

Plant Archives

Journal homepage: http://www.plantarchives.org DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.1.045

FT-IR ANALYSIS OF DISCOLOURED GRAINS AND BROWN SPOT INFECTED RICE CULTIVAR ADT 36

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(Date of Receiving-22-11-2023; Date of Acceptance-28-01-2024)

FT-IR analysis was done for healthy, moderately discoloured rice grain, completely discoloured grain, chaffed grains/ seeds, healthy and brown spot infected rice leaves of rice cultivar ADT 36 through methanolic solvent. All the above samples were collected from the pot culture and the chemical compounds were analysed through the FT-IR analysis. The results indicated that, the FT–IR spectrum of healthy and brown spot diseased leaves have similar chemical functional groups only with minor differences of phenolic compounds. From the FT-IR spectrum observed in grains analysis, it has been clearly mentioned that more amount of methyl, methylene groups of lipids, fatty acids and starch present in healthy grains, when compared to other infected samples.

Key words : FT- IR analysis, Grain discolouration, Brown spot, Rice.

Introduction

Brown spot is more prevalent in all rice fields that are deficient in nutrients especially nitrogen and poor practices in rice cultivation (Mew et al., 2018). The disease produces a characteristic nature of sesame shaped spot in leaves on all the rice varieties and the causal organism is capable of affecting all crop growth stages of rice. Also, the same pathogen is major responsible for grain discolouration (Savary et al., 2000). An efficient analytical report based on Fourier Transform Infrared Spectroscopy (FT- IR) would offer the improvement of a rapid and sample analysis of cereals. With FT-IR report, the intensity level and infrared peak condition can identify the chemical bonds and chemical structures. The present studies were undertaken to investigate the FT- IR analysis of brown spot, healthy rice leaves and various stages of grain discolouration.

Materials and Methods

Crop	Oryza sativa L. (Rice)
Variety	ADT 36
Source of rice seeds	TRRI (Tamil Nadu Rice Research Institute), Aduthurai, 612101, Tanjore district, Tamil Nadu, India.

Bipolaris oryzae inoculation studies under pot culture

Nearly four-week-old seedlings (most susceptible rice variety of brown spot, ADT 36) were transplanted in the cement pots and then inoculated with the *Bipolaris oryzae* (AUBo₃; NCBI Accession Number: ON601149). conidial suspension with adequate cfu (5,00,000 spores / 10 ml) on tenth day after transplanting. Plants were inoculated with the pathogen using an atomizer in the evening time. *B. oryzae* inoculated rice crops were

sheltered with polythene sheets to maintain a high RH (Jaiganesh, 2012).

FT-IR analysis for identifying chemical functional groups

Healthy, moderately discoloured grains, completely discoloured grains, chaffed rice grains/ seeds, healthy and brown spot infected rice leaves (methanolic solvent) were collected from the pot culture treatments and identifying the chemical compounds were analysed through the FT-IR analysis. Two mg of the testing sample were mixed with two hundred mg Potassium bromide (FT-IR grade - KBr) and pressed into a pellet. FT-IR spectra for the testing samples were recorded in the range 4000 to 400 cm⁻¹. The chemical functional groups were identified based on peak value and wavelength (cm⁻¹) of Infrared Radiation (Talari *et al.*, 2017).

These FT IR analyses were carried out in the Food Testing Laboratory (FTL), National Institute of Food Technology Entrepreneurship and Management (NIFTEM-T) (formerly called as IIFPT), Ministry of Food Processing Industries, Government of India, Tanjavur, Tamil Nadu, India.

Instrument details

Instrument model and serial Number	Nicolet iS50 and AUP1910262
Software used for FT – IR analysis	OMNIC 9.9.549
Number of scans and resolution	32 and 4
Detector	DTGS KBr
Source	IR
Beam splitter	KBr



Plate 1 : Rice brown leaf spot symptoms.

Results

FT-IR analysis of healthy and brown spot infected leaves

The results mentioned that, the FT – IR spectrum of healthy and brown spot diseased leaves were similar chemical functional groups identified only with minor differences of phenolic compounds (1367 cm⁻¹) (Table 1, Fig. 1 and Plate 1). The remarkable differences were observed in peak positions and transmittance percentage was also observed between these samples. Comparison of FT-IR spectrum of rice healthy leaves to brown spot infected leaves revealed a clear variation in Sugars or carbohydrates chemical functional groups that were present in healthy sample (off-peak line wider/ larger in Healthy (1036 cm⁻¹) sample whereas smaller in diseased leaves (1027 cm⁻¹).

FT-IR analysis of rice grains

The FT-IR spectrum of healthy, moderately and



Sporulation in grains

Completely discoloured grain

Plate 2 : Symptoms on grains (from Pot culture treatment).

Healthy grain

Healthy rice leaves			Brown spot infected rice leaves	
Peak no.	X (cm ⁻¹)	Chemical Functional groups	X (cm ⁻¹)	Chemical Functional groups
1.	433.03	S-S stretch, disulfides	424.99	S-S stretch, disulfides
2.	456.69	S-S stretch, disulfides	455.90	S-S stretch, disulfides
3.	786.15	C-Cl stretch, Aliphatic Chloro compound	518.16	S-S stretch, disulfides
4.	1035.53	Carbohydrates, Saccharides	794.72	C-Cl stretch, Aliphatic Chloro compound
5.	1367.06	Phenolic groups O-H Bend	1026.90	Carbohydrates, Saccharides
6.	1621.38	C=C, Ketone compounds	1462.80	C-H, C=C-C, Aromatic compounds
7.	2848.73	Stretching C–H, Aliphatics, Fatty acid, Lipids	1646.40	C=C, Amide I
8.	2916.64	Phospholipids, O-H stretch, Carboxylic acid	2848.75	Stretching C–H, Aliphatics, Fatty acid, Lipids
9.	3274.48	O–H(water), N-H (protein), Hydroxy compound	2916.66	Phospholipids, O-H stretch, Carboxylic acid
10.		_	3331.23	O–H(water), N-H (protein), Hydroxy compound

Table 1 : FT-IR Peak value of healthy and brown leaf spot infected rice leaves.

Table 2 : FT-IR Peak value of healthy, moderately and completely discoloured and chaffy grains of rice var. ADT 36.

Healthy grains		Moderately discoloured grains		
Peak no.	X (cm ⁻¹)	Chemical Functional groups	X (cm ⁻¹)	Chemical Functional groups
1.	418.30	S-S stretch, disulfides	405.35	S-S stretch, disulfides
2.	430.29	S-S stretch, disulfides	418.44	S-S stretch, disulfides
3.	514.92	S-S stretch, disulfides	440.25	S-S stretch, disulfides
4.	860.23	C-H bending P-O-C stretch, Aromatic phosphates	995.60	C=C bending, alkene, Silicate ion
5.	995.56	C=C bending, alkene, Silicate ion	1076.00	PO ₃ Stretch, Phosphate ion
6.	1076.21	PO ₃ Stretch, Phosphate ion	1147.75	C-O stretching, Aliphatic ether, glycogen
7.	1147.59	C-O stretching, Aliphatic ether, glycogen	1239.54	C-O Stretch, Acid
8.	1239.27	C-O Stretch, Acid	1363.43	O-H bend, Phenolic group
9.	1336.23	O-H bend, Phenolic group	1636.90	C=O Stretching, Ketone compounds
10.	1540.97	Amide II	1737.98	C=O stretching band mode of the fatty acid ester
11.	1639.82	C=O Stretching, Ketone compounds	2849.74	Symmetric stretching of -CH(CH2), Fatty acid, lipid and Protein
12.	1744.09	C=O stretching band mode of the fatty acid ester	2918.25	Asymmetric stretching of -CH(CH ₂), Saturated aliphatic compound, Lipids
13.	2922.21	Saturated aliphatic compound, Lipids	3275.43	H- bonded, OH Stretch, Hydroxy compound
14.	3274.19	H- bonded, OH Stretch, Hydroxy compound		1
Completely discoloured grain		Chaffy grains		
Peak no.	X (cm ⁻¹)	Chemical Functional groups	X (cm ⁻¹)	Chemical Functional groups
1.	404.91	S-S stretch, disulfides	403.72	S-S stretch, disulfides
2.	417.67	S-S stretch, disulfides	410.07	S-S stretch, disulfides

Table 2 continued....

		1		
3.	424.46	S-S stretch, disulfides	417.47	S-S stretch, disulfides
4.	432.64	S-S stretch, disulfides	428.30	S-S stretch, disulfides
5.	1000.84	C=C bending, alkene, Silicate ion	448.65	S-S stretch, disulfides
6.	1075.76	PO ₃ Stretch, Phosphate ion	517.72	S-S stretch, disulfides
7.	1540.69	Amide II	996.52	C=C bending, alkene, Silicate ion
8.	1644.31	C=O Stretching, Ketone compounds	1076.37	PO ₃ Stretch, Phosphate ion
9.	2849.84	Symmetric stretching of -CH(CH ₂), Fatty acid, lipid and Protein	1147.15	C-O stretching, Aliphatic ether, glycogen
10.	2918.69	Saturated aliphatic compound, Lipids	1365.62	O-H bend
11.	3273.56	H- bonded, OH Stretch, Hydroxy compound	1541.07	Amide II
12.			1643.13	C=O Stretching, Ketone compounds
13.			1744.36	C=O stretching band mode of the fatty acid ester
14.			2851.32	Symmetric stretching of -CH(CH ₂), Fatty acid, lipid and Protein
15.			2921.12	Saturated aliphatic compound, Lipids
16.			3277.52	H- bonded, OH Stretch, Hydroxy compound

Table 2 continued....



Plate 3: Symptoms on grains.



Brown spot infected rice leaf

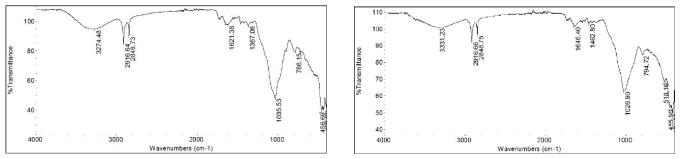


Fig. 1 : FT IR spectrum of healthy and brown spot infected rice leaves var. ADT 36.

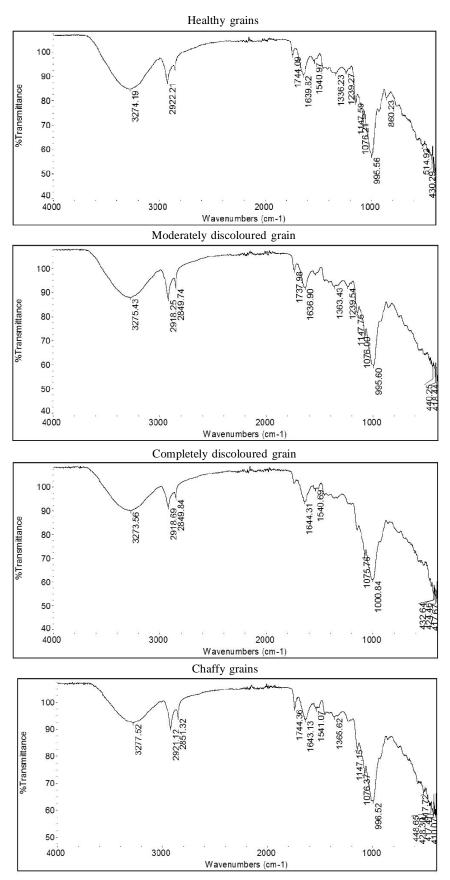


Fig. 2 : FT-IR spectrum of healthy, moderately and completely discoloured and chaffy grains of rice var. ADT 36.

completely discoloured grains, chaffy grains were shown in Fig. 2. Spectrum of all these four grains contain similar bands of C=C bending, C=O Stretching, Amide II, H- bonded and OH Stretch although the intensities of chemical functional groups were varied. The spectrum band in the range of 3273 -3277 cm⁻¹ was assigned to the -OH stretching and may be considered as indication of water present in all the four types of grains. From the FT-IR spectrum observed in Table 2 (Plates 2, 3), it has been clearly mentioned that more amount of methyl, methylene groups of lipids, fatty acids and starch present in healthy grains when compared to other samples (500 - 1500/cm).

Discussion

FT-IR analysis of Healthy and brown spot infected leaves

The IR regions of 735-795 cm⁻¹ and 900 - 1185 cm⁻¹ in the area attributed that common chemical group such as carbohydrates and polysaccharides. All manner of carbohydrate chemical groups are starch, cellulose, sugars and polysaccharides (Lammers et al., 2009). Stretching frequency of 1367 cm⁻¹, confirmed the presence of phenolic groups (Phenolic acids, Flavonoids, Isoflavonoids) in healthy rice leaves, which give the information about defense action and resistance against pathogenic infection. A parallel tendency was also made by Kumar et al. (2015) with regard to bacterial leaf blight infection in Mulberry leaves. In the present analysis, the chemical profile of functional group in brown spot diseased and healthy leaves are given, these results are useful for the development of quick brown spot diagnostic kit under field conditions in future.

FT-IR analysis of grains var. ADT 36

For the first time of this FT-IR Spectroscopic analysis have been employed to observe the rice grains infected by *B. oryzae* and a complex infection (grain discolouration). FT-IR analysis suggests the modification of proteins and starch in the completely, moderately discoloured grains, chaffy grains (samples from integrated treatments) after the infection of pathogen (a complex infection) with respect to healthy rice grains. The protein and starch content recorded in the diseased grains can be relevant to the plant defence response that crops activate during the pathogenic invasion (Sharma *et al.*, 2020).

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