

New Markers for Potato Late Blight Resistance and Susceptibility Using FTIR Spectroscopy

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Abstract

The potato (*Solanum tuberosum*) is the 3rd most important crop worldwide, and *Phytophthora infestans* is the most devastating pathogen to potato crops. In this study, it has identified markers for resistance and susceptibility to late blight using potato genotypes that differ in their resistance/susceptibility to *Phytophthora infestans*. Using Fourier transform infrared spectroscopy, many absorbance bands have been identified as specific to resistant potato plants, and others were specific to susceptible potato plants. For each case (resistance/susceptibility), three bands were identified: 941-1180 cm⁻¹, 1336-1483 cm⁻¹, and 1483-1703 cm⁻¹ and 1056-1294 cm⁻¹, 1442-1585 cm⁻¹, and 1585-1832 cm⁻¹, respectively. As it is a simple, rapid, and inexpensive technology, FTIR (Fourier Transform Infrared) spectroscopy offers an excellent opportunity for studying potato resistance/susceptibility to late blight.

Keywords: FTIR, markers, MAS, *Phytophthora infestans*, resistance, *Solanum tuberosum*, susceptibility

Introduction

Late blight is the major disease of potato crops and is also important in tomato and other *Solanaceae* species. The disease is caused by the oomycete *Phytophthora infestans*, which is a heterothallic hemibiotrophic pathogen. When *S. tuberosum* is the host, the asexual life cycle of *P. infestans* can be completed rapidly, with the production of a massive number of sporangia that are readily dispersed. Such a process explains why entire fields can be transformed from slightly diseased to almost completely destroyed within only a few days (Flor, 2008).

P. infestans uses a multitude of effectors to produce a successful infection. As defined by Kamoun (2006), effectors are molecules that manipulate the host cell structure and function, thereby facilitating infection (infection factors or toxin) and/or triggering defence responses (avirulence factors or elicitors).

Late blight control is mainly based on fungicides. However, potato resistance plays an important role in controlling late blight and also in optimising fungicide protection (Sedláková *et al.*, 2011). Potato breeding for late blight resistance has been based on the introgression of resistance (*R*) genes from the related wild species *S. demissum*. To date, 11 *R* genes have been introduced in different potato cultivars around the world; however, *P. infestans* was able to overcome the resistance conferred. Resistance breeding is also based on resistance genes from other wild species, such as *S. bulbocastanum* and *S. phure*; very recently, gene

pyramiding (gene stacking) has been used to introduce several (at least 2) *R* genes from different *Solanum* species into varieties of cultivated potatoes.

Marker-assisted selection (MAS) offers many advantages to breeders: it reduces the time necessary for testing the plant materials and the effort and money required to accomplish a successful gene introgression in the genotype of interest. In general, MAS uses molecular markers as tools for the identification of resistance genes and resistant materials. However, molecular techniques remain expensive, time consuming and not all laboratories can afford them. Therefore, the development of a new simple, inexpensive and rapid procedure for selection is vitally important.

Fourier Transform Infrared (FTIR) spectroscopy is a simple method, and its applications can be easily and quickly used. Furthermore, FTIR can be used as a non-destructive technique and is a promising alternative to the molecular techniques used in MAS. FTIR has not been used thus far in MAS, and FTIR has seldom been utilised to study plant pathology (Bertoluzza *et al.*, 1999; Stewart *et al.*, 1994; Taoutaou *et al.*, 2010).

In this work, it has attempted to identify specific markers for potato resistance/susceptibility to the late blight agent *P. infestans* using a quick and inexpensive technique, FTIR spectroscopy. This report is a follow up to a previous study regarding the application of FTIR to studying interactions in the *P. infestans*-*S. tuberosum* pathosystem (Taoutaou *et al.*, 2010).

Materials and methods

Plant material

The potato plants used in this study were divided into two groups: resistant and susceptible. The first group was represented by *Solanum demissum* (dms), *Solanum bulbocastanum* (blb), and 2 accessions of *Solanum tuberosum* ('R4', a potato plant with the resistance gene *R4*, and '21', which has three *R* genes *R2*, *R3*, and *R4*). The susceptible potato genotypes are all *S. tuberosum* cultivars: the cultivars 'Desiree' and 'Bintje', which lack resistance genes, and four accessions with different resistance genes, *R1*, *R2*, *R3*, and *R5*, named for the gene they possess.

Pathogen

Two isolates of *P. infestans* have been used to inoculate the potato genotypes: A2.2 (NL08009) and A2.3 (88133). The *P. infestans* isolates were kindly provided by WG Flier, GBM van den Bosch and GJT Kessel from Plant Research International BV.

Inoculation

The inocula preparation and leaf inoculation were performed according to Cip manual (1997) using the detached leaf test.

FTIR procedure

In the fifth day post-inoculation, the leaves were ground into a fine powder using a mortar and pestle with liquid nitrogen and then transferred to a 2 ml Eppendorf tube. After adding 1 ml of 70% methanol, the mixture was sonicated for 15 minutes and then centrifuged. A 100 μ l aliquot of the supernatant was used for the analysis. The infrared profile was determined using the Shimadzu Prestige 2, Apodization: Happ-Genzel spectrophotometer in the wavelength range of 4000-500 cm^{-1} .

Results and discussion

As expected, the pathogen isolate was able to infest the susceptible potato plants. The reactions to inoculation were different for the resistant potato genotypes: a hypersensitive reaction was detected for genotype '21', but it has observed no hypersensitivity or other symptoms for 'R4'. The symptoms are shown in Fig. 1.

The FTIR spectra for the potato genotypes are shown in Fig. 2 for the susceptible genotypes and in Fig. 3 for the resistant genotypes. Overall, the FTIR spectra for the susceptible plants exhibit the same shape (Fig. 2); however, differences are detectable when the spectra are studied in detail and by specific intervals.

These results were also found for the resistant genotypes, with the exception that the 'R4' spectra of the control (non-inoculated) and inoculated plants can be nearly superposed (Fig. 3).

When the spectra of the resistant and susceptible genotypes are combined, specific bands for each group are easily identifiable (Fig. 4), with the following major bands: 941-1180 cm^{-1} , 1336-1483 cm^{-1} , and 1483-1703 cm^{-1} for the resistant genotypes and 1056-1294 cm^{-1} , 1442-1585 cm^{-1} , 1585-1832 cm^{-1} for the susceptible genotypes (Fig. 4).

In addition to these major specific bands, it has also examined many smaller bands and/or peaks that were specific to the resistant or susceptible genotypes. Bands at 631-637 and 683-770 cm^{-1} were detected in inoculated 'R4' and *S. demissum*, all of the treatments of '21', and the control and infected *S. bulbocastanum*. The band at 949-1016 cm^{-1} was also specific to the resistant potato genotypes, with the exception of the peak at 691 cm^{-1} , which was detected only in the uninfected 'Bintje'. Near to the last band (the band at 949-1016 cm^{-1}), the susceptible plants had absorbances at 1072-1076 cm^{-1} , whereas the resistant plants had absor-



Fig. 1. Reactions of different potato genotypes to the inoculation with *P. infestans* and the symptoms of the susceptible genotypes. 1-Control; 2-'R2'; 3-'Bintje'; 4-'R4'; 5-'21'; 6-*S. demissum*. On 'R2' and 'Bintje', the mycelia can easily be observed on the leaves; '21' reacted with a hypersensitive response, whereas no reactions were detected for 'R4' and *S. demissum*

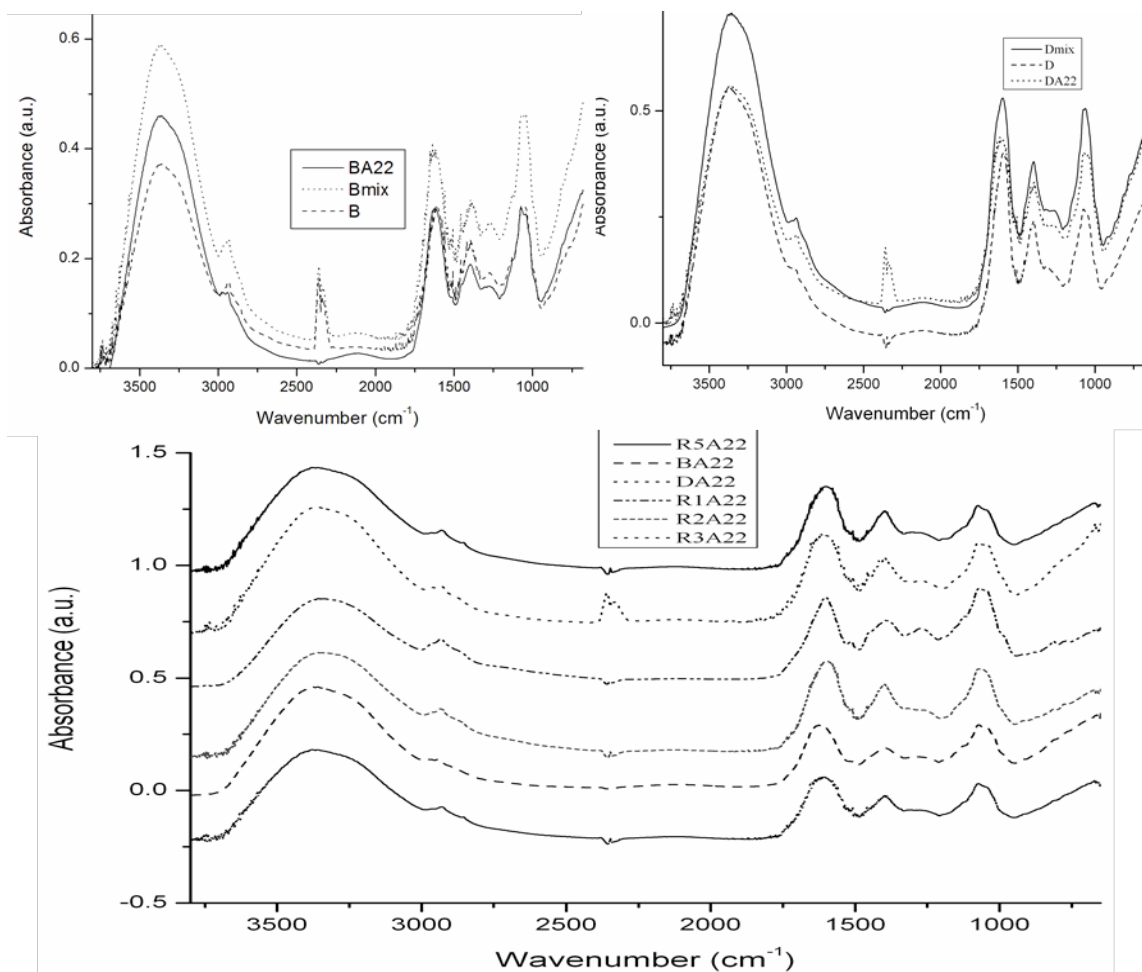


Fig. 2. FTIR spectra of susceptible potato genotypes (inoculated ‘Bintje’ and ‘Desiree’ with A22 and a mix of A22+A23) and the control and a compilation of the spectra of different inoculated genotypes with A22. The spectra have the same shape with minor differences. The major bands are common between all genotypes; however, the absorption intensities differ in the function of the genotype

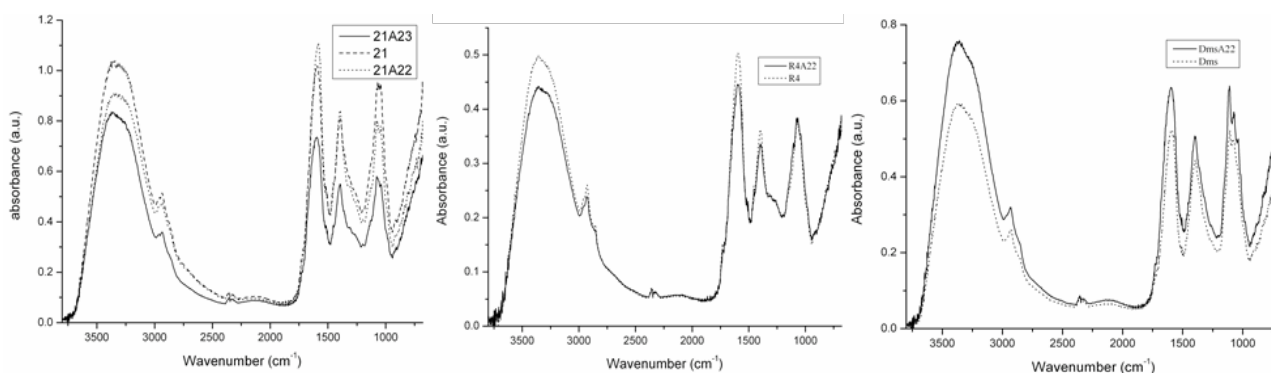


Fig. 3. FTIR spectra of different resistant genotypes with different treatments (control and inoculated). The spectra are approximately superposed in the case of ‘R4’; in the other cases, only the absorption intensities differ

bances at 1078-1109 cm^{-1} . The 1196-1200 cm^{-1} band was common to most of the plants, regardless of their resistance/susceptibility status. An interesting band specific to *S. tuberosum* was found at 1396-1400 cm^{-1} and was not detected in *S. demissum* or *S. bulbocastanum*. Another band specific for resistant plants is the band at 1238-1265 cm^{-1} . Whereas the resistant *S. tuberosum* and inoculated R1 had

absorbances at 1506 cm^{-1} , the susceptible plants showed absorbances in the 1524-1553 cm^{-1} and 1558-1572 cm^{-1} intervals. Other resistance bands have been found: 1576-1589 cm^{-1} , 2291-2313 cm^{-1} , 2590-2932 cm^{-1} (for the last interval, some exceptions were found for the control R1 at 2590 cm^{-1} and the infected R3 at 2617 cm^{-1} and 2855 cm^{-1}), 3015-3262 cm^{-1} , and 3271-3275 cm^{-1} . Other sus-

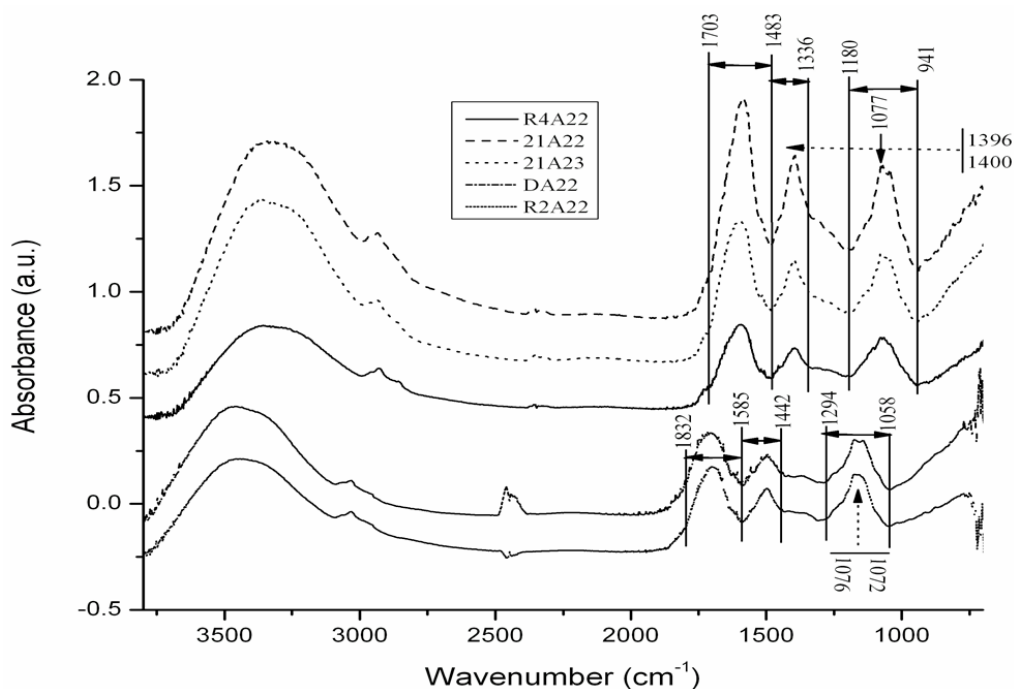


Fig. 4. FTIR spectra with characteristic bands of resistant and susceptible potato plants to the late blight agent. All the spectra show the same behavior; however, for susceptible plants, there is a shift to a longer wavelength of the bands for the susceptible genotypes. Resistant: 941-1180 cm^{-1} , susceptible: 1056-1294 cm^{-1} ; resistant: 1336-1483 cm^{-1} , susceptible: 1442-1585 cm^{-1} ; resistant: 1483-1703 cm^{-1} , susceptible: 1585-1832 cm^{-1}

ceptibility bands have been detected at 1603-1630 cm^{-1} , 1969-2041 cm^{-1} , 2062-2152 cm^{-1} , and 2270-2284 cm^{-1} .

FTIR is a simple, easy and rapid technique that provides a large amount of information for the study of plant pathology. For single-celled organisms, such as bacteria and some fungi, FTIR measures the total composition of the cell in a non-destructive manner, producing an IR spectrum with bands for all of the cellular components (e.g., membranes, proteins, nucleic acids) (Oust *et al.*, 2004). However, the situation is somewhat different in the case of plants. Compounds, such as matrix heteropolymers, pectins and hemicelluloses, phenolic oxidation products, dyes, alcohols, terpenes, tannins, additional waxes, oils, resins, salt crystals, and mucilage, which are often excreted on the plant cell surface, create difficulty when using nondestructive spectroscopic methods for the evaluation of the plant status (Ivanova and Singh, 2003). However, even with these difficulties, FTIR is still a simple, rapid, inexpensive and reagent-free technology.

An FTIR spectrum can be divided into five zones. The first is called the fingerprint zone, in the 600-900 cm^{-1} range. The second is situated in the interval of 900-1200 cm^{-1} and is specific for polysaccharides and carbohydrates. The third zone in the interval 1200-1500 cm^{-1} is a mixed region detecting proteins, fatty acids, DNA, RNA, and groups with phosphorus molecules. The fourth zone, at 1500-1800 cm^{-1} , is characteristic of amides and proteins (e.g., amide I, amide II, proteins, peptides) and phenols and polyphenols. The fifth and last region is the fatty acid zone in the interval of 2800-3200 cm^{-1} .

For the resistant genotypes, the specific markers are situated within the polysaccharide and carbohydrate zones, the mixed zone and the amide and phenol regions. In the case of susceptible plants, the first specific band is part of the polysaccharide and mixed zones, and the second band is contained within the mixed region but also within the amide and phenolics zones. The third band is situated in the amide/protein and phenolics regions. Nevertheless there is a delay of bands in the case of the susceptible genotypes. This delay indicates a low concentration of the metabolites and molecules implicated in resistance, which are present in the resistant genotypes. The susceptible genotypes synthesise other molecules from the same group, but the molecules have no effect on the outcome of the potato-*Phytophthora* interaction. Metlitsky and Ozheretkovskaya (in Dyakov and Dzhavakhiya, 2007) found that the synthesis of terpenoids in resistant and susceptible potato varieties proceeds in a similar manner up to the stage of C15 farnesyl pyrophosphate production. After this point, two molecules of farnesyl pyrophosphate condense (catalysed by squalene synthetase) in susceptible varieties, with the production of C30 squalene from which phytosterols are produced. In contrast, under the effect of another enzyme (cyclase) in the resistant varieties, the farnesyl pyrophosphate molecules close, forming two rings and producing bicyclic sesquiterpenes, which are highly toxic phytoalexins. Thus, instead of providing a required nutrient, the plant furnishes the parasite with a toxin (Dyakov and Dzhavakhiya, 2007).

The small band at 631-637 cm^{-1} is important and could be considered as a marker for resistance, especially because it is situated in the fingerprint zone. This band was detected all of the resistant genotypes, both in the control and inoculated leaves.

In addition, these bands and peaks could be considered as markers for compatible *Solanum* spp.-*P. infestans* interactions in the case of the susceptible genotypes and as markers for incompatible interactions in the case of the resistant genotypes.

If the advantages of MAS could be combined with the FTIR technique, it would offer significant reductions in cost and time to breeders and researchers. MAS is currently used for gene pyramiding and for pyramided QTLs (Ashikari and Matsuoka, 2006). Gene pyramiding is the accumulation of resistance genes (in this case) into a single genotype or cultivar (Tan et al., 2010), such as genotype '21' in the present study.

In this study, it has demonstrated that FTIR can be used in plant pathology research. FTIR is not yet popular among plant pathologists; however, the potential opportunities offered by this technique are manifold (Bertoluzza et al., 1999; Ivanova and Singh, 2003; Stewart et al., 1994; Taoutaou et al., 2010). FTIR is used also in many fields similar to plant pathology, such as mycology and human medicine. In their review, Santos et al. (2010) describe FTIR as a powerful technique for the identification and characterisation of filamentous fungi and yeast. Using synchrotron FTIR spectromicroscopy, Jikline et al. (2008) were able to study the chemical composition of filamentous fungi at the single-cell level.

Conclusions

The bands identified in this study could be considered as markers for resistance (941-1180 cm^{-1} , 1336-1483 cm^{-1} , and 1483-1703 cm^{-1}) or at least as markers for an incompatible *Solanum* spp.-*Phytophthora infestans* interaction. Conversely, the 1056-1294 cm^{-1} , 1442-1585 cm^{-1} , and 1585-1832 cm^{-1} bands could be considered as markers for susceptibility in this pathosystem or at least as markers for a compatible interaction.

Despite its lower popularity among plant pathologists, FTIR spectroscopy offers an important opportunity for studying potato late blight resistance and/or susceptibility, as it is a simple, rapid and inexpensive technique.

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