University of Agricultural Sciences
and
Veterinary Medicine
Cluj-Napoca

Taoutaou A, Socaciu C.
Pamfil D., Fetea F., Balazs E.,
Botez C. & Chis A.

FTIR MARKERS FOR LATE BLIGHT RESISTANCE AND SUSCEPTIBILITY

Introduction

- Potato: the 4th crop worldwide.
- *P. infestans*: the most important pathogen on potato.
- Loses: billions of US$/year
- Control:
  - Chemical
  - Resistance

Introduction

- Fourier-Transform Infrared (FTIR) Spectroscopy: is a sample quick technique.
- An infrared spectrum is commonly obtained by passing infrared radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy.
- The technique measures the total composition of an organism cell in a nondestructive manner, producing an IR spectrum with bands from all cellular components (membranes, proteins, nucleic acids...).

Introduction

- FTIR spectrum can be divided into several domains. Each domain is specific for a group of organic molecules in the biological systems:
- 500-900 cm^{-1}: fingerprint zone, specific spectral patterns.
- 900-1200cm^{-1}: polysaccharides, carbohydrtes.
- 1200-1500 cm^{-1}: mixed region: proteins, fatty acids, DNA, RNA, groups with a phosphor molecule.
- 1500-1800 cm^{-1}: amid region, amide I, amide II, proteins, peptides.
- 2800-3200 cm^{-1}: fatty acid zone.

Introduction

Frequency regions cm^{-1}	Characteristic frequencys cm-1	Assignment
Region 1 <1000 cm^{-1}	976	Phosphates (P=O)
	964	Compoud with double conjugated bonds:
	961	polyinsaturated fatty acids and carotenoids
Region 2 : 1000-1200 cm^{-1}	1035	Glucose
	1065	Fructose
	1079	Galactose,Oligo-and
	1103	Starch
Region 3: 1200-1500 cm^{-1}	1120	Phosphorilated glycosides
	1240, 1341	Secondary amines
	1400	Carboxyl groups, specific cu organic acids and amino acids
Region 4: 1500-1700 cm^{-1}	1566, 1574	Proteins, peptides,
	1595	Phenolics, chlorophylls
	1650, 1662	Amides I, II
	1655	
Region 5: 2800-3000 cm^{-1}	2854	Fatty acids
	2920	Cholesterol
	2934	Carotenoid pigments

Materials & Methods

- Potato plant:
  - Resistant: S. tuberosum: R4, 21(R2R3R4), S. demissum, and S. bulbocastanum
  - Susceptible: Bintje, Desiree, R1, R2, R3, R5.
- Pathogen: 2 isolates of *P. infestans* : A2.2 (NL08009) and A2.3 (88133). kindly given to us by W.G. Flier, G.B.M. van den Bosch and G.J.T Kessel from Plant Research International BV.
- Detached leaf test: conform to CIP manual 1997

Materials & Methods

- **FTIR procedure**:

In the 5th day,

the leaves have been ground into a fine powder in a mortar and pestle under liquid nitrogen,

then transferred to an eppendorf 2 ml tube. 1 ml of 70 % methanol was added, and

mixed by sonication for 15 minutes, then centrifuged.

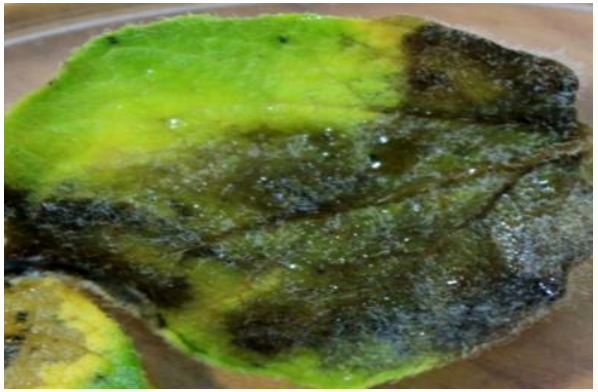
100 µl of supernatant was used for analysis.

Infrared profile was realized using the Shimatzu Prestige 2, Apodization: Happ-Genzel spectrophotometer, the profile was recorded in the wavelength range of 4000-500 cm^{-1}.

Results & Discussions

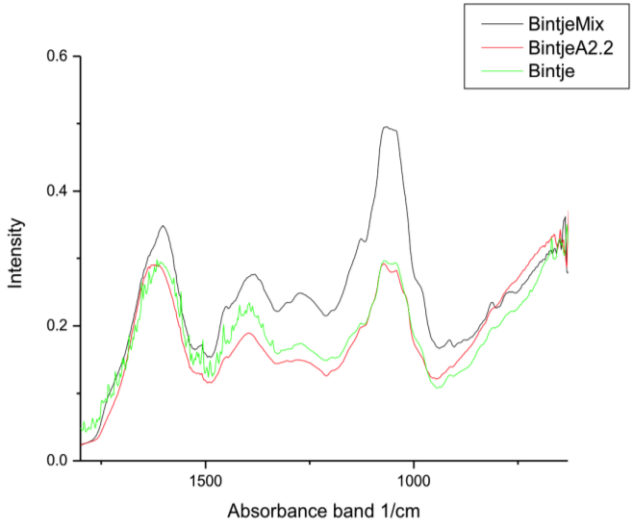
- Resistant genotypes: R4, 21, *S. demissum*, and *S. bulbocastanum*.

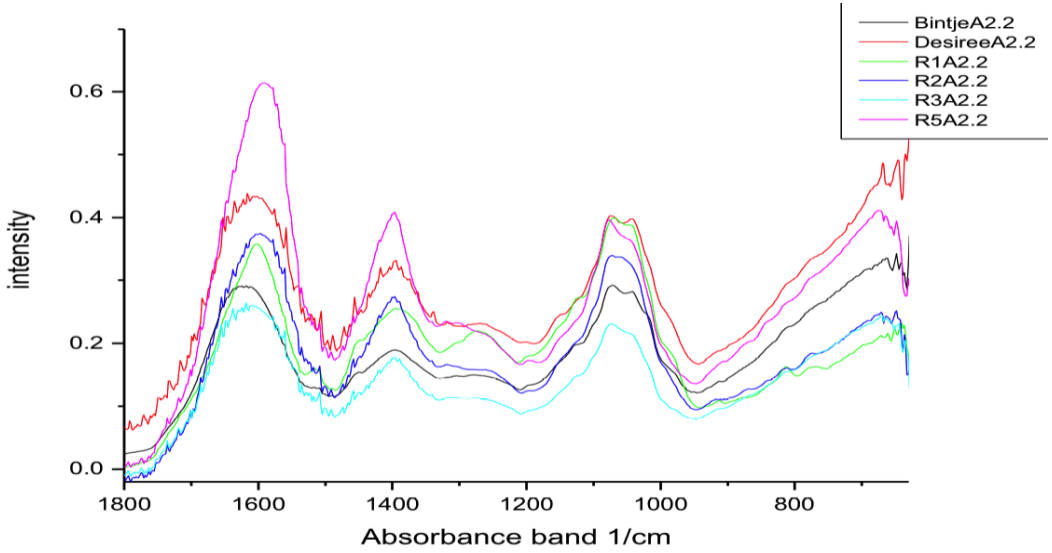

- Susceptible: Bintje, Desiree, R1, R2, R3, and R5

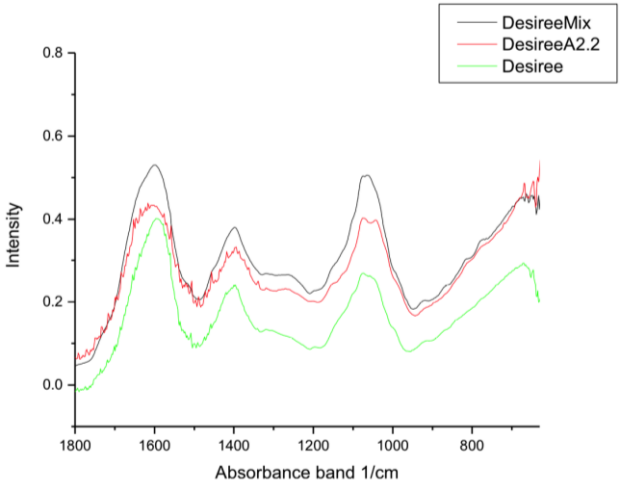



Results & Discussions

The spectra of susceptible genotypes, share the same shapes
The difference is detected in the intensity of the peaks,
Also, in many peaks are induced or suppressed after inoculation

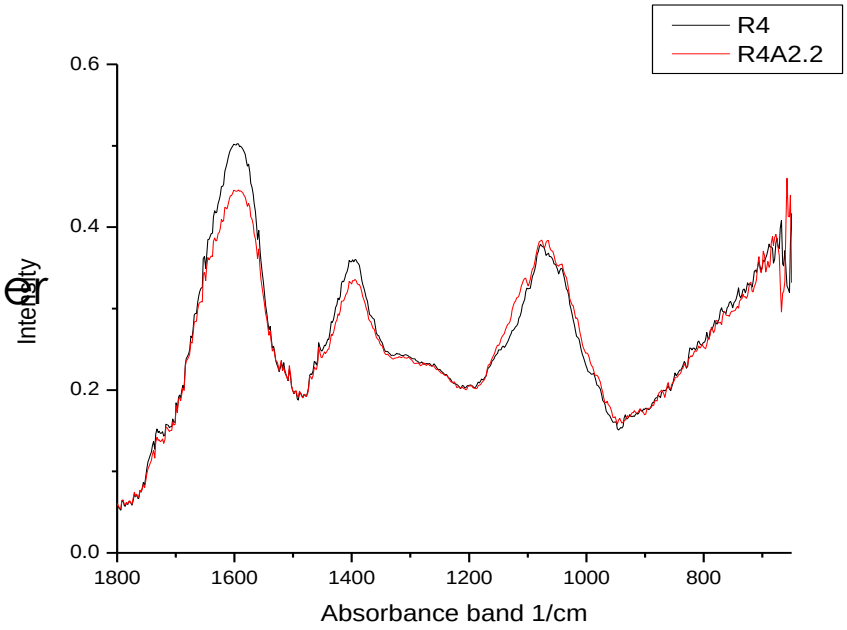


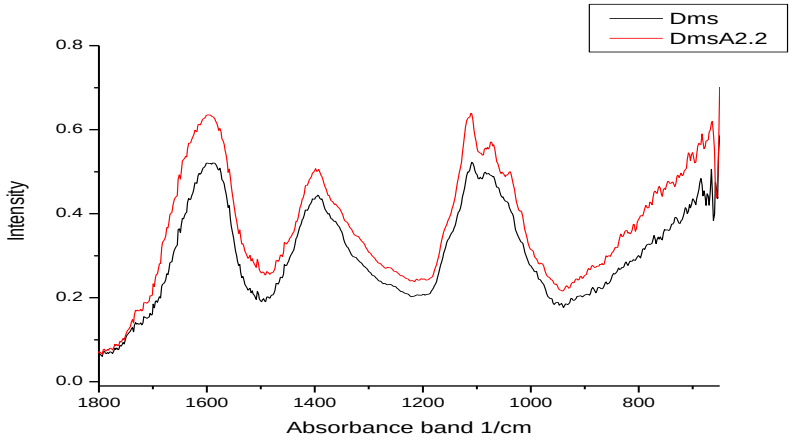


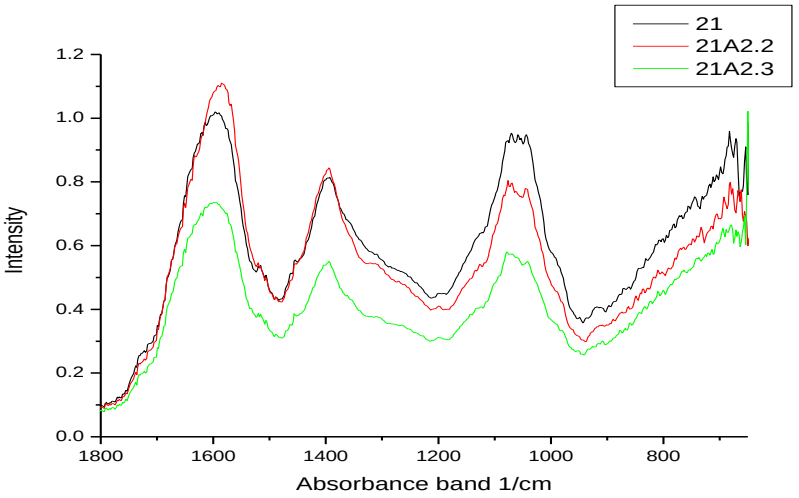


Results & Discussions

In the case of resistant genotypes,
Case R4: the spectra of the control and the inoculated are approximately superposed.
Case 21: the intensity of absorption differs from one treatment to another
Case S. demissum: idem to 21.

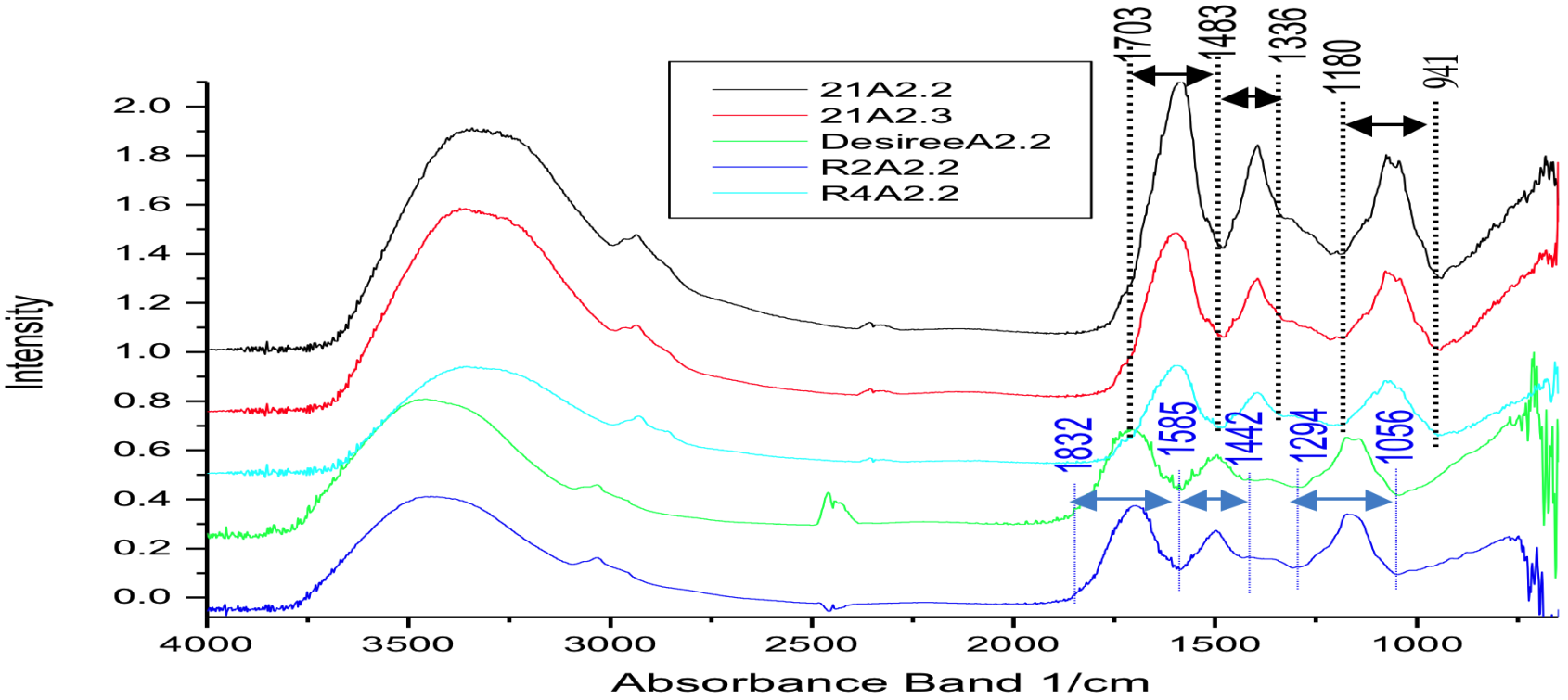






Results & Discussions

When we put all the spectra from the susceptible and resistant genotypes together:
A delay of the bands of absorption is detected in the case of susceptible plants
Resistant: 941-1180 cm^{-1}, 1336-1483 cm^{-1}, 1483-1703 cm^{-1}.
Susceptible: 1056-1294 cm^{-1}, 1442-1585 cm^{-1}, 1585-1832 cm^{-1}.



Conclusions

- The bands: 941-1180 cm^{-1}, 1336-1483 cm^{-1}, 1483-1703 cm^{-1} could be considered as markers for resistance or at least for an incompatible interaction *P. infestans-Solanum* spp.

- the bands:1056-1294 cm^{-1}, 1442-1585 cm^{-1}, 1585-1832 cm^{-1}could be considered as markers for resistance or at least for an compatible interaction *P. infestans-Solanum* spp.

- FTIR spectroscopy is a promising technique for with a huge potential in plant pathology, it is cheap, quick, can be used also in a non-destructive mode and reagent free.

Acknowledgement

- The authors are grateful to W.G. Flier, G.B.M. van den Bosch and G.J.T Kessel from Plant Research International BV for *P. infestans* isolates, also to Dr. Oltean Gh. Research manager of Potato Institute, Brasov, Romania, for plant material. This project is financed by the ministry of education and Research, Romania, in the frame of the PN II program, project no. 1050/1009, code CNCSIS 1496.

Thank you!