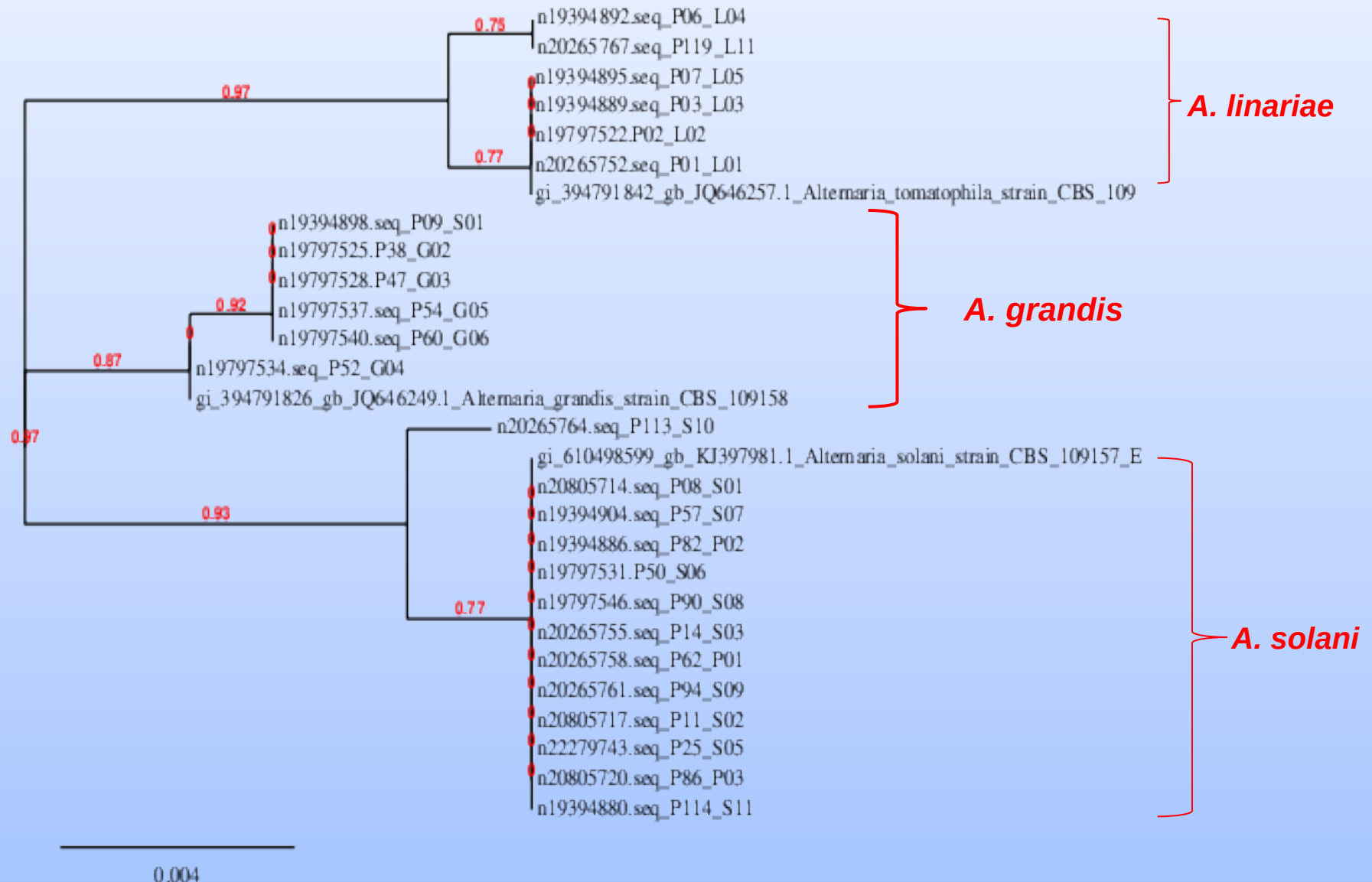
Discrimination between the two species (*A.solani* and *A.grandis*)

PCR-RFLP



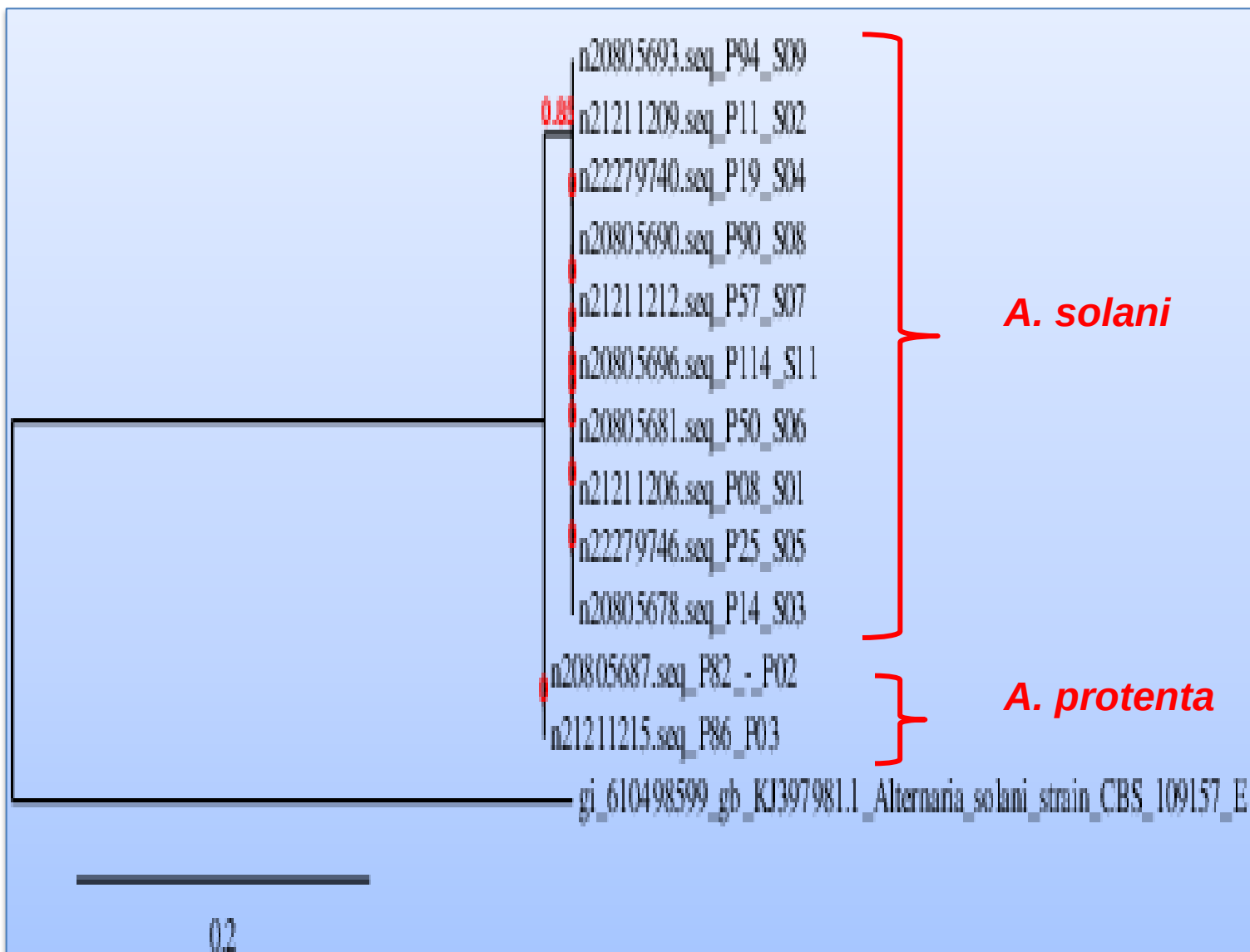
Used for rapid discrimination of the two species *A.solani* and *A.grandis* by DNA double digestion with *RsaI* and *HaeIII* of PCR products from the calmodulin locus

Cluster analysis of sequence at the Calmodulin loci



**Discrimination between *A. solani*
and *A. protenta* using sequence at
the Rpb2 loci**

Cluster analysis of sequence at the Rpb2 loci

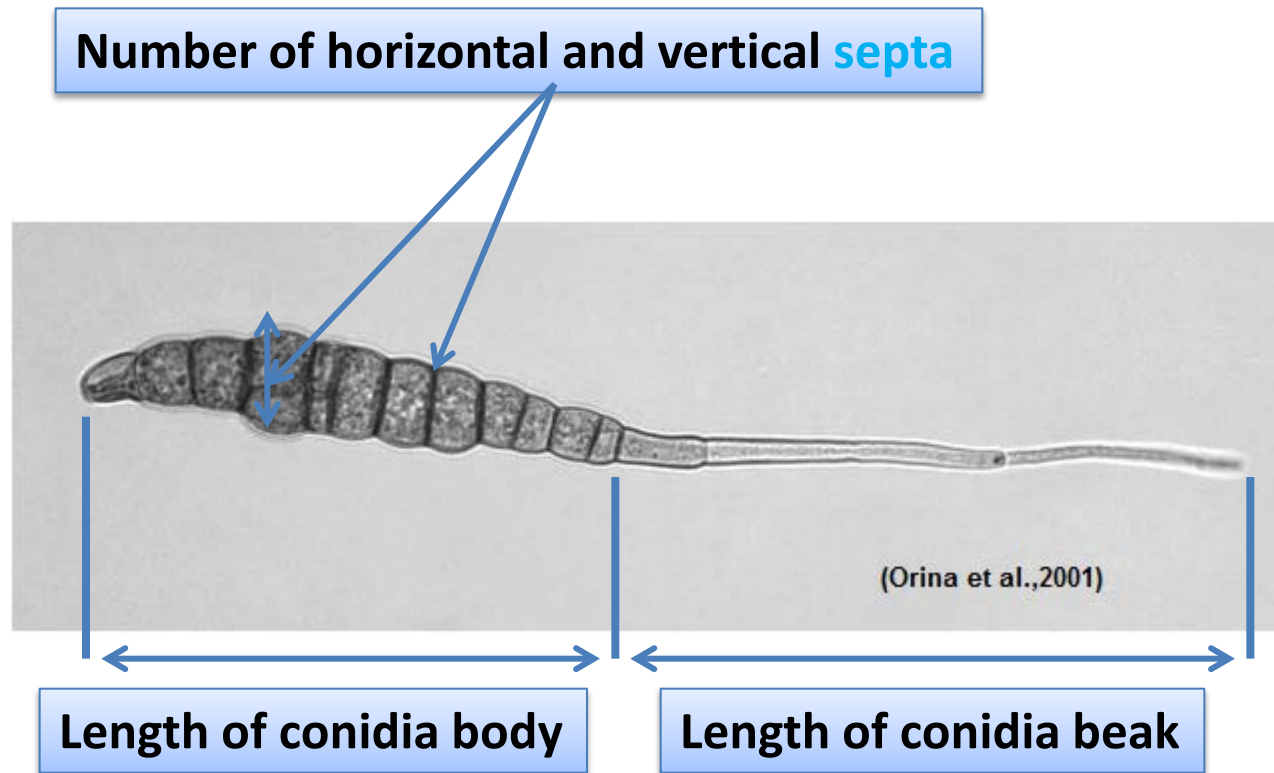


**Morphological characterization
of large-spored *Alternaria*
*species***

Collection of 23 isolates of large-spored choosed for the present study

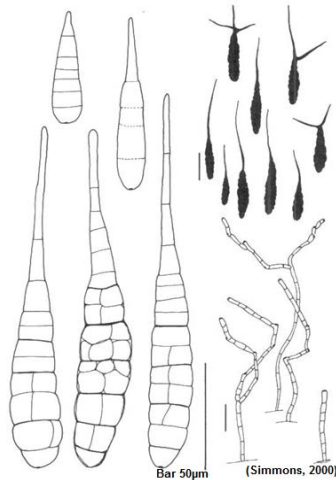
Code 1	Code2	place of isolation	Location	species
P01	L01	Fouka	Tipaza (North)	<i>A.linareae</i>
P02	L02	Fouka -Zirou	Tipaza (North)	<i>A.linareae</i>
P03	L03	Fouka marine	Tipaza (North)	<i>A.linareae</i>
P06	L04	Staoueli-domaine 1.0	Alger (North)	<i>A.linareae</i>
P07	L05	Staoueli-Ferme N°67	Alger (North)	<i>A.linareae</i>
P09	G01	Staoueli-Ferme N°69	Alger (North)	<i>A.grandis</i>
P38	G02	Ouled Belhadj	Alger (North)	<i>A.grandis</i>
P47	G03	Mostaghanem	Mostaghanem (west)	<i>A.grandis</i>
P52	G04	Rouina	Ain Defla (west)	<i>A.grandis</i>
P54	G05	Bourahla	Mostaghanem (west)	<i>A.grandis</i>
P60	G06	Bourahla	Mostaghanem (west)	<i>A.grandis</i>
P08	S01	Staouéli	Alger (North)	<i>A.solani</i>
P11	S02	Fouka	Alger (North)	<i>A.solani</i>
P14	S03	Staoueli -ITCMI	Alger (North)	<i>A.solani</i>
P19	S04	Bouira	Bouira (East)	<i>A.solani</i>
P25	S05	Bouira	Bouira (East)	<i>A.solani</i>
P50	S06	Rouina	Ain Defla (west)	<i>A solani</i>
P57	S07	Ghris	Mascara (west)	<i>A.solani</i>
P90	S08	Mabrouk Jar Ellah	El Oued (South)	<i>A.solani</i>
P94	S09	ENSA-Echantillon2	Alger (North)	<i>A.solani</i>
P114	S11	Fedjoudj	Guelma (East)	<i>A.solani</i>
P82	P02	Ourmes	El Oued (South)	<i>A.protenta</i>
P86	P03	Sahn Gazel	El Oued (South)	<i>A.protenta</i>

Morphological criteria of large-spored *Alternaria* species

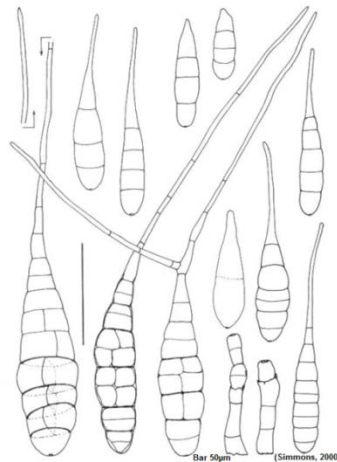


Morphology of the large-spored *Alternaria* species causing early blight of potato and tomato

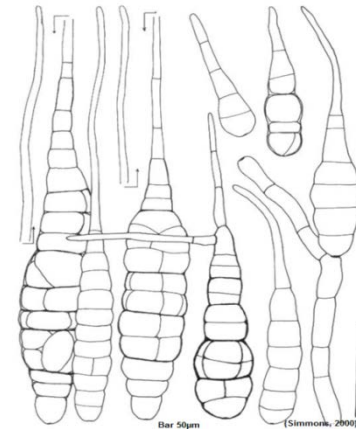
A. solani



A. linariae



A. grandis



Morphological characteristic ¹	Species		
	<i>A. solani</i> ²	<i>A. tomatophila</i> ¹	<i>A. grandis</i> ³
Conidium body length	85 - 100	70 - 99	102 - 184
Conidium body width	18 - 22	12 - 20	14 - 17
Beak length ⁴	83 - 110	99 - 197	135 - 206
Number of transversal septa	8 - 12	7 - 12	9 - 14
Number of longitudinal septa	1 - 3	1 - 4	0 - 3
Number of beaks ⁵	1	1 and 2	1

Morphological characterization of isolates belonging to *A. solani*



Hyphal width 4.23 μm – 7.57 μm

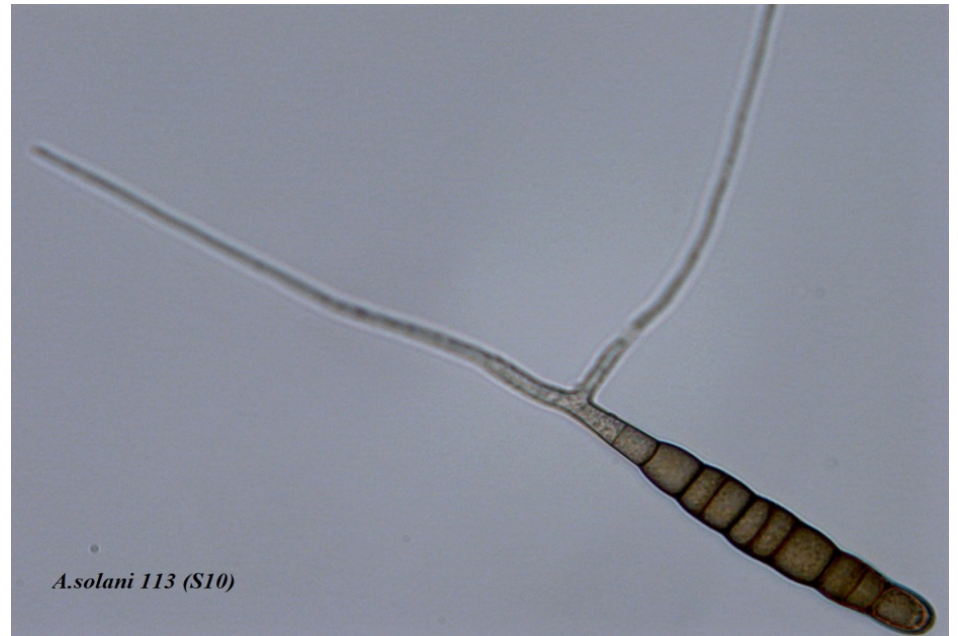
Body conidia length 46.23 μm – 80.86 μm

Body conidia width 13.47 μm – 25.54 μm

Beak length 30.86 μm – 114.40 μm

H septation 1–9

V septation 1–12



Morphological characterization of isolates belonging to *A. grandis*



Hyphal width 4.40 μm – 8.74 μm

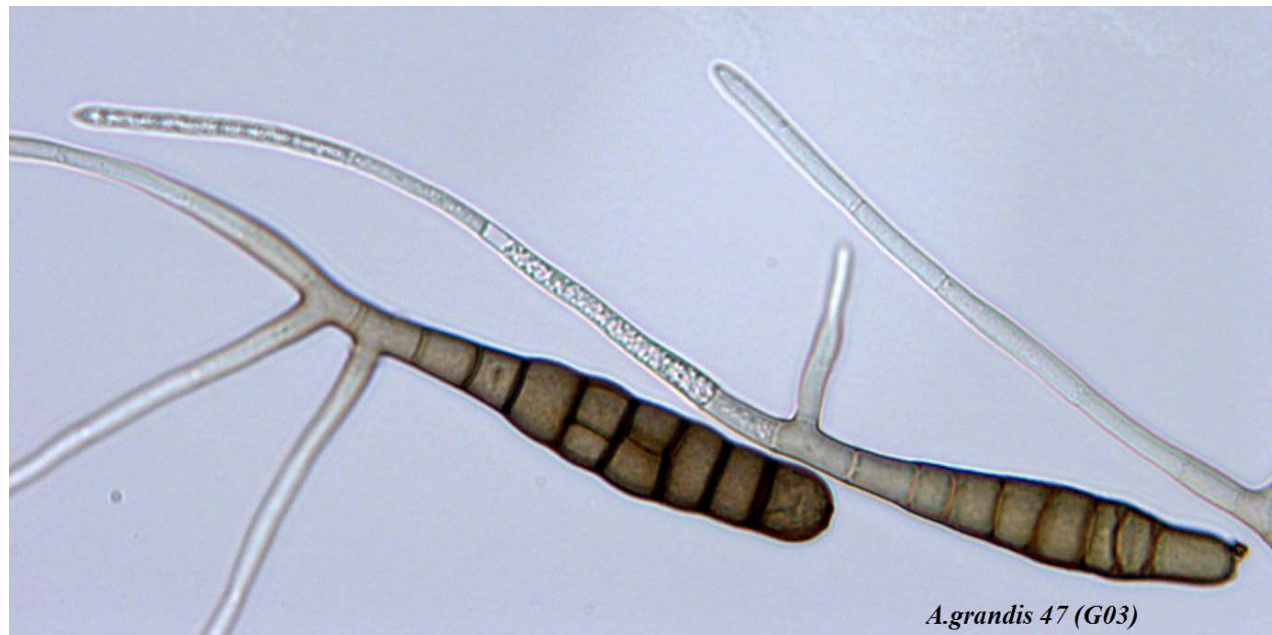
Body length 36.17 μm – 97.157 μm

Body width 10.66 μm – 21.70 μm

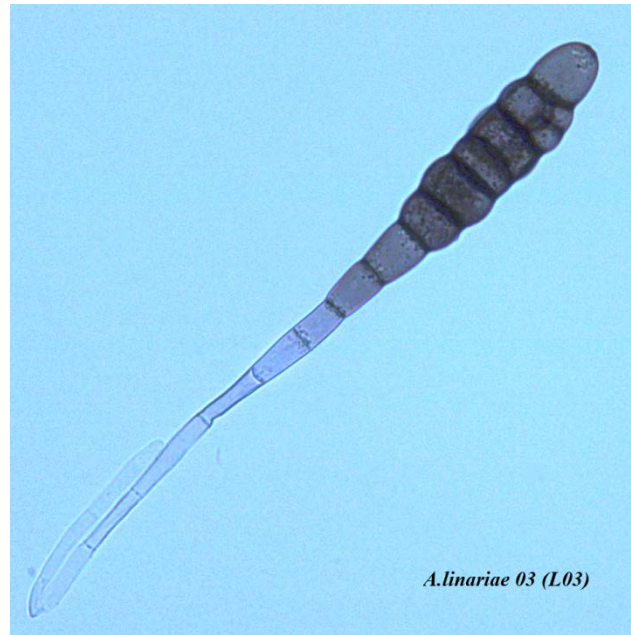
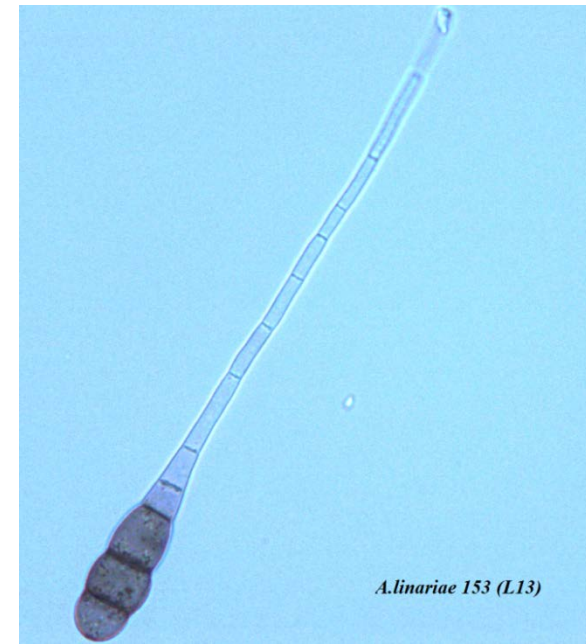
Beak length 61.28 μm – 186.57 μm

H septation 1–7

V septation 2–11



Morphological characterization of isolates belonging to *A. linariae*



Hyphal width 3.31 μm – 8.37 μm

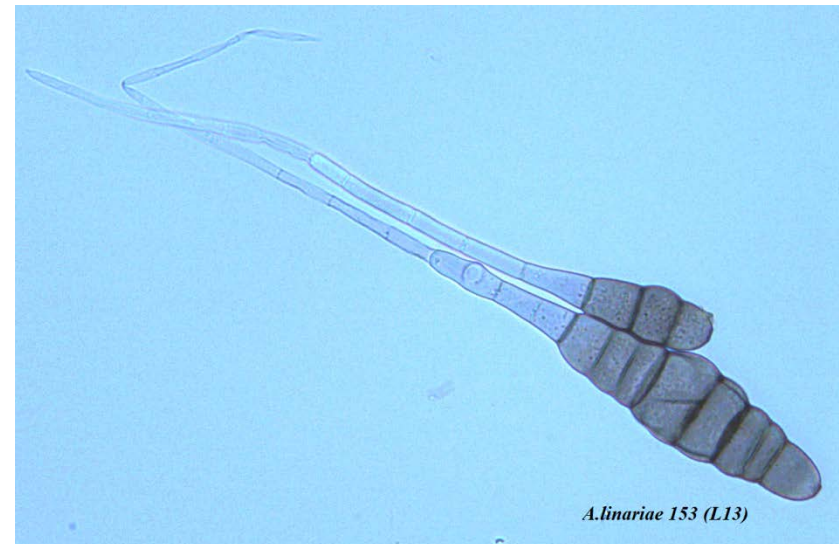
Body length 41.08 μm – 108.275 μm

Body width 8.47 μm – 18.12 μm

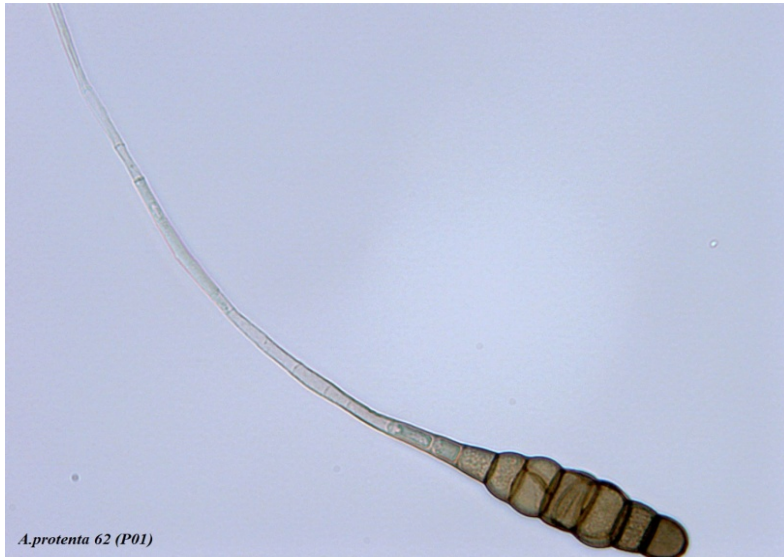
Beak length 56.84 μm – 224.78 μm

H septation 0– 3

V septation 1– 19



Morphological aspect of isolates identified as *A. protenta*



Hyphal width 3.40 μm – 6.67 μm

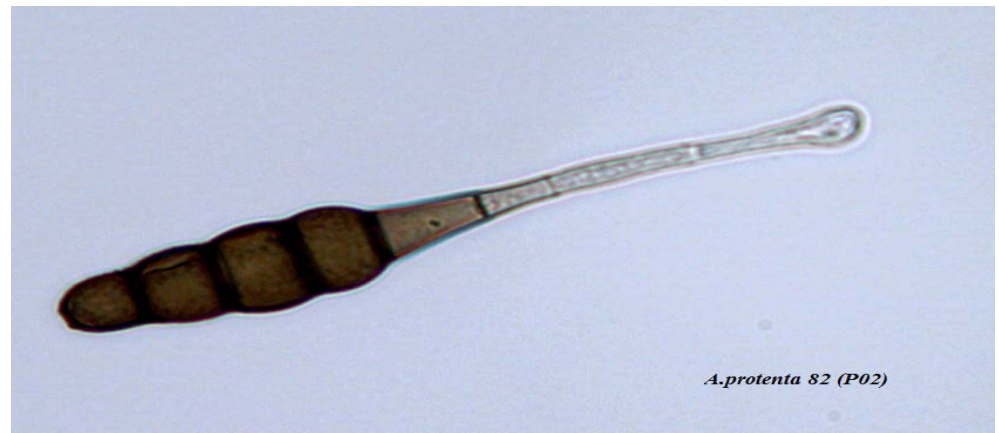
Body length 28.14 μm – 93.907 μm

Body width 10.30 μm –22.733 μm

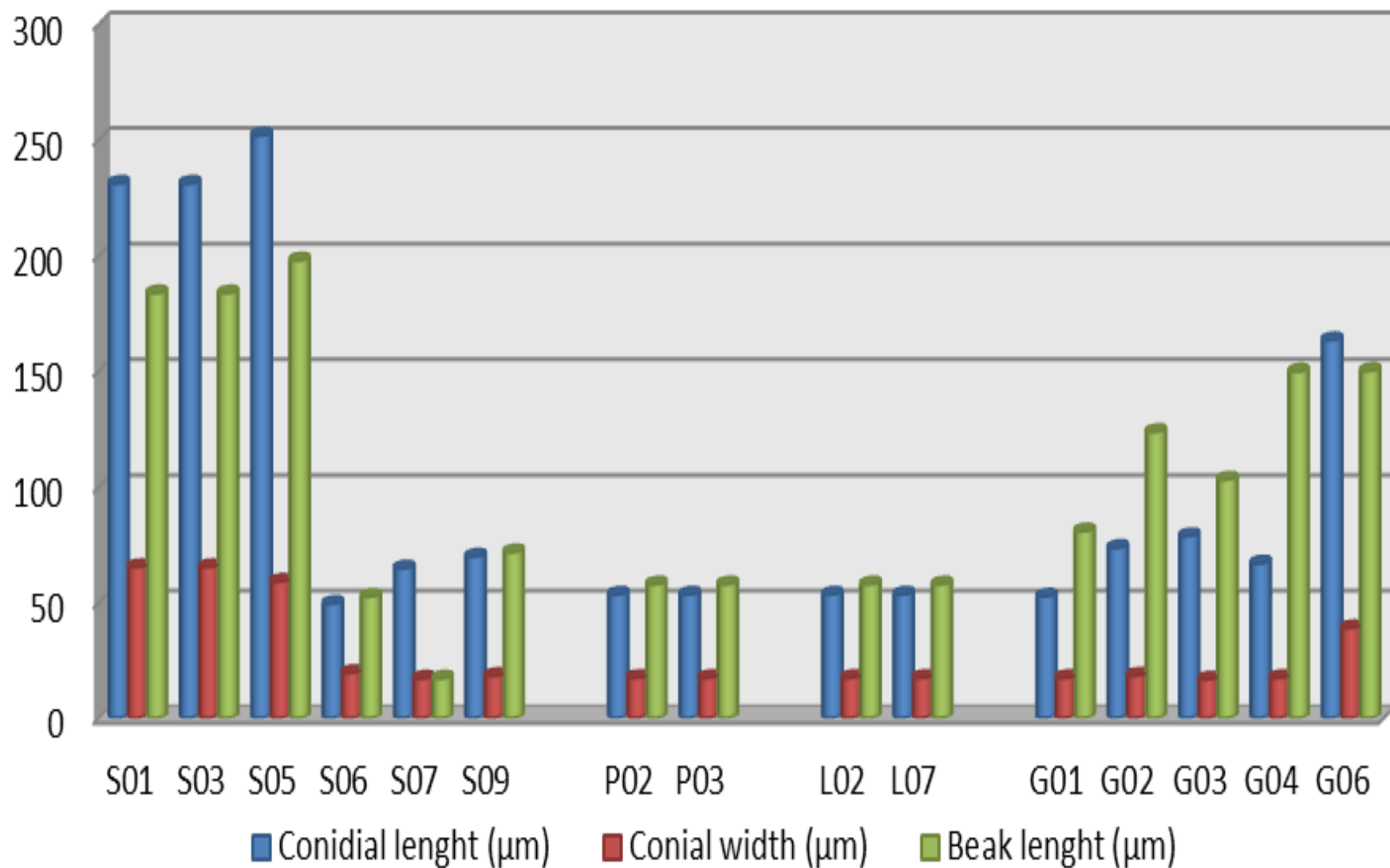
Beak length 34.24 μm –130.49 μm

H septation 0–8

V septation 1–10



Conidial size diversity of large-spored *Alternaria* species



➤ Conclusions on morphological characterization

- ❖ High morphological variability within the large-spored *Alternaria* in the isolates collection from diseased potatoes
- ❖ Overlapping of morphological characters (conidia and beak size, septation) so species identification has to be confirmed by molecular tools

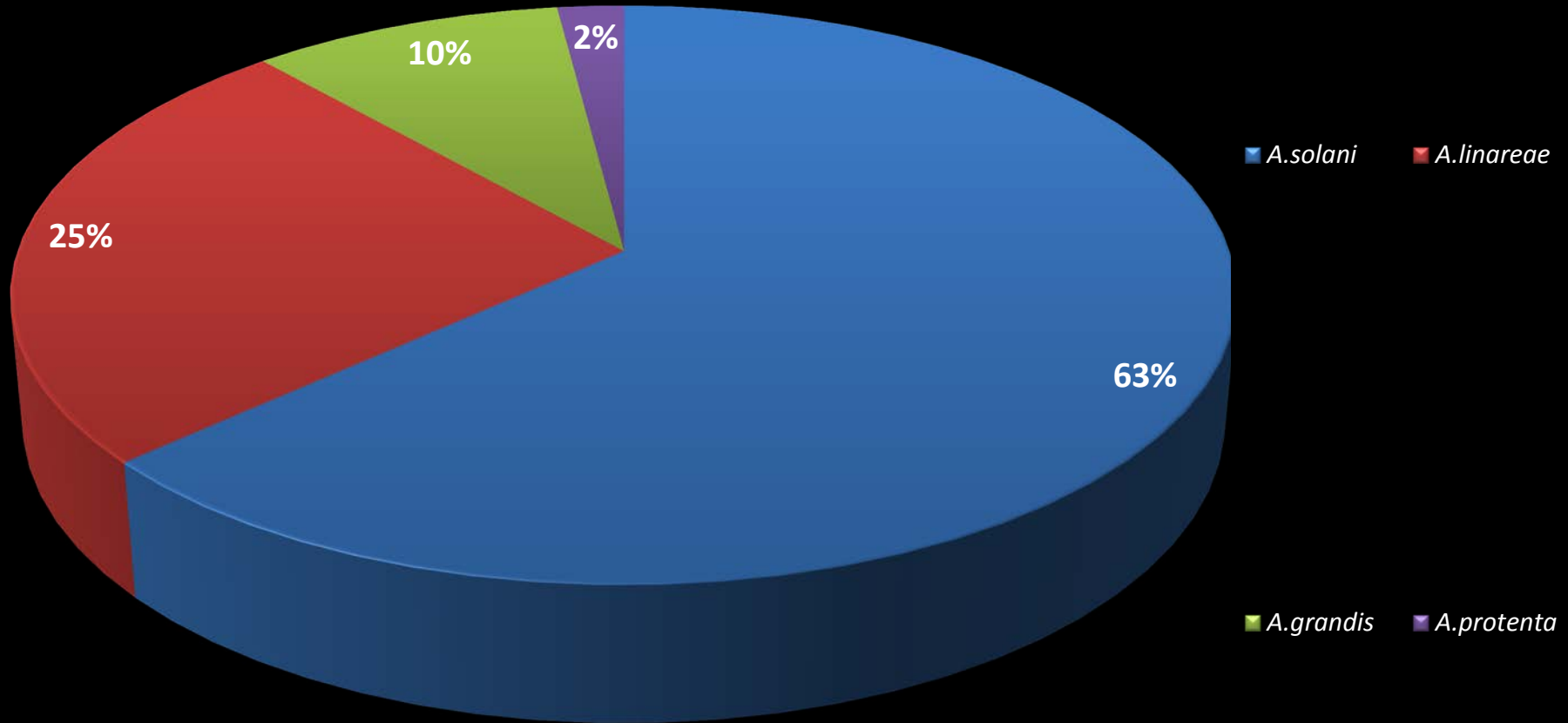
➤ Conclusions on identification of *A. protenta*

- ❖ Morphological characters are not sufficient for clear distinction between *A. solani* and *A. protenta* isolates
- ❖ Only nucleotide polymorphism at selected loci (Rpb2) allows specific identification of *A. solani* and *A. protenta* isolates

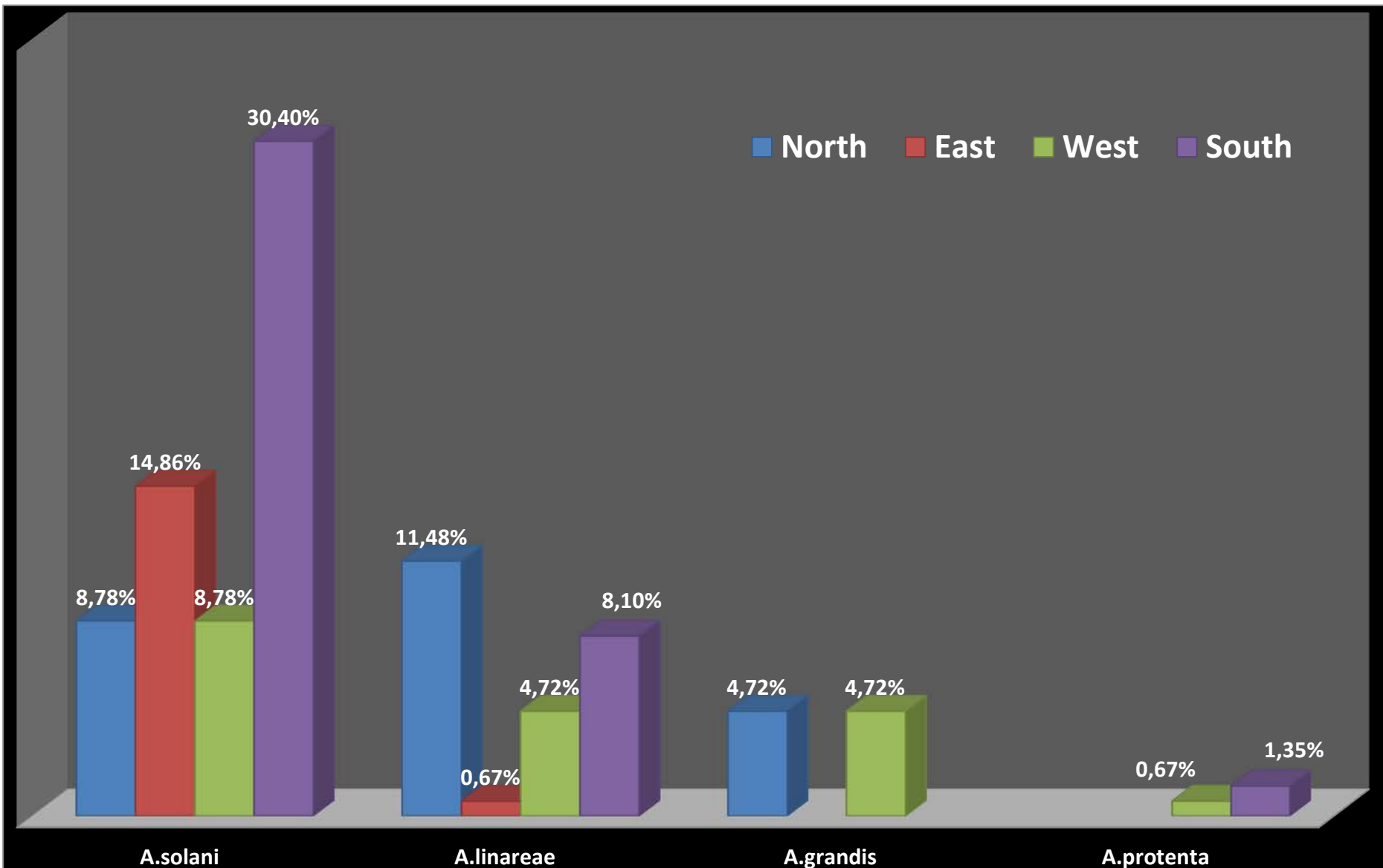
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Occurrence and prevalence of Large-spored *Alternaria* species found on potato and tomato field in Algeria

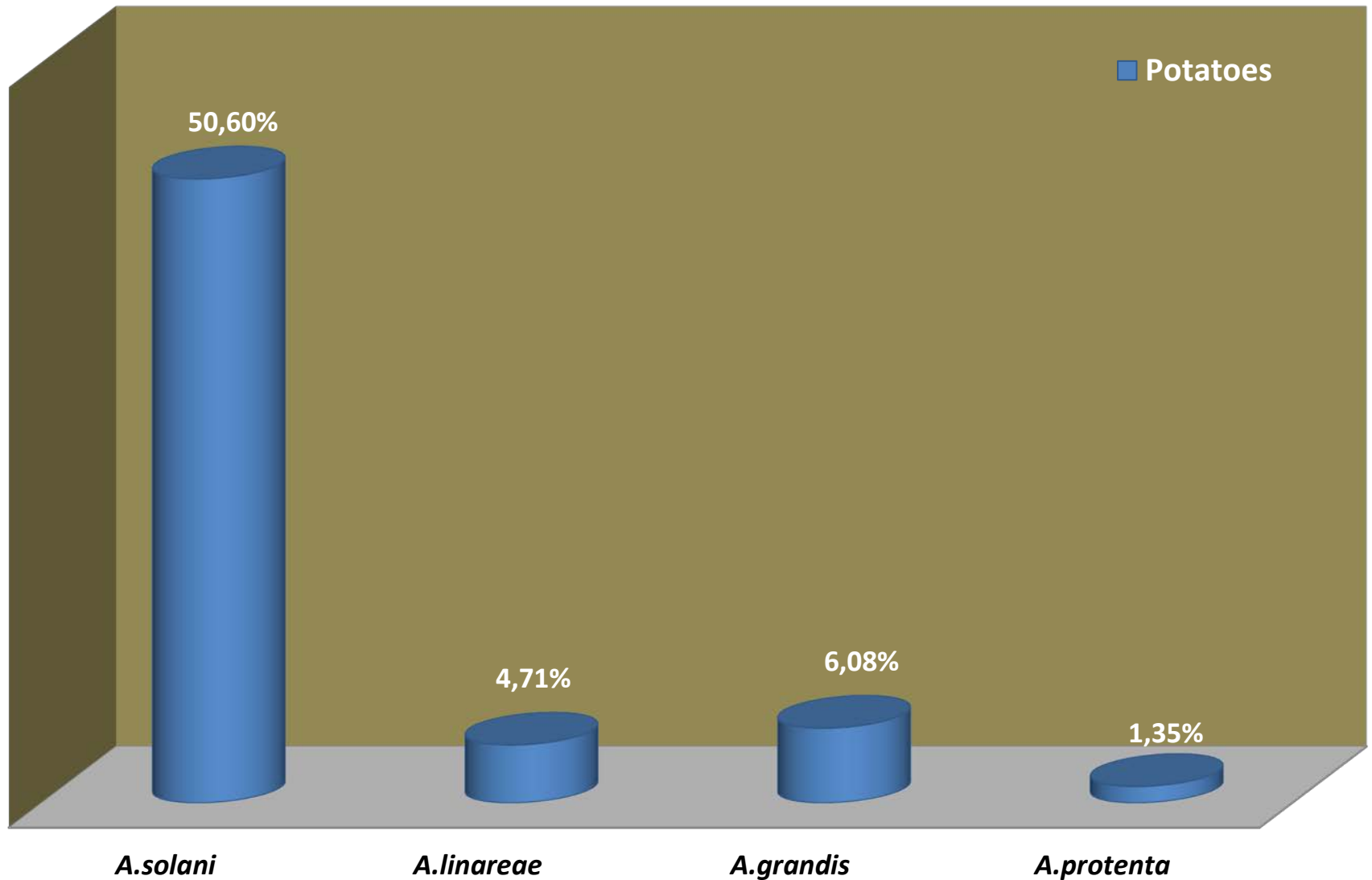
Occurrence and prevalence of large-spored *Alternaria* species on potato during 2012-2015



Distribution of *Alternaria* spp. on potato and tomato in some algerian regions (center, east, west and south)



Distribution of large-spored *Alternaria* spp. in Algeria on potato



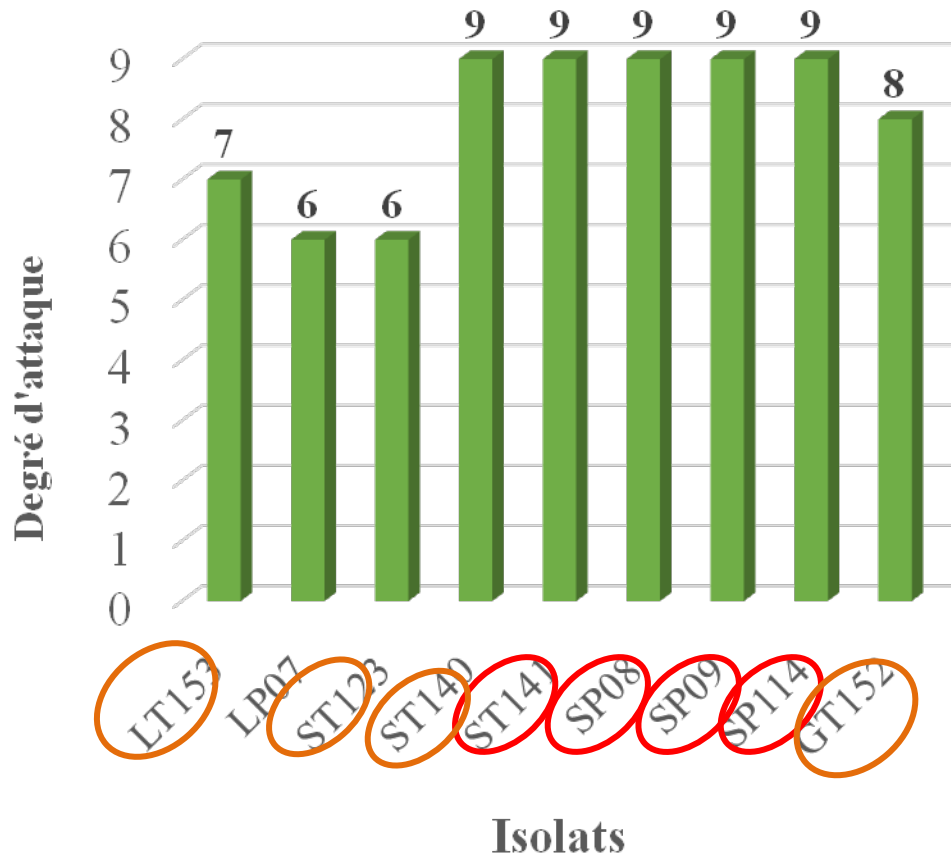
➤ Discussion on the occurrence and prevalence of the large-spored *Alternaria* spp. in Algeria

- ❖ The survey carried out through the 12 growing regions of potato showed the presence of 4 large-spored *Alternaria* species in Algeria : *A. solani*, *A. grandis*, *A. linariae* and *A. protenta* at variable levels from one region to another
- ❖ Our results confirm therefore 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., 2016), 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. protenta* and *A. linariae* on potato.

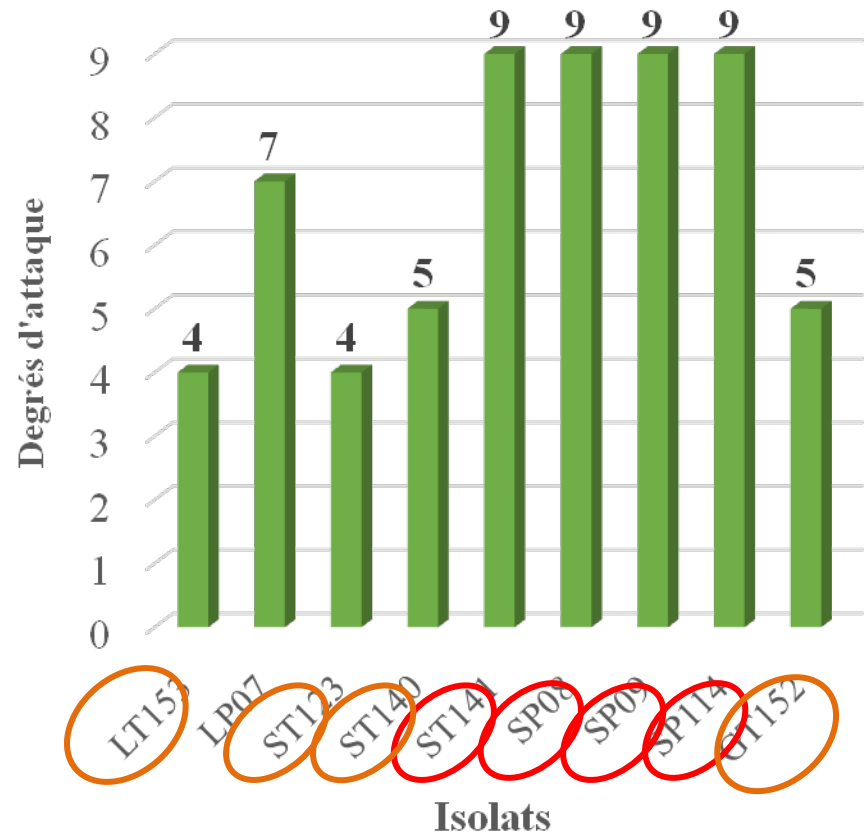
**Aggressiveness and parasitic
specialisation of *Alternaria* species on
their natural hosts**

Potato and tomato plants inoculated by isolates of *A. solani*, *A. grandis* and *A. linariae*

Tomato



Potato



Potato and tomato plants inoculated by isolates of *A. solani*, *A. grandis* and *A. linariae*



Isolate SP08



Isolate ST141



Isolate SP08



Isolate ST141



Isolate GP09



Isolate GP09



Isolate GP60



Isolate GT99



Isolate GT153



Isolate GT99



Isolate LP02



Isolate LP07



Isolate LT152



Isolate LP07



Isolate LP02

Discussion on parasitic specialisation

- ❖ The high degrees of aggressiveness obtained on whole seedling plants of potato and tomato, shows that the three *Alternaria* ssp. isolated from potato or tomato are also aggressive on both host plants.
- ❖ These behaviours are also in agreement with those realized *in vitro* conditions by Gilbert and Webbs (2007), and Rogrigues (2009) for *A. linariae* and *A. grandis* respectily on tomato and potato. 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.
- ❖ These results allow us to confirm the absence of parasitic specialisation in the three pathogens with respect to their two hosts : potato and tomato.

Conclusions

- ❖ Morphological characterization remains insufficient for the distinction of the large-spored *Alternaria* spp. ; molecular tools remain the only possibility for the species distinction.
- ❖ Beside *Alternaria alternata* species group, *A.solani* is the most common large spored species on potato; however others species with large conidia as *A. linariae*, *A. grandis* and *A. protenta* can also infect potato in all growing areas in Algeria.
- ❖ We confirmed that *A. grandis* is formally considered as responsible of early blight on potato and also on tomato in Algeria.

Conclusions

❖ *A. protenta* and *A. linariae* have been found on potato for the first time in Algeria and are pathogenic on this crop.

❖ Our results show that *A. linariae* is not specific to tomato as previously reported in the literature; it has been isolated from potato for the first time in Algeria under field conditions.

Early blight of potato

Group of large-spored species

Potato = *A.solani*

A. solani
A. protenta
A. grandis
A. linariae

Thank you for your attention!

