Pak. J. Phytopathol., Vol. 25 (02). 105-109

Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

FOURIER TRANSFORM-INFRARED SPECTROSCOPY TO MONITOR MODIFICATIONS IN CANOLA BIOCHEMISTRY CAUSED BY ALTERNARIA DESTRUENS

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A B S T R A C T

Alternaria destruens (Simmons) is a serious pathogen of *Brassica napus* L. as well as the other members of mustard family resulting in significant yield loss worldwide. Fourier transform-infrared spectroscopy is a new, rapid, easy technique to study the compositional and metabolic changes in the plant cell wall after any biotic or abiotic stresses. In current research, FTIR technique was utilized to observe the biochemical changes in canola caused by *A. destruens*. The mid-infrared region of the spectrum revealed dramatic changes in protein, lipid and carbohydrate composition of the infected leaves and stem when compared to healthy leaves. Disturbed biochemistry of diseased plants could lead to a disaster for the yield of this major oil producing crop, therefore effective management strategy should be adopted on emergency basis.

Keywords: Alternaria blight, biochemistry, canola, functional groups, FTIR.

INTRODUCTION

Fourier transform-infrared (FT-IR) spectroscopy is a physico-chemical analytical technique that provides a snapshot of tissue metabolic composition at a specified period under diverse environment (Griffiths and de Haseth, 1986; McCann *et al*., 1997; Martin *et al.,* 2005). FTIR generates a spectrum by the vibrations of bonds within chemical functional groups that can be considered as a biochemical or metabolic "fingerprint" of the sample. Assessing the width, position and intensity of infrared light absorption, the configuration of molecular functional assemblies can be evaluated (Yee *et al*., 2004). In most cases, the structure of the biomass being already known, the absorption peaks of the molecular bonds can be found in the literature and changes in some of these absorption peaks, due to the presence of the any stress can be easily detected (Freitas *et al*., 2008). Applying metabolomic techniques to plant pathology is a new approach, generally used as a complementary method to transcriptomeand proteome analyses (Martin *et al*., 2005).

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A. destruens is pathogen of Alternaria blight or Alternaria black spot disease that affected many members of mustard family including canola (*B. napus*) resulted in drastic yield loss globally (Shrestha *et al.*, 2005). The characteristic symptoms produce by the disease areappearance of brown to black circular spots on leaves, stems and pods that expand under favorable condition into gray colored lesions with concentric rings of black border. Pods with infected pedicels fail to develop while severely spotted pods dry, shrink, and may split open prematurely, allowing shrunken seeds to drop off. This disease leads induced a drastic alteration in plant biochemistry that lead to reduced photosynthetic area, defoliation and accelerated senescence (Allen *et al*., 1971). This study focused on detecting biochemical differencesin diseased and healthy canola plants by using FTIR spectroscopy.

MATERIAL AND METHODS

Survey of Kenzo Farm, Shiekupura (35 km northwest of Lahore) was conducted during April, 2013 to collect healthy and diseased canola plants 7 days before harvesting. At the farm, canola was grown over area of 209 m^2 (row to row distance: 30 inches; plant to plant distance: 6-9 inches) on sandy loam soil having 1.2%

organic matter, 7.5 pH and 1150 µS/cm electrical conductivity. The highest and lowest temperatures during sampling were 26-35°C and 21-25°C, respectively. Canola was cultivating in same filed from last two years. Plant sowing was done in November. Soil was sprayed with herbicides 1-2 week of seed sowing. Disease was appeared in late January and persisted till April. Typical symptoms of Alternaria disease i.e. black, necrotic lesion on leaves, pods and stems (Cook *et al*., 2009) were observed uniformly throughout the filed. To assess disease incidence, plot was divided in to 4 sub plots each of 50 m^2. Disease incidence was noticed using following formula:

Disease incidence (%) = (number of infected plants/total number of plants) x 100

About 85% of the plants were found be infected with Alternaria blight disease. Ten samples of each healthy and diseased canola were up rooted randomly from the each sub plot (Fernando e*t al*., 1997).

Plants were brought to the laboratory in clean and sterilized polythene bags. Infected leaves, stem, roots and pods of canola plant were cut into 1cm pieces, surface sterilized by submerging in 0.1% sodium hypochlorite solution for 2-3 minutes and then rinsed 3-4 times with sterilized distilled water. After this treatment, 8-10 pieces were placed on each plates containing malt extract 2% malt extract agar and incubated at 25ºC ± 3. From all (100%) inoculated samples, mycelium of the same fungus started emerging. The resulting mycelium was sub-cultured and purified fungus was identified through cultural basis and morphological basis by compound microscope (Simmons, 2007).

For FTIR, leaf and shoot samples of diseased and healthy canola plants were prepared by method of Naumann *et al*. (1991). A measured amount of dry sample (1 mg) was homogenized with 2.0 mg of dry potassium bromide (KBr) in pestle and mortar. Diffuse reflectance infrared spectrum were obtained from the finally prepared pellet for replicate samples. Each IR spectrum was observed in the mid infrared range (4000-400 cm^{-1}) at room temperature (26°C ± 3°C). All spectra are exhibited in terms of absorbance as calculated from the reflectance-absorbance spectrum by the Hyper-IR software.

RESULTS

Obvious spectral differences in the mid-infrared region (4000-400cm^{-1}) between healthy and diseased leaves and stem samples of canola are presented in Fig. 1 A&B. A summary of the characteristic bands and their assignments are presented in Table 1.

Table 1: Important IR bands of healthy and diseased canola leaf and stem samples along with their possible assignments.

Healthy leaves cm^{-1}	**Diseased leaves cm^{-1}**	**Healthy stem cm^{-1}**	**Diseased stem cm^{-1}**	**Functional group assignment to wave number**
3730	3823, 3730	3798, 3730	3799	**3700-3000 cm^{-1}**: Protein and carbohydrate **3700 cm^{-1}**: OH stretching
3362	3375	3370	3375	**3000-3600^{-1}**: NH stretching
2924	-	2926	2924	**3000-2880 cm^{-1}**: Lipid region (-OCH_2-)
2347	2349	2349	2349	C≡C, C≡N
-	1911	1906	1908	C=O
1623	1607	1607	1612	**1600 -1400 cm^{-1}**: Protein region (Amide-I and II) **1685-1621 cm^{-1}:** Amide I band mainly (C=O)
1393	1394, 1325	1379, 1325	1385, 1325	**1350-1000 cm^{-1}**: Region of the phosphate vibration carbohydrate residues attached to collagen and amide III vibration (in collagen)
1250	1260	1243	1242	**1244-1245 cm^{-1}**: PO^{-2} asymmetric (phosphate-I)
1072	1076	1063	1058	**1255 cm^{-1}**: Amide III **1020-1050 cm^{-1}**: Glycogen, Collagen
912	-			Phospodiester region

The changes in protein under disease stress: It is evident from the spectra that β-sheet structure located around 1606-1622 cm^{-1} (amide-I) compared with the control have changed in diseased plant. Intensity of wave number was increased from 1606 cm^{-1} (healthy leaves) to 1612 cm^{-1} in infected leaves and band intensity decreased from 1622 cm^{-1} to 1606 cm^{-1} in infected stem (Table 1, Fig. 1&B).

The changes in lipid under disease stress: The bands around 2925 cm^{-1} characterizes stretching vibration of C-H asym- or sym-, of $-CH_2$ group of lipids. The band at 2924 cm^{-1} in healthy leaves disappeared in diseased leaves, whereas band intensity of 2926 cm^{-1}drop to 2924 cm^{-1}in diseased stem (Table 1, Fig. 1&B).

The changes in carbohydrates under disease stress: The IR spectra between 1200 and 1000 cm^{-1} in the fingerprint region indicate C-H deformation and C-O or C-C stretching (Table 1, Fig. 1&B).

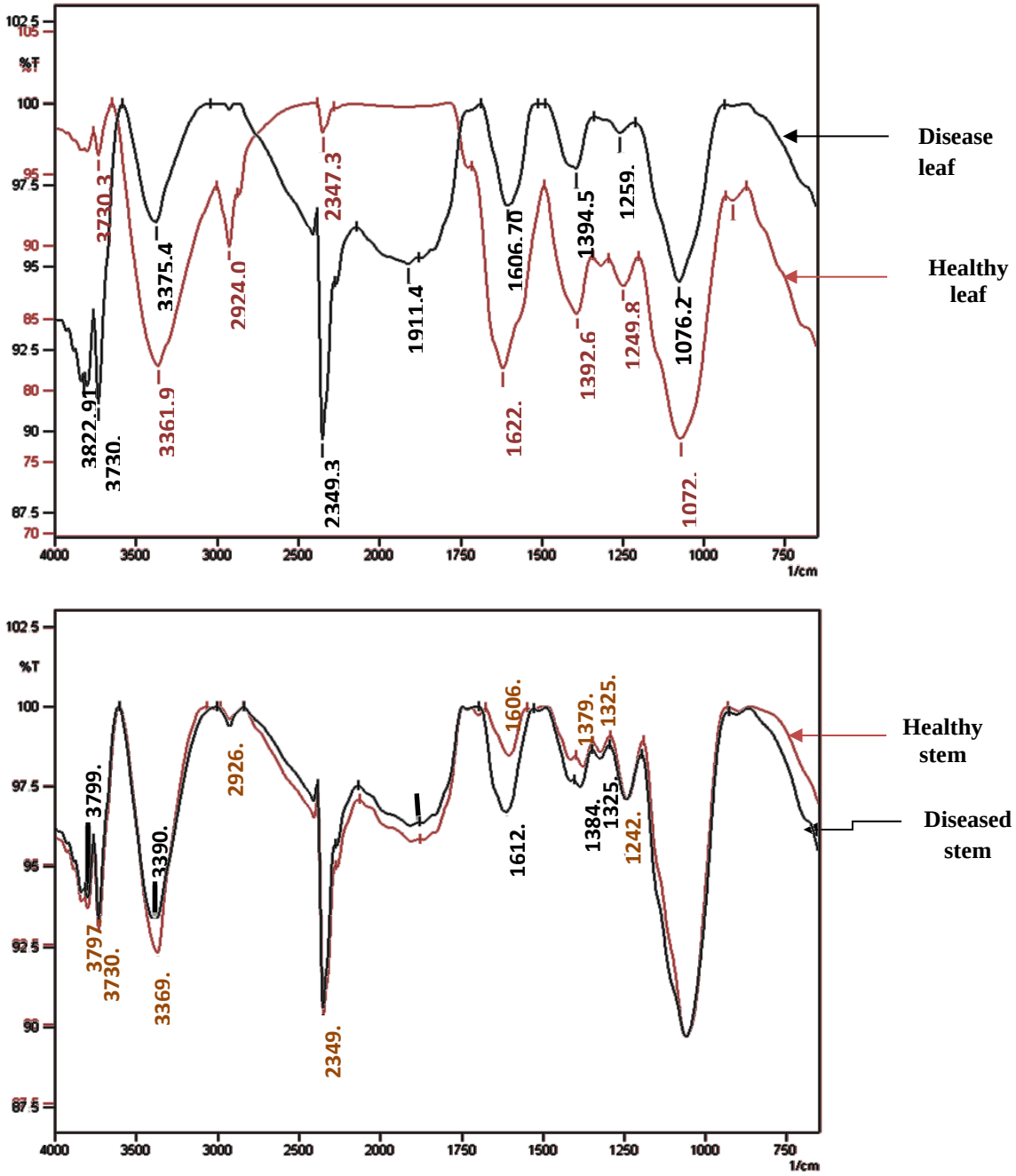

Fig. 1 A& B: Comparison and characterization of infra-red spectra of canola leaves (A) and stem (B).

DISCUSSION

FTIR spectrum of leaves corresponded to black spot diseased caused by *A. destruens* showed absorption band at 3822 cm^{-1}, 3730 cm^{-1}, 3375 cm^{-1}, 2349 cm^{-1}, 1911 cm^{-1}, 1606 cm^{-1}, 1394 cm^{-1}, 1325 cm^{-1}, 1269 cm^{-1} and 1076 cm^{-1}. Likewise, diseased stem represented band at 3788 cm^{-1}, 3730 cm^{-1}, 3380 cm^{-1}, 2924 cm^{-1}, 2349 cm^{-1}, 1907 cm^{-1}, 1612 cm^{-1}, 1384 cm^{-1}, 1325 cm^{-1}, 1242 cm^{-1} and 1058 cm^{-1}. The following bands conforming to the compounds belong to carbohydrates, carotenoid, glycogen, amino acids, amides, phosphates, lipids, glycogen and cellulose indicated the responsibility for the disease (Valchos *et al*., 2006; Gokulakumar and Narayanaswamy, 2008).

The alteration in amide-I region of protein could be ascribed to a modification in whole protein composition. Crucial role of protein in the physiology of living organisms cannot negate. Any variation in the protein turnover may impart an adverse effect on the vital and complex groups of biological materials. These biological materials comprised of the nitrogenous constituents thus execute diverse biological events to maintain cell's homeostasis (Baseri and Baker, 2011).Consequently, the cell's protein content is a diagnostic tool to assess the physiological phases of a cell (Movasaghi *et al*., 2008).

Change in lipid contents of diseased plant indicated the reduction in-CH_2/peroxides & hydroperoxides and could be considered as biomarker for lipid peroxidation. It is concluded that infected plant suffered oxidative stress of lipid and resulting fungal pathogen exhibited black necrotic lesion on whole plant.

Carbohydrates in the leaves are the major constituents of these absorption bands (Li *et al*., 2004). Increase or decrease in bands strength in this region after disease stress directed the alteration in carbohydrate structure and sensitivity of carbohydrate synthesis in the canola plants.

CONCLUSION

Various spectral data acquired by FTIR can be efficiently utilized to analyze changes in various important biochemical compounds in the canola plants caused by Alternaria blight.

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