



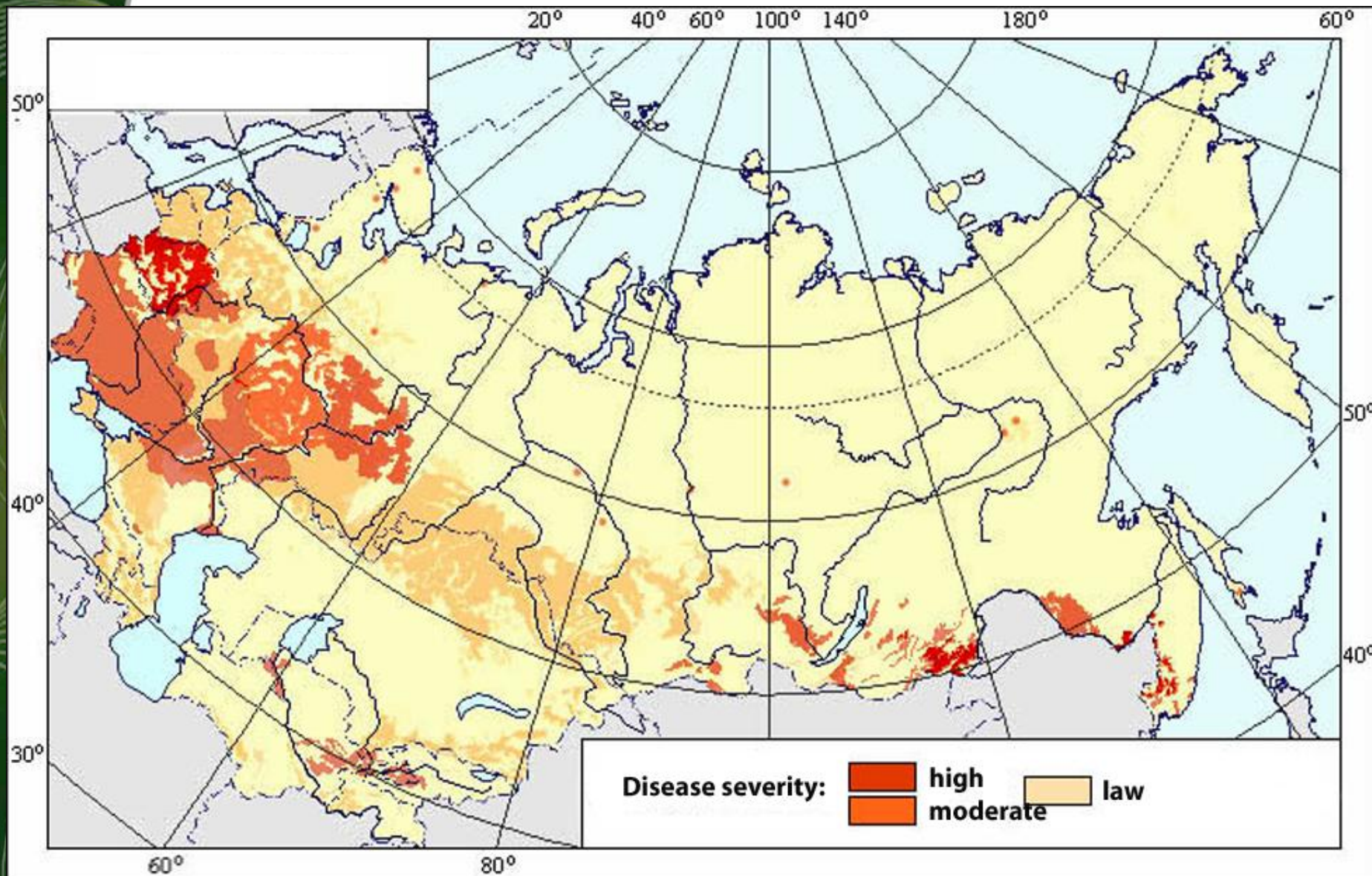
Lomonosov Moscow State University

# Species of *Alternaria* causing early blight of potato and tomato in Russia

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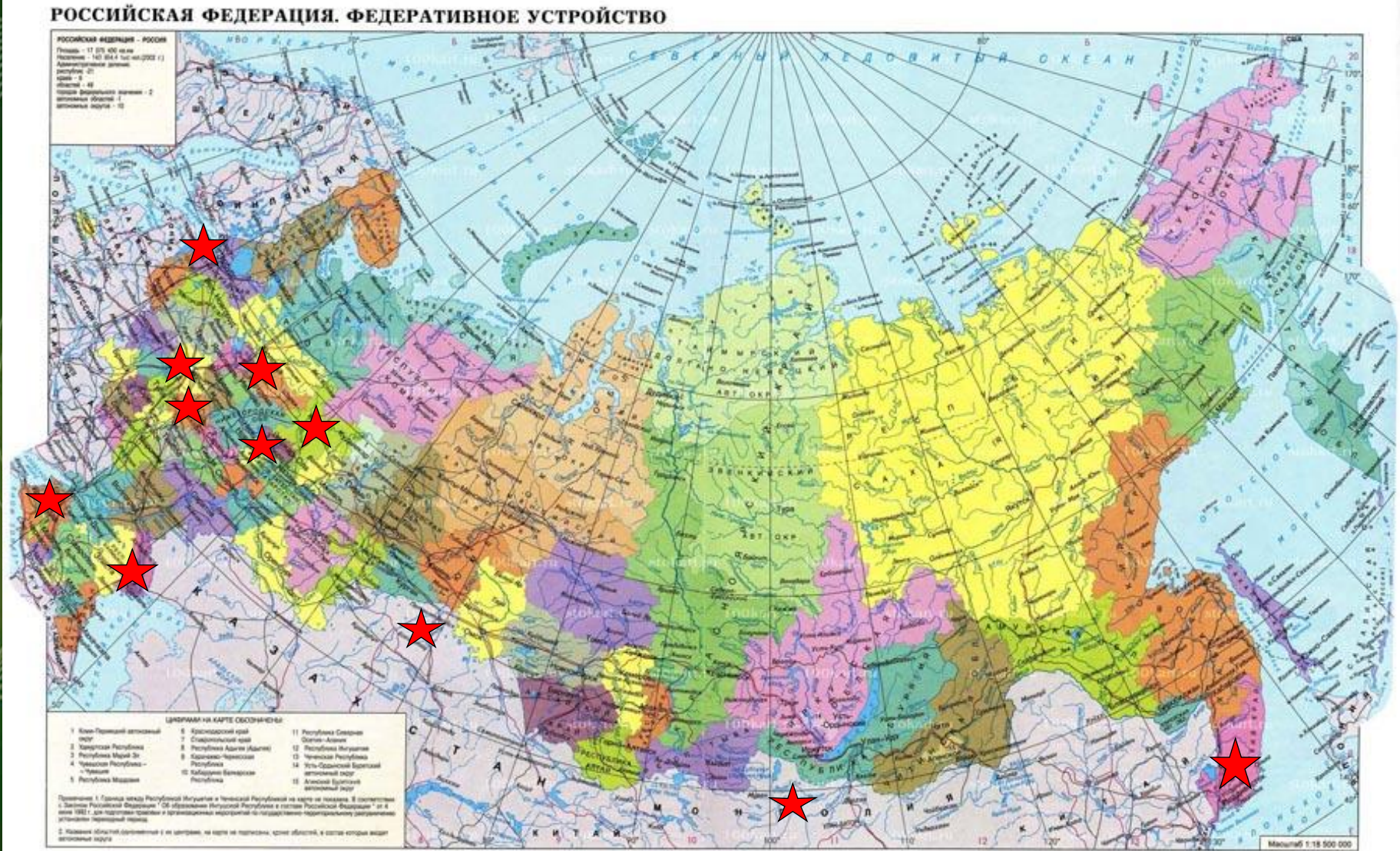
**Euroblight Workshop, Limassol,  
Cyprus, 2013**

# Distribution of potato and tomato early blight in Russia





# Locations of sampling sites



**143 samples from 12 locations were tested**

**The aim of the study was the species composition study and development of molecular identification method of early blight pathogens.**





# Traditional method

## isolation of pure cultures

Infected leaves  
of potato and  
tomato



Dried  
samples



Incubation in  
moist chamber

isolation of  
pure cultures

morphological  
diagnostics

molecular  
methods



**Difficulties: fresh leaves transport, isolation in pure culture, contamination with secondary mycobiota, sterile mycelium**

# Elaborated method

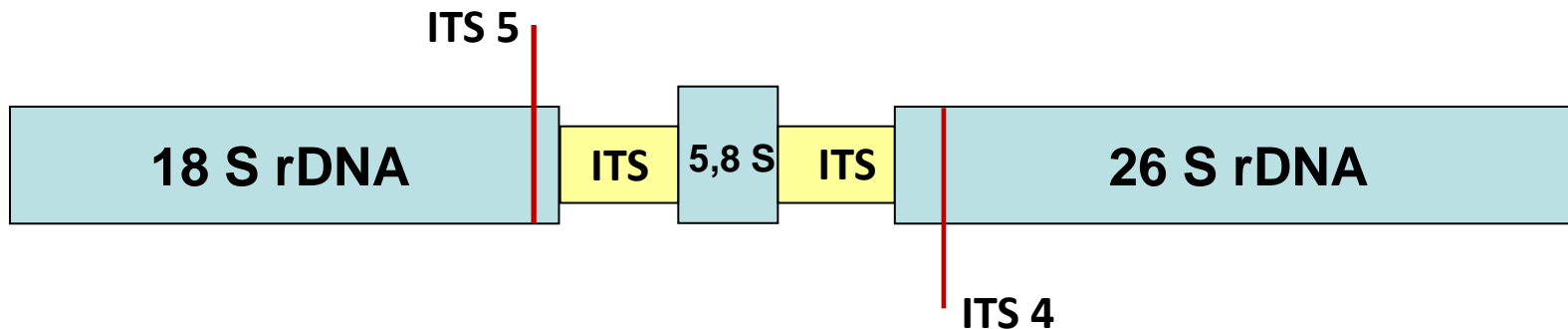
**Without isolation of pure cultures**



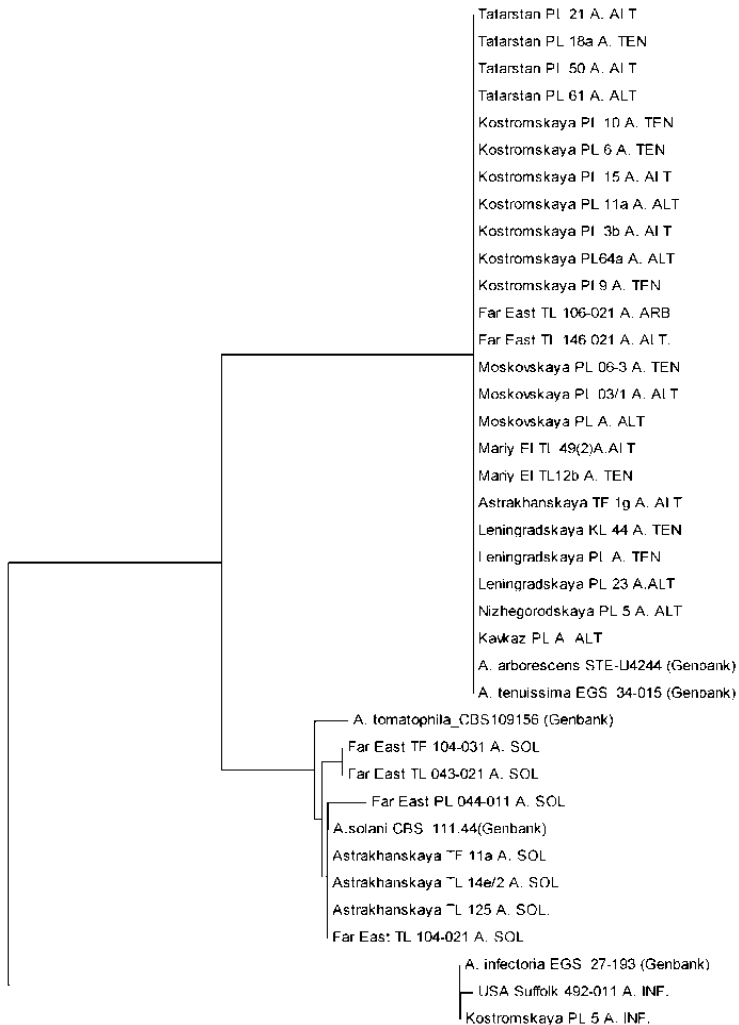
**In working method diagnostic of the species composition was carried out in samples of leaves, fixed immediately after collection in 70% ethyl alcohol.**

# Molecular identification of species

To identify the species composition a comparative study of nuclear DNA sequences coding ribosomal genes and intergenic transcribed spacers ITS5-ITS4 was conducted strains, isolated from infected tomato and potato plants, growing in seven distant regions of the European part of Russia and Russian Far East.



# Molecular identification of species



Small-spored strains  
(*A.alternata* s.l.)

**3 groups separated:**

***Alternaria solani* Sorauer,**

***A. infectoria* E.G. Simmons**

**group of small – spored species**

**(*A. alternata sensu lato*)**

Large-Spored strains

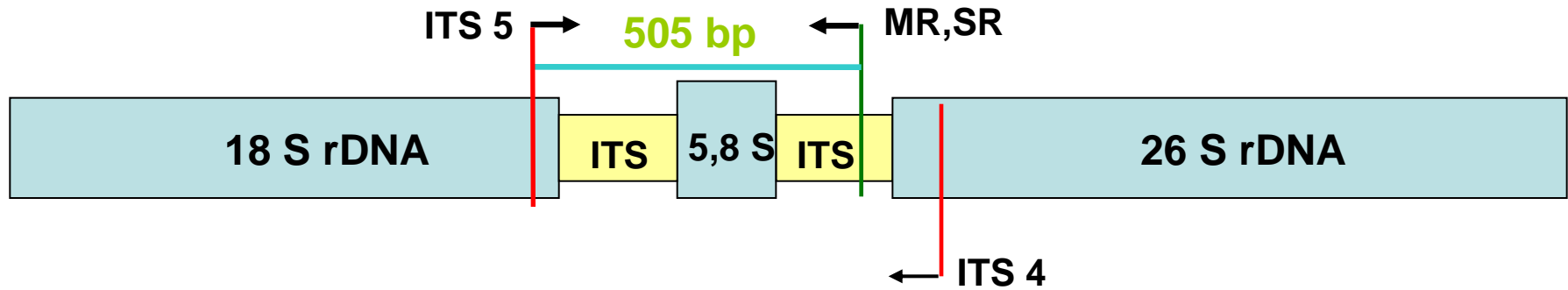
*A. infectoria*

0.01

Dendrogram, obtained by the maximum-likelihood method for the structure of the ITS5-ITS4region.

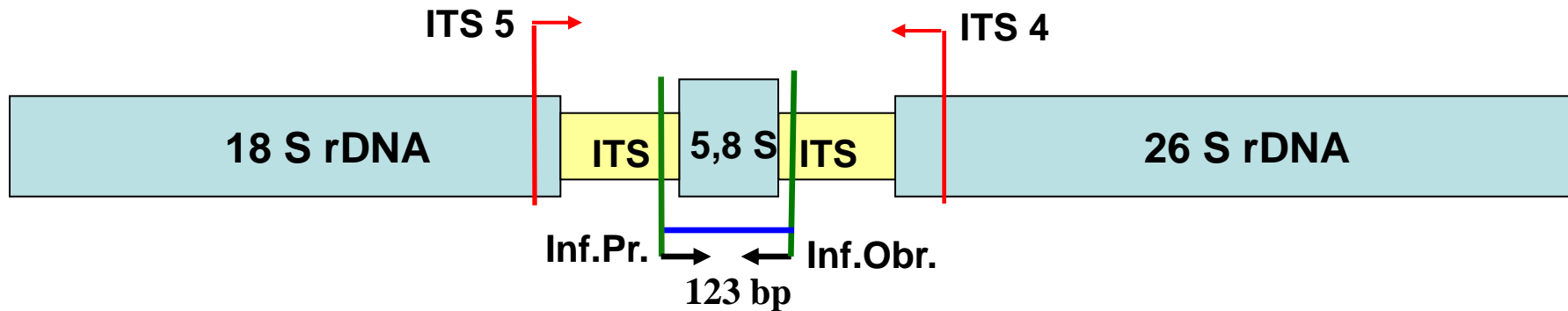


# Diagnostic primers for the identification of small-spored isolates and *A. solani*



Name	DNA sequence
Forward primer ITS5	5' – GACACCCCCCGCTGGGGCACTGC
Reverse primer for small-spore (MR)	5' – GACCTTTGCTGATAGAGAGTG
Reverse primer for <i>A. solani</i> (SR)	5' – GGTTGGTCCTGAGGGCGGGCGA

# Diagnostic primers for the identification of *A. infectoria*



Name	DNA sequence
Forward primer for <i>A. infectoria</i> (Inf.pr)	5' - GACACCCCCCGCTGGGGCACTGC
Reverse primer for <i>A. infectoria</i> (Inf.obr.)	5' - GGTTGGTCCTGAGGGCGGGCGA

# *Alternaria* species identification in fixed leaves with diagnostic primers

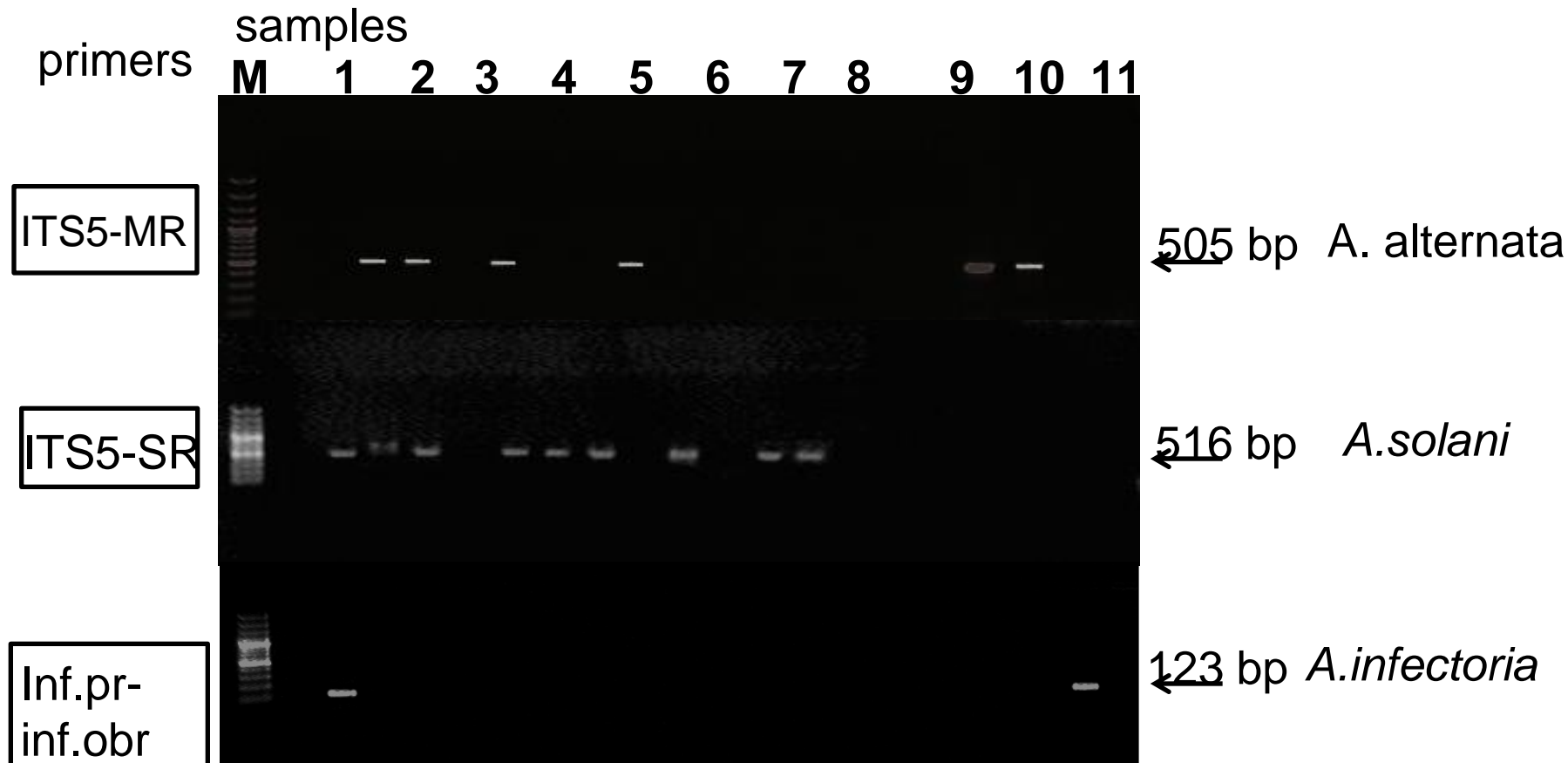


Figure 2. Specific amplification of *Alternaria* genome fragments using designed primer pairs.



# Identified *Alternaria* species

Species	Number of samples with species-specific DNA regions					
	Moscow region		Kostroma region	Mongolia	Ryazan region	Tatar region
Host plant	Pot.	Tom.	Pot.	Pot.	Pot.	Tom.
<i>A. solani</i> only	<b>22</b>	<b>2</b>	<b>5</b>	<b>1</b>	<b>2</b>	<b>3</b>
<i>A. alternata</i>	<b>18</b>	-	<b>15</b>	<b>2</b>	<b>5</b>	<b>2</b>
<i>A. infectoria</i> only	<b>4</b>	-	<b>3</b>	<b>1</b>	-	-
<i>A. solani</i> + <i>A. infectoria</i>	<b>2</b>	-	<b>3</b>	-	-	-
<i>A. solani</i> + <i>A. alternata</i>	<b>10</b>	-	<b>4</b>	<b>1</b>	<b>3</b>	<b>2</b>
<i>A. alternata</i> + <i>A. infectoria</i>	<b>2</b>	-	<b>2</b>	-	-	-
All three groups	<b>3</b>	-	<b>1</b>	-	-	-
All samples studied	<b>83</b>	<b>2</b>	<b>35</b>	<b>5</b>	<b>10</b>	<b>8</b>

*Study of infected potato and tomato samples from Moscow, Kostroma Ryazan regions, and Mongolia showed the presence A. solani, A. infectoria and A. alternata in all the regions*

# Conclusions

- The elaborated method of *Alternaria* species PCR diagnostics in infected leaves allow to identify different species inside the leaf and one necrosis.
- The designed primers can be used for the specific amplification of DNA of *Alternaria* species, providing their successful identification in the case of any problems with their morphological identification.
- Primary infection of potato leaves can be caused by different species of the genus *Alternaria*, and the symptoms are not differ