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Resistance of Sugarcane Clones (*Saccharum* spp.) to Red Rot Disease (*Collectorichum falcatum* Went) and Analysis of Resistant clone by FTIR

V. Ravichandran [a*], S. Ganapathy [b], M. Shanmuganathan [c], J. Jayakumar [d], G. Gayathry [d], P. A. Saravanan [a] and P. Veeramani [a]

[a] Tapioca and Castor Research Station, Tamil Nadu Agricultural University, Yethapur, India.
[b] Agricultural College and Research Institute, TNAU, Vazhavachanur, India.
[c] Agricultural College and Research Institute, TNAU, Kudimiyanmalai, India.
[d] Krishi Vigyan Kendra, TNAU, Vriddhachalam, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Sugarcane (*Saccharum* spp.) is an important industrial crop and mainly grown for the sugar and jaggery production. The major constraint in the sugarcane cultivation is the outbreak of red rot disease and many high yielding and high sugar varieties succumb to the disease-causing huge reduction in the cane yield and also quality of cane. The disease is very difficult to manage as the pathogen is deep seated in the stalk. Exploitation of the host resistance in viable way to contain the disease. The sugarcane clones developed were screened for resistance to red rot disease by

**Corresponding author: E-mail: ravichandranpath@gmail.com;*

artificial inoculation. Among the 52 clones screened three clones *viz*., C15632, 16G032 and 16G031 behaved resistant and thirteen clones C15004, C15006, C15011, C15021, C15095, C15157, C15559, C15603, 16G006, 16G087, 16G046, 15G060 and 15G028 behaved moderately resistant to red rot. In the FT-IR analysis of the leaf samples of resistant type clones spectral peaks were found in wave length of 1046 cm^{-1}, 1044 cm^{-1} and 1068 cm^{-1} associate with the functional group of the molecule Aliphatic fluoro compound, C-F stretch / Primary alcohol, C-O Stretch / Primary amine, CN stretch / Phosphate / Silicate ions and these functional group of components may be associated for the resistance to the red rot.

Keywords: Sugarcane; red rot; Colletotrichum falcatum; resistance; FTIR.

1. INTRODUCTION

Sugarcane (*Saccharum* spp.) is important commercial crop grown in India with an area of 4.60 million ha with the production of 370.5 million tonnes 2019-20 [1]. Major limitations of sugarcane productivity were the epidemics of diseases and it was reported more than 55 diseases in India that affects the sugarcane. In the sugarcane cultivation the occurrence of red rot is the major constraint and it affect both the yield of the cane and also the juice quality. Red rot epidemics resulted in the loss of important commercial varieties as they become susceptible [2]. Prevalence of red rot disease was documented in all sugarcane growing area. The outbreak of red rot diseases not only reduces yield of the cane but also reduces juice extraction and sucrose content. Red rot disease incited by *Colletotrichum falcatum* Went affects all the parts of the plants *viz*., leaves, buds, nodes, stalk and root with characteristic symptoms of reddening of internal tissue of the stalk with typical white spot either restricted or progressive in nature longitudinally along the stalks. The other symptoms include yellowing and drying of the leaves, presence of reddish spots with dark center along the midrib region, rotting of the inter nodal region, rotting of the nodes, darkening of the nodal region, breaking of the stalk in the nodal region and death of the plants. Among the different management practices, utilization of host resistance is the viable and feasible way to overcome the red rot disease and hence the sugarcane clones developed were evaluated for resistance to red rot disease. Weather conditions, genotypes, the presence of a virulent pathogen, and the time for disease development influences severity of the disease. These factors must be thoroughly investigated in order to achieve effective disease control. It has been observed that once the disease has appeared in the field, control is impossible. The use of resistant varieties has largely been responsible for effective red rot control. Regardless of the fact that the genetics of red rot resistance is not well established, significant advances have been made in the production of red rot resistant varieties [3]. In India sugarcane varieties with resistance to red rot disease were recommended for commercial cultivation since screening is the integral part of varietal development programme. The most common method for screening red rot resistance is plug method of inoculation of the pathogen. The red rot pathogen adopts and maintained the virulence on the new cultivars of the host and hence screening for red rot resistance is being taken up with designated pathotypes [4]. The biochemical composition of the plant cell viz., carbohydrates, polysaccharides and fatty acids can be determined by FTIR spectroscopy [5]. Phytoalexins were involved in resistance to red rot [2]. Hence this was formulated to study red rot disease resistance in sugarcane clones developed and to identify functional group in resistant clone through FTIR.

2. MATERIALS AND METHODS

2.1 Screening for Red Rot Resistance

The field experimental trail was laid at Sugarcane Research Station, Cuddalore, India (latitude; 11º 46' North; longitude: 79º.46' East; altitude: 4.60 m MSL) during 2020-21 season. Sugarcane clones were obtained from Sugarcane Research Station, Cuddalore (30 Nos.) and Sugarcane Research Station, Melalathur (23 Nos.) along with the susceptible check CoC 671. The crop was raised as per the recommended package of practices. Red rot pathogen *C. falcatum* CF 06 obtained from ICAR - Sugarcane Breeding Institute, Coimbatore was used in the present study for testing sugarcane clones for resistance to red rot disease. The red rot pathogen was multiplied in oat meal agar medium and utilized for the preparation of inoculum for inoculation in the test clones. The test clones (7 to 8 months old) were inoculated with the spore suspension

(spore load of 10^6 cfu/ml) of *C. falcatum* by using IISR inoculator. The inoculated canes were split open longitudinally along the point of inoculation after 60 days and evaluated based on yellowing /drying of the foliage, white spot, lesion width and nodal transgression, using the 0-9 scale and the disease reaction was considered as Resistant (0.0 to 2.0), Moderately resistant (2.1 to 4.0), Moderately susceptible (4.1 to 6.0), Susceptible (6.0 to 8.0) and Highly Susceptible (>8.0) [6].

2.2 FTIR Analysis of Leaves

The leaf sample of three clones *viz*., C 15632, 16G031 and 16G032 and check variety CoC 671 was collected from third leaves from whorl leaves and subject to FTIR analysis. In transmission FT-IR spectrometer, the sample (1.0 ml) was pelleted using Potassium bromide (KBr pellets) and placed in between two infrared-transparent plates of the sample holder of the FT-IR chamber. The FTIR spectrum of the sample were analyzed at wave numbers of mid IR range from 4000 to 400 cm^{-1} with the resolution of 0.1 cm^{-1} [7]. The spectrum generated by FTIR on the vibrations of bonds within functional groups based on the peak width, position, and the intensity of absorption, the configuration of molecular functional assemblies was grouped [8].

3. RESULTS AND DISCUSSION

3.1 Reaction of Sugarcane Clones for Inoculation of *C. falcatum*

Among the 53 clones screened, three clones *viz*., C 15632, 16G032 and 16G031 behaved resistant in reaction to red rot. Thirteen clone clones *viz*., C 15004, C 15006, C 15011, C 15021, C15095 C 15157, C 15559, C 15603, 16G006, 16G 087, 16G046, 15G060 and 15G028 behaved moderately resistant to red rot. All the other clones behaved moderately susceptible to highly susceptible in reaction to red rot inoculation. Tabassum et al. [9] reported that a total of 142 C2 generation clones under artificial inoculation with two prevalent races (CF08 and CF09) of the red rot pathogen separately by plug method identified 39 clones with resistant/moderately resistant reaction against both the races over the three seasons. Similarly, Ganapathy et al., [10] identified two clones having high CCS yield, sucrose percent and resistant to red rot disease. The breakdown of resistance in commercial varieties was due to the evolution of newer pathotypes in red rot pathogen. *Saccharum spontaneum* has been employed in breeding programme for the development of resistant varieties against the red rot [11].

3.2 FT-IR Analysis

The FT-IR spectroscopy, with a spectral range from 4000 to 400 cm^{-1} was used for analyzing organic compounds containing -OH, -NH, and –CH functional groups in sugarcane clones showing red rot disease resistant (C 15632, 16G031 and 16G032) and susceptible (CoC 671) clones. Infrared spectroscopy was used to analyses the vibrations of atoms in a molecule and infrared spectrum obtained by passing infrared radiation through the sample was used to determine how much of the incident radiation is absorbed at each energy level. The frequency of a vibration of a part of a sample molecule corresponds to the energy at which any peak in an absorption spectrum appears. The spectrum generated by FTIR on the vibrations of bonds within functional groups based on the peak width, position, and the intensity of absorption, the configuration of molecular functional assemblies was grouped [8,12].

In the FTIR spectrum a peak was observed in the wave length of 1324 (cm^{-1}) in the susceptible variety CoC 671 and it was absent in all the resistant clones. The functional group of the components in that peak was presented in the Fig. 1 to 4. In the resistant types spectral peaks were found in wave length of 1046 cm^{-1} (C15632), 1044 cm^{-1} (16G032) and 1068 cm^{-1} (16G031) associate with the functional group of the molecule Aliphatic fluoro compound, C-F stretch / Primary alcohol, C-O Stretch / Primary amine, CN stretch / Phosphate / Silicate ions were absent in the susceptible variety (CoC 671). Similarly, the peaks were in the FTIR spectrum of resistant varieties *viz*., C15632 of 560 cm^{-1}, 16G032 at the wavelength of 575 and in 16G032 at the wavelength of 580 cm^{-1} associated with the functional groups of Aliphatic iodo compounds, C-I stretch was absent in the Susceptible variety (CoC 671) and these functional group of components may be responsible for the resistance reaction to the red rot disease. It has been reported that the average spectrum for the resistant tree sample had higher absorbance peaks than the spectra from susceptible tree indicating increased formation of lignin and suberin indicating the usefulness and sensitivity of the FT-IR technique

Table 1. Reaction of sugarcane clones to red rot disease (plug method)

Clone	Score	Disease reaction	Clone	Score	Disease reaction
C15004	3.2	Moderately Resistant	C 15639	4.2	Moderately Susceptible
C 15006	3.5	Moderately Resistant	C 15642	5.6	Moderately Susceptible
C 15011	3.7	Moderately Resistant	C 15645	4.9	Moderately Susceptible
C 15021	2.8	Moderately Resistant	C15683	4.1	Moderately Susceptible
C 15079	9.0	Highly Susceptible	15G010	9.0	Highly Susceptible
C 15081	9.0	Highly Susceptible	15G012	9.0	Highly Susceptible
C 15086	9.0	Highly Susceptible	15G028	3.4	Moderately Resistant
C 15088	9.0	Highly Susceptible	15G032	9.0	Highly Susceptible
C 15095	3.1	Moderately Resistant	15G044	5.1	Moderately Susceptible
C 15151	8.8	Highly Susceptible	15G060	3.8	Moderately Resistant
C 15157	3.7	Moderately Resistant	16G006	2.9	Moderately Resistant
C 15175	4.9	Moderately Susceptible	16G011	5.1	Moderately Susceptible
C 15176	9.0	Highly Susceptible	16G012	7.5	Susceptible
C 15181	5.9	Moderately Susceptible	16G013	5.8	Moderately Susceptible
C 15192	4.5	Moderately Susceptible	16G021	9.0	Highly Susceptible
C 15195	5.2	Moderately Susceptible	16G031	1.6	Resistant
C 15210	5.6	Moderately Susceptible	16G032	1.5	Resistant
C 15708	9.0	Highly Susceptible	16G038	9.0	Highly Susceptible
C 15810	4.5	Moderately Susceptible	16G045	6.2	Susceptible
C 15827	5.7	Moderately Susceptible	16G046	3.0	Moderately Resistant
C 15499	7.3	Susceptible	16G051	9.0	Highly Susceptible
C 15525	6.0	Moderately Susceptible	16G064	5.9	Moderately Susceptible
C 15559	3.4	Moderately Resistant	16G077	4.7	Moderately Susceptible
C 15603	3.3	Moderately Resistant	16G080	4.5	Moderately Susceptible
C 15607	5.3	Moderately Susceptible	16G083	9.0	Highly Susceptible
C 15632	1.5	Resistant	16G087	3.7	Moderately Resistant
CoC671 (Check)	9.0	Highly Susceptible			

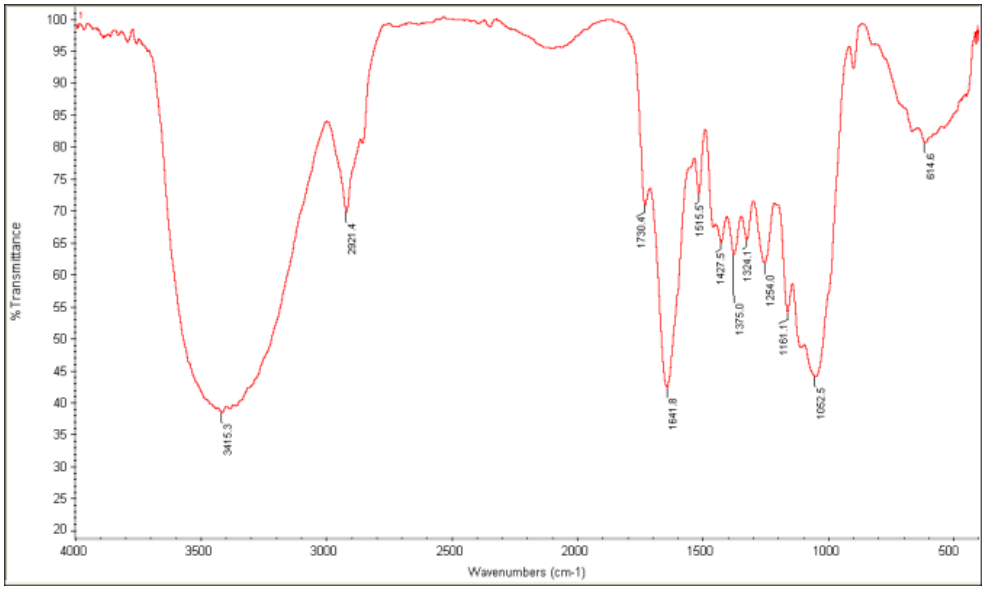

Fig. 1. FT-IR Spectroscopy of leaf samples of sugarcane variety CoC 671

for evaluating host resistance in Dutch elm disease complex [13]. The mechanism governing red rot resistance in sugarcane is unknown. Details about the host pathogen interaction, role of phytoalexins, pathogenesis related protein and molecular basis of diseases resistance was

discussed by [2]. The resistance in canola for club root disease was activation of a basal defence gene through the phenylpropanoid pathway, and lignin accumulation may contribute to the clubroot resistance and the role of cell wall components in defence response to club root was indicated through FTIR spectroscopy [14]. The biochemical changes in Xylem tissues of *Ulmus minor* was used for the identification of resistance and susceptibility to *Ophiostoma novo-ulmi* based on the changes in the lignin composition using Fourier transform-infrared spectroscopy [13]. In the Quercus suber roots FT-IR spectroscopy and chemometrics was utilized to deduct the changes in the metabolic patterns based on the differences in the intensity of certain spectral bands. The use of vibrational spectroscopic and chemometric method have used to find the disease susceptibility in tress based on chemical fingerprint data [15]. The FT-IR spectrum has been used to find the resistance in wave length of 560 $cm^{-1}$to 580 cm^{-1};1046 $cm^{-1}$to 1068 cm^{-1} and the functional group of compounds associated with these spectra may be exploited for studying the biochemical basis of resistance to red rot.

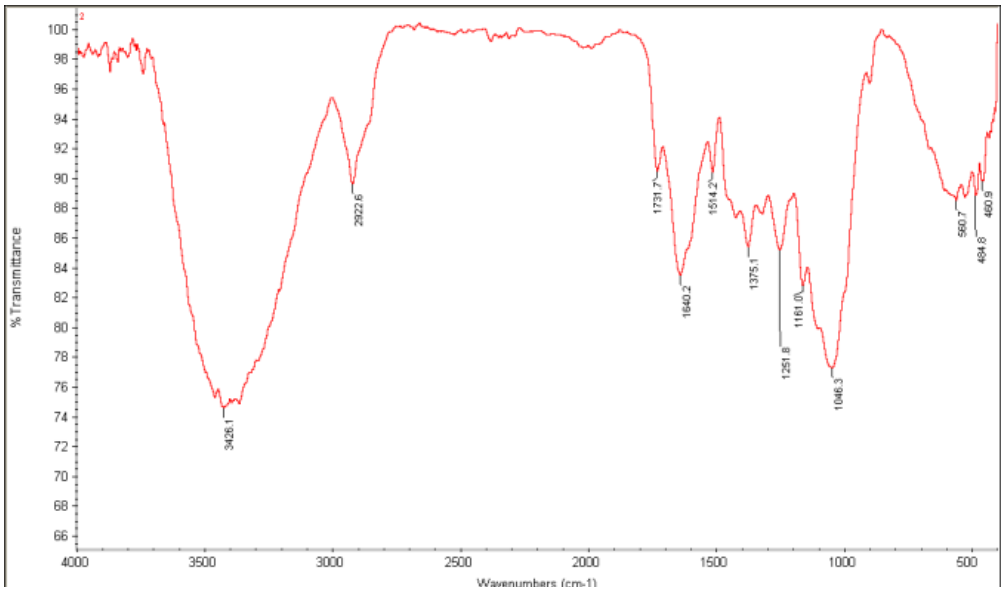

Fig. 2. FTIR spectroscopy of leaf samples of sugarcane variety C 15632

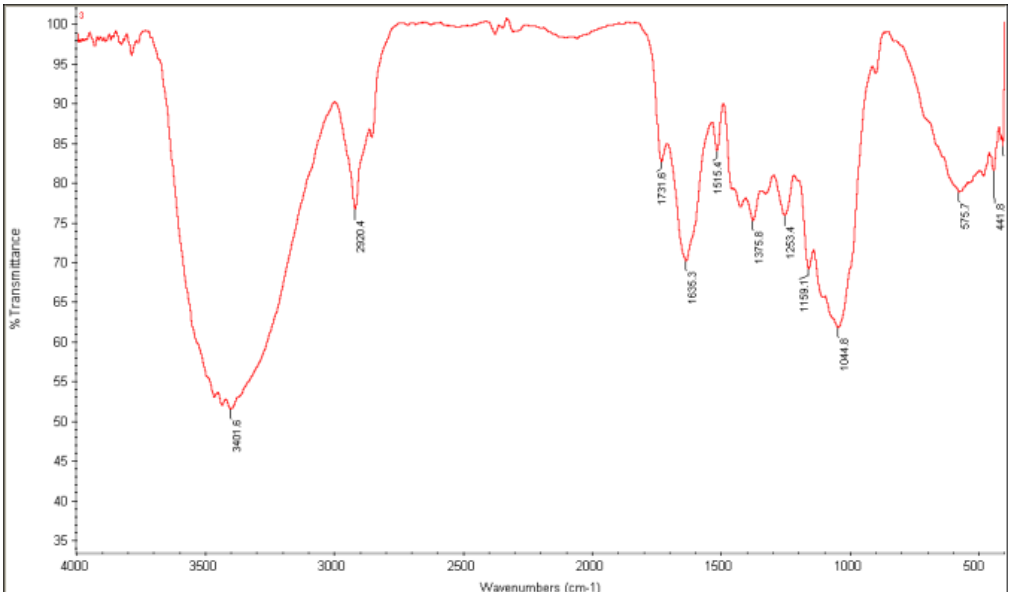

Fig. 3. FTIR spectroscopy of leaf samples of sugarcane variety 16G031

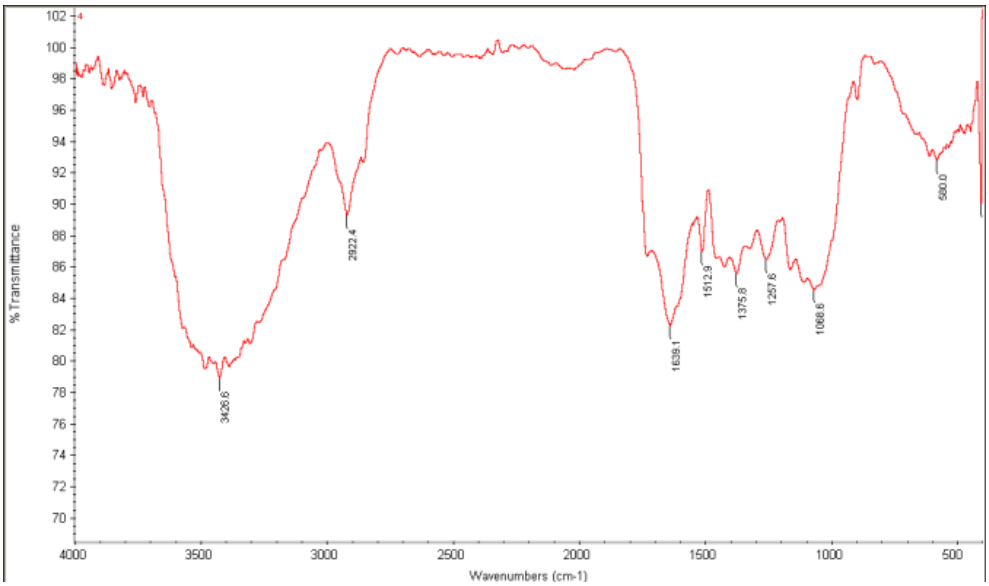

Fig 4. FTIR spectroscopy of leaf samples of sugarcane variety 16G032

4. CONCLUSION

In the present study three sugarcane clones with resistance to red rot *viz*., C15632, 16G032 and 16G031 and thirteen clones viz., C15004, C15006, C15011, C15021, C15095, C15157, C15559, C15603, 16G006, 16G087, 16G046, 15G060 and 15G028 with moderate resistance was identified and may be utilised for further studies to release for commercial cultivation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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