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Taxonomic discrimination of *Solanum nigrum* and *S. giganteum* by Fourier transform infrared spectroscopy Data

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ABSTRACT:

Fourier transform infrared spectroscopy (FTIR) provides biochemical profiles containing overlapping signals from a majority of the compounds that are present when whole cells are analyzed. Leaf samples of higher plant species and varieties were subjected to FTIR to determine whether plants can be discriminated phylogenetically on the basis of biochemical profiles. The results showed that the infrared spectra of *Solanum were* fingerprint-like patterns which were highly typical for different taxa. The principal component analysis of Fourier Transform Infrared (FTIR) data confirmed most of morphological classifications of the species proposed in previous works. The protein absorption bands located between 1800-1500 and the bands between 1500-1000 cm^{-1} (finger print region) showed variation between the two species *S. nigrum* and *S. giganteum*. Infrared spectra of leaves are of taxonomic value in genus *Solanum,* and this technique can be widely used for identification and classification of other taxa when standard spectra are available.

Keywords:
Solanum, analysis, infrared spectra, taxonomic significance.

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INTRODUCTION

The genus *Solanum* L. consists of over 2000 species distributed worldwide (Knapp, 1991) is the largest in Solanaceae and is one of the largest among flowering plants (Olmstead and Palmer, 1997). The species are a common source of vegetables (Omidiji, 1982), medicinal herbs (Caicedo and Schaal, 2004) and contain unique alkaloids and other biochemical constituents used for the treatment of diverse ailments (diabetes, cholera, bronchitis, high blood pressure) and as laxatives (Lester and Seck, 2004). In spite of the economic and medicinal value of *Solanum* species, no serious attention has been paid to diversity, characterization and taxonomical identification at the biosystematic level. This is a prerequisite to the exploitation of the vast genetic variability available for the improvement of the quality and quantity of their drug contents. Although the species discovered in this genus have been sorted out, classified and revised many times during generic revision, there is much disagreement concerning combination of species depending on the taxonomic authority (Gracelin *et al*., 2011). Hence, it is necessary to further investigate the classification of the species using other technologies.

Chemotaxonomy has strongly influenced the entire field of biology, which is also useful for plant systematics. Fourier Transform Infrared (FTIR) Spectroscopy is a rapid, noninvasive, high-resolution analytical tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint". This technology allows detecting the whole range of infrared spectrum in measurements of biological specimens (Griffiths and de Haseth, 1986). Thus, these "fingerprints" are made up of the vibrational features of all the cell components, i.e., DNA, RNA, proteins, and membrane and cell-wall components. The biochemical profiles of FTIR from whole cell samples are extremely high density data sets and, consequently, FTIR data must be analyzed by means of multivariate analysis when multiple samples are compared. FTIR has been shown to be a valuable tool for differentiating, classifying and discriminating closely related microbial strains (Lamprell *et al*., 2006; Rebuffo *et al*., 2006). In plant classification, Kim *et al* ., (2004) have proposed this approach is robust in chemotaxonomic classification of flowering plants, and we previously have used this method to identify the species in *Hypericum* L. and *Triadenum* Raf. (Lu *et al*., 2004). All these previous studies showed that this approach is a valid representation of phylogenetic relationships between plant taxa even closely related.

In this report, we conducted a comprehensive FTIR analysis of carbohydrates, proteins, lipids, and cell wall pectin from *Solanum nigrum* and *S. giganteum* leaves. De-convolution and curve-fitting analysis of IR spectrum could acquire accurate data, thus helping for quantitatively analyzing some functional groups. The presence of secondary metabolites and the value of FTIR method in this field were also considerate in this study.

MATERIALS AND METHODS

Plant materials

Fresh specimens of two species of *Solanum* such as *S.nigrum* and *S.giganteum* were collected from Kerala, India, and were identified by comparison with the voucher specimen from Kerala Forest Research institute (KFRI, Kerala). One voucher was deposited at the herbarium of the Department of Botany, University College, Kerala.

IR spectroscopy

The leaves (approximately 3-4cm) were taken from different plants and were pooled as one sample. Then the samples were immediately dried in an oven for 2days at 60°C. Tablets for FTIR spectroscopy were prepared in an agate mortars, by mixing leaves powder (2 mg) with KBr (1:100 p/p). The absorbance spectra

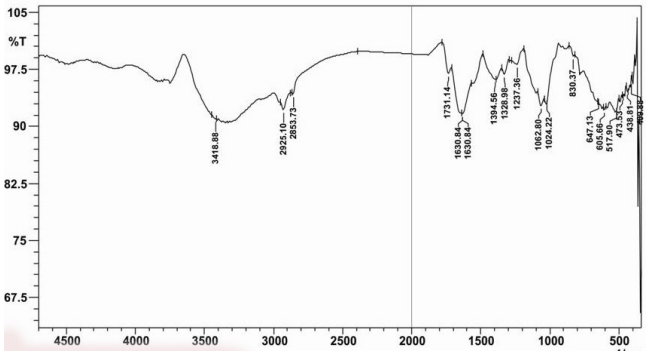

Figure 1. IR spectrum of *Solanum nigrum*.

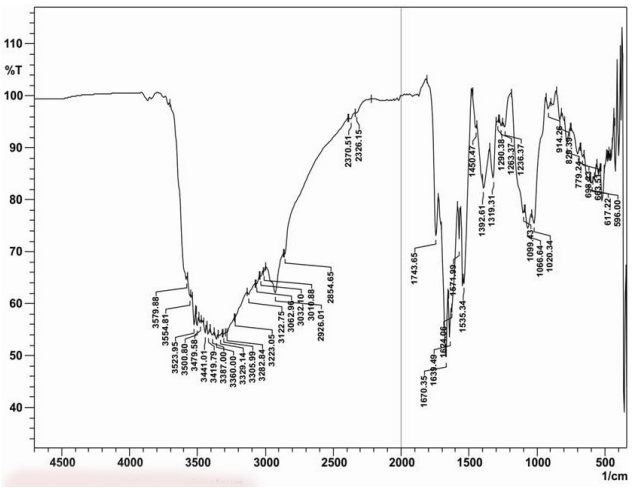

Figure 2. IR spectrum of *S. giganteum*.

were measured between 300 and 4500 cm^{-1}. At least three leaves were collected and at least three spectra were obtained from each sample.

A FTIR spectrometer (FTIR Shimadzu Prestige 21) was used to collect spectra. Spectra were obtained in 32 scans co-added, 4000 resolution, and 2.0 gains. The parameters for the Fourier self-deconvolution were a smoothing factor of 15.0 and a width factor of 30.0 cm^{-1}. De-convolved and second-derivative spectra were calculated for Fourier self-deconvolution and the bands were selected and normalized to unity with Omnic 7 software. Curve-fitting of the original spectra was performed with Origin 7 software. The band position of functional groups was monitored with Knowitall 7.8 software. The spectral region between 3000 and 2800 cm^{-1} was selected to analyze lipids. The spectral region between 1800 and 1500 cm^{-1} was selected to analyze proteins. The spectral region between 1200 and 1000 cm^{-1} was selected to analyze carbohydrates.

RESULTS AND DISCUSSION

FTIR spectra of *Solanum* species

FTIR detected all compounds, including polymers and low-molecular weight compounds in whole cell samples, subsequently providing biochemical profiles of extremely high-density data sets. Representative baseline-corrected and normalized FTIR spectra for *Solanum nigrum* and *S. giganteum* are shown in Fig. 1 and 2. Absorption bands in the range of 4000–1500 cm^{-1} are due to functional groups (e.g., –OH, C=O, N–H, CH_3, etc.), while the region 1500–675 cm^{-1} is referred to as the fingerprint region, which is highly specific for each taxon (Pan *et al*., 2000). Similarly, in mid-IR region (2000-1000cm^{-1}) appeared large numbers of sharp peaks, indicating that the leaves have a rich chemical composition, such as carbohydrates, proteins and lipids. However, this region yielded broad and overlapped bands.

Knowitall software was used to find the functional groups for preliminarily analyzing IR spectra collected. The bands around 3370 cm^{-1} represent O-H and N-H stretching vibrations that are mainly generated by proteins and carbohydrates (Wolkers *et al*., 1998). The bands between 3000 and 2800 cm^{-1} represent C-H stretching vibrations that are mainly generated by lipids (Wolkers and Hoekstra, 1995). The proteins absorption bands mainly located between 1800 and 1500 cm^{-1} contained amide-I and amide-II bands (Stehfest *et al*., 2005), but overlapped with other absorption bands within this region. Amide III, the function group of nucleic acid and carbohydrates contributed to these absorption bands in the leaves. Amide-I and amide-II bands are particularly useful for determining the protein IR absorption changes. Amide-I region (1700-1600 cm^{-1}) mainly represent C=O stretching vibrations of polypeptide, which can detect

Table 1. IR bands obtained in *S. nigrum* and *S.giganteum*

Solanum nigrum	*Solanum giganteum*
409.88	596
438.81	617.22
473.53	663.51
517.9	698.23
605.66	779.24
647.13	829.39
830.37	914.26
1024.22	1020.34
1062.8	1066.64
	1099.43
1237.36	1236.37
	1263.37
	1290.38
1328.98	1319.31
1394.56	1392.61
	1450.47
	1535.34
	1571.99
1630.84	1624.06
1630.84	1639.49
	1670.35
1731.14	1743.65
	2326.15
	2370.51
2853.73	2854.65
2925.1	2926.01
	3010.88
	3032.1
	3062.96
	3122.75
	3223.05
	3282.84
	3305.99
	3329.14
	3360
	3387
3418.88	3419.79
	3441.01
	3479.58
	3500.8
	3523.95
	3554.81
	3579.88

changes of the overall protein conformation and content (Surewicz *et al*., 1993). *S.nigrum* displayed a band at 1630.84. Meanwhile, *S.giganteum* showed five bands viz. 1535.34, 1571.99, 1624.06, 1639.49 and 1670.35 (Table 1). The protein banding pattern show diversity between the two species and this may be used to demark the plants at species level. Further analysis by de-convolution and curve fitting process in amide-I region between 1700 and 1600 cm^{-1} can give additional information about the protein structure: the band around 1685 cm^{-1} assigned to the turn structure, the band around 1656 cm^{-1} are assigned to the α-helix structure, and the band around 1621 cm^{-1} are assigned to the β-sheet structure.

The bands around 2850 cm^{-1} and 2921 cm^{-1} represents C-H asymmetric or symmetric stretching vibration, which belongs to the $-CH_2$ group of lipids. The results show the total band areas (3000-2800 cm^{-1}) were similar. This implies that lipid profiles in the species are similar. The IR spectra between 1200 and 1000 cm^{-1} mainly occur from carbohydrates. The band size at 1024.22 in *S.nigrum* more or less matches with that of *S.giganteum* (1020.34). The band around 1743 cm^{-1} represents -COOR stretching vibration (Fig 1.), which belongs to characteristic group of cell wall pectin. *S.giganteum* possess the characteristic band width at 1743.65 while, *S.nigrum* showed only a lower band width of 1731.14.

The peak 3200- 3300 may represent NH group of *Solanum* alkaloids. Similarly the peak at 1635 forms C=N group containing alkaloids. *S.giganteum* showed peaks at 3223.05 and 3282.84 but the peaks in this range were absent in *S.nigrum*. Both the species possess peaks at 1635 region. The bands at 617.22, 779.24, 914.26, 1099.43, 1263.37, 1290.38 and 1450.47 are unique and can be used as IR finger prints to identify *S.giganteum* from *S.nigrum*. Parallely, bands from 1328-1394 are shared between the species (Fig. 1 and 2).

FTIR spectroscopy allows detecting the whole

range of infrared spectrum simultaneously providing speed and accuracy in measurements of biological specimens (Griffiths and de Haseth, 1986). With this technique, Sheng *et al*., (2006) reported the effect of MG132 on the change of FTIR spectra of cell wall during pollen germination and pollen tube growth, and Wu *et al*., (2003) studied the chemical characterization of casparian strip in needles of Pinus bungeana. The application of a combination with numerical methodologies, FTIR is recommended and has many advantages. This technique has been successfully exploited for classifying normal and aged soybean seeds (Kusama *et al*., 1997) and distinguishing cell wall mutants from wild-type Arabidopsis (Chen *et al.*, 1998; Mouille *et al*., 2003). These studies, including determination ofthe fruit content in processed foods (Wilson *et al*., 1993) and discrimination of the genuineness of Chinese medicines (Hong *et al*., 2006), have also been conducted. In plant taxonomic classification related studies, Sene *et al*., (1994) showed differences in the plant cell walls of five angiosperms. Further, Kim *et al*., (2004) proposed that FTIR was an excellent method for determining phylogenetic relationships between flowering plants, and Lu *et al*., (2004) used this method to identify the species in Hypericum and Triadenum.

The species differ in many morphological characteristics. *S.nigrum* plants are unarmed, small erect herbs with white flowers while, tall tree like armed plants and purple flowers in *S.giganteum*. In the past the taxonomic status of *Solanum nigrum* remained highly controversial (Jennifer and James, 1997). Clarke, (1885) did not separated *Solanum nigrum, S. americanum* and *S. villosum* from each other and considered all of them along *S. nigrum*. Rechinger, (1958) findings were contradicted to it. According to him a plant sample with white flowers and black berry must be identified as *S. nigrum* Whereas, Edward (1990) mentioned *S. nigrum* with orange colour fruit.

S. nigrum and *S.giganteum* have fused petals strongly adnated to androecium, anthers open by apical pores which are the characteristic of genus. Previous systems are based on the macro-morphological taxonomy, while the relationships of these species in our study are investigated on IR spectral characteristics. Hence, the difference may have some relationship with what the characteristics are studied. However, we still cannot exclude the possibility that the population size in the species may lead to the discrepancy, since chemical components may be varied a little in different population sizes.

In conclusion, the infrared spectra of *Solanum* are fingerprint-like patterns which are highly typical for different taxa. The FTIR data shows relationship between species that are in agreement with most of the previous proposed morphological classifications. Differences in cell compositions of the species by infrared spectroscopy thereby can provide the basis for chemotaxonomy of species. Infrared spectra of leaves appear to have taxonomic value and be more useful for discriminating closely related species or varieties in the genus, and this technique can be widely used for identification and classification of other taxa when standard spectra are available. In our studies, the sections and the interspecific relationships we concluded are based on the chemical bonds of total mixture of leaf cells, reflecting the interspecific differences on chemical components. Therefore, additional evidence, such as DNA data, is still needed for interpreting the classification of Solanum.

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