Raman spectroscopic analysis of paddy rice infected by three pests and diseases common in Northeast Asia

Xin Yue¹, Yong Tan¹, 5, Wenzhong Fan², Shaozhong Song³, Hongyu Ji⁴ and Bingbing Li⁴

¹School of Science, Changchun University of Science and Technology, Changchun 130022, Jilin, P.R. China
²Jilin Agricultural Science and Technology University, Jilin 132101, Jilin, P.R. China
³School of Information Engineering, Jilin Engineering Normal University, Changchun 130052, Jilin, P.R. China
⁴Jilin Business and Technology College, Changchun 130507, Jilin, P.R. China
⁵Email: laser95111@126.com

Abstract. Pests and diseases seriously affect the yield and economic benefits of growing rice, and the key to inhibiting rice's pathogenesis is to find early identification of rice infection. The characteristic Raman spectrum of healthy leaves, and leaves infected with rice blast, paddy rice bakanae and infected by Chilo suppressalis (Walker) were tested by TriVista555CRS laser Raman spectrometer (900cm⁻¹-1700cm⁻¹). At the same integration time, compared with healthy plants, the Raman peak of infected plants not only changed significantly, but also the signal intensity decreased. The results show that there are clear Raman peaks at the three characteristic wavenumbers of 1002.87cm⁻¹, 1156.5cm⁻¹ and 1522.36cm⁻¹. Especially in the leaves of rice blast, it was found that the degree of fungal infection affects the peak width at half height of the characteristic peak. The research shows that Raman spectroscopy provides an effective method for the early detection of rice pests and diseases which may have economic benefits.

1. Introduction
Paddy rice is important food for people living in Northeast Asia for humans. However, the lower agricultural productivity rate caused by paddy rice blast, paddy rice bakanae and Chilo suppressalis (Walker), which produce a serious economic loss [1-3]. Compared with the traditional detection methods, Raman scattering spectroscopy is widely applied to detect the disease of rice as it has the advantage that it is nondestructive [4-6].

In previous publication, Raman spectroscopy can well be used to study the spatial distribution of globulin within the rice kernel. [7]. Transmission Raman spectroscopy is also a highly useful analytical method, which can improve the accuracy of analysis due to the presence of more selective and less moisture-insensitive spectral features compared to those provided by NIR spectroscopy. Meanwhile, Transmission Raman spectroscopy has been effectively utilized for the discrimination of rice samples according to geographical origin [8]. With the help of chemometric techniques, Raman spectral data also can be used to detect rice paraffin adulteration rapidly [9]. Raman spectrosopies reveal many changes about the interaction between protein and starch, as well as the secondary and tertiary structural transformation of protein caused by rice aging. These changes can
be attributed to the difference in the characteristics of fresh and aged rice [10]. The spectral pretreatment of transgenic rice and non-transgenic rice can be classified more accurately by the PLSDA model based on Vis-NIR and Raman spectroscopy [11]. For Raman analysis, it can be observed from the study that detection time has a significant impact on Raman characteristics, and proper testing time tends to produce good spectral characteristics [12]. Moreover, surface-enhanced Raman spectroscopy (SERS) can be used to detect diphenyl phosphorus residues in rice. When the concentration of diphenyl sulfurate in the rice extract is greater or equal to 0.1 mg/L, the characteristic signal can still be detected [13]. We use Raman spectroscopy to analyze the relationship between paddy rice pests and vibrational spectroscopy. Furthermore, the collected Raman spectroscopy information is analyzed by means of feature selection and extraction. This work has provided an effective method for early detection of paddy rice pests and diseases.

2. Theoretical analysis

Actually, the Raman scattering process shows the loss of the incident light \( \nu_o \) and the gain of the scattered light \( \nu_s \) by the paddy rice leaves. When the gain is higher than the loss, and the stimulated Raman scattering occurs at this time [14].

Stimulated Raman scattering is a third-order optical effect associated with the two-photon transition process. The generation of anti-Stokes scattering is not the result of a downward transition from a high-energy state as in the normal Raman effect, it is considered to be the result of consuming two incident \( \nu_o \) photons. Therefore, according to the energy conservation law. The requirement of the law of conservation, by \( 2\nu_o = (\nu_s + \nu_m) + (\nu_o - \nu_m) \), it is shows that the anti-Stokes scattering process also produces a stokes photon.

Stimulated Raman scattering has significant characteristics of stimulated Raman. For example, the excitation has a distinct threshold, and the scattered beam has the characteristics of coherence, orientation, high monochromaticity and high intensity of the stimulated radiation. Thus, the experimental observation of stimulated Raman scattering is directional. During stimulated Raman scattering, a significant portion of the excitation light power is converted to \((\nu_o \pm m\nu_m)\) radiation (there into \( n = 1, 2, 3, \cdots \)). Most of the incident radiations whose wavenumber is \( \nu_o \), they are converted to the first Stokes wavenumber \( \nu_o - \nu_m \) and resulting in a significant population of the first vibrational level corresponding to the \( \nu_m \).

When describing light scattering by microscopic theory. If described by quantum field theory, the entire scattering system is composed of quantized particles. That is the incident and scattered beams are the quantum of the optical wave field (ie photons). The scattering targets are quantized particles or quasi-particle composition. The scattering process is the process of generating and annihilating incident photons, target particles (paddy rice plant particles) and scattered photons under the interaction of photons and target particles.

In order to describe the mechanism of molecular light scattering in rice leaf plants, which is considered that the vibrational light scattering of molecules are caused by the vibration of the atoms in the molecule near the equilibrium position, and the induced dipole moment \( P_k \) of the vibration mode \( Q_k \) generated by the external electric field \( \vec{E} \).

\[
P_k = \alpha_k \cdot E = \alpha_0 \cdot E_0 \cos \omega_o t + \frac{1}{2} Q_{k0} \alpha_k' \cdot E_0 \cos \left[ (\omega_o - \omega_k) t + \varphi_k \right]
+ \frac{1}{2} Q_{k0} \alpha_k' \cdot E_0 \cos \left[ (\omega_o + \omega_k) t + \varphi_k \right]
\]
Where $E_0$ is the amplitude vector of $E$, the frequency is the light wave of $\omega_0$, $Q_{\omega_0}$ is the amplitude of the vibration, $\omega_k$ and $\varphi_k$ are the frequency of the vibration and the initial phase, respectively, $\alpha_0$ and $\alpha_k$ are the corresponding polarizabilities. The radiation generated by the induced electric dipole moment is the scattered light of the molecular vibration.

If the induced electric dipole moment $P^G$ of the molecular vibration is used, the transition matrix element of the atomic electric dipole moment $P$ of $P_{nk} = \langle \varphi_n (r) | P | \varphi_k (r) \rangle$ can be replaced.

$$P^G_{nk} = \langle \varphi_n (r) | P^G | \varphi_k (r) \rangle = \langle \varphi_n (r) | \alpha \cdot E | \varphi_k (r) \rangle = \langle \varphi_n (r) | \alpha | \varphi_k (r) \rangle \cdot E$$

(2)

Introduce the $[\alpha]_{nk}$ to express the polarized transition matrix element in the above formula.

$$[\alpha]_{nk} = \langle \varphi_n (r) | \alpha | \varphi_k (r) \rangle$$

(3)

From the Equation (2) of the transition matrix element $P^G_{nk}$ of the electric dipole moment and the expression of the polarization ratio transition matrix element $[\alpha]_{nk}$, therefore we can conclude that, if the polarizability $\alpha$ is the constant $C$, they can be transferred to the front integral number. Due to the orthogonality of the wave function $\varphi_n (r)$ and $\varphi_k (r)$, there is

$$P_{nk} [\alpha]_{nk} = \begin{cases} C, & n = k \\ 0, & n \neq k \end{cases}$$

(4)

$n = k$ or $n \neq k$ respective indicate that the transition occurs between the energy levels of the same and different energy levels. If light scattering occurs, it is bound to correspond to the Rayleigh scattering with constant incident light energy and the Raman scattering of energy changes. Furthermore, the changes of $r$ for electrons and polarizability $\alpha$ for molecules are the important condition to produce Raman scattering of atoms and molecules. The change is the electrons in the atom vibrate near the equilibrium position and the atoms in the molecule vibrates near the equilibrium position. Thus, quantum mechanics theory explains the Raman scattering mechanism of paddy rice leaves at the level of microscopic motion.

3. Experimental

The samples of fresh paddy rice plants were obtained from the experimental field of Jilin Agricultural Science and Technology University, Jilin Province. Samples of paddy rice leaves collected included three samples from healthy paddy plants, and three samples of infected leaves for each of the three diseased plants. A total of twelve samples were collected.

The experimental instrument used was a TriVista CRS555 micro-confocal Raman spectrometer. (the resolution is 0.6cm⁻¹; the grating size is 68×84mm; the repeatability is ±1 pixel.) This experiment uses the 550nm LD laser. The shift and intensity in spectroscopy were affected by the experimental environment [15]. Spectral shift induced by different Raman spectroscopy, different environment or different measured time can decrease the accuracy of the results [16-17]. Therefore, the experiment was carried out in a superclean room with a constant temperature, and the sample was in focus [18]. Experiments can be carried out when the liquid nitrogen cooling system temperature was around −120°C degrees. The plane light path diagram is shown in Figure 1. The principle of the optical path was the light beam from the ① 532 nm laser through the focusing action of the ② Diaphragm, and the reflection lens entering the ③ Objective lens were reflected onto the rice leaf. At this moment, energy exchange between the photons and the leaf molecules occurs, with the direction of photons changes. Moreover, a part of the energy in the photon was transmitted to part of the molecules in the blade or a part of the energy was transmitted to the molecules in the blade by the vibration and rotation of the molecules, so that the frequency of the photons changes. The photon information scattered from the blade at this time includes the desired Raman scattering. The photons scattered enter the grating.
system through the 532 nm filter in the ③ Objective lens. The Raman scattered photons were first reflected by the ④ Reflector to the ⑤ collimating lens, and the collimated optical paths were reflected by the ⑥ Grating to the ⑦ focusing lenses. The rear optical path enters the ⑨ CCD photodetector through the ⑧ Reflector. Finally, the spectral data was acquired by connecting the Raman spectrometer software in the ⑩ computer. Raman spectroscopy was performed on the samples in the range of 900 cm⁻¹ to 1700 cm⁻¹ to obtain the original data.

**Figure 1.** TriVista CRS555 Raman spectrometer plane light path diagram.

4. Results and discussion

4.1. Typical peak confirmation

The Raman spectroscopy of healthy paddy rice leaves and noise were collected by Raman spectroscopy under the medium laser intensity (120 mW), visible light band 532 nm and non-polarization conditions. The wave number ranged from 900 cm⁻¹ to 1700 cm⁻¹ was collected. Raman spectrum of healthy paddy rice leaves and Noise are shown in Figure 2. By Raman shift and attribution confirmation, 1002.77 cm⁻¹ is identified as the in-plane rocking vibration of methyl -CH₃ [19] in the rice leaf spectrum, 1156.5 cm⁻¹ is the telescopic vibration of C-C [19], 1522.36 cm⁻¹ is the vibration of C=C [19]. Because the double bond vibration of C=C in rice leaves was an amorphous solid state vibration in water filling, and the plant leaves form a dense and compact type of amorphous solid state vibration during growth [20]. To some extent, the chemical bond force constant of the C=C can be enhanced, and the Raman spectral vibration characteristic frequency can shift to a higher frequency. The main frequency shifts and assignments of the normal, healthy and rice leaves are shown in Table 1.
Figure 2. Raman spectrum of healthy paddy rice leaves and noise.

Table 1. Main frequency shift and attribution of paddy rice leaves.

| Raman shift (cm⁻¹) | Assignment                      |
|--------------------|---------------------------------|
| 1002.87            | -CH₃ plane vibration swing      |
| 1156.5             | C-C stretching vibration        |
| 1522.36            | C=C stretching vibration        |

4.2. Comparative analysis of Raman characteristic frequency of internal groups

The Raman spectrum of healthy rice plants and plants infected by pests and diseases were obtained by Raman spectroscopy using a medium laser intensity of 532 nm without polarization. Mainly due to the green powerful reflection peak region of chlorophyll near the wavelength of 550nm [21], chlorophyll is dominant in magnitude compared to other pigments (the carotenoids and phycobilins). Therefore, samples used diseased plants include paddy rice blast, paddy rice bakanae and paddy rice infested by Chilo suppressalis (Walker).

4.2.1. Paddy rice blast. In the comparison of different paddy rice blast disease rice leaves and healthy paddy rice leaves, the disease white spot is used as reference. Discussion about the characteristic Raman spectroscopy of four regions on paddy rice blast leaves, as follows: between the edge and center of the white spot (BECWS), white spot center (WSC), white spot edge (WSE) and beyond the white spot (BWS). The specific positions are shown in Figure 3.

Figure 3. Specific position segmentation map of paddy rice leaves.
The fluorescence-treated Raman spectrum are shown in Figure 4. We select the spectrum of the first frame for analysis and comparison from three samples of healthy leaves and rice blast affected leaves. The Raman spectra of the white spot edge of rice blast were compared with healthy leaves. In terms of intensity, it is apparent that the spectral intensity of the leaves of the rice blast disease is significantly lower than healthy leaves. The specific quantitative information is shown in Table 2. Particularly, the relative light intensity is from high to low as the diseased part from minor to severely, and both are negatively correlated. The intensity of the Raman characteristic peak between the BECWS and the WSE is relatively low. However, the intensity of the Raman characteristic peak is relatively increased at the BWS, but still lower than the characteristic peak intensity of healthy leaves. In the middle peak and the right peak of Figure 4, a difference between the healthy leaves and the infected leaves is apparent, but the difference between the left peaks is weak. The phenomenon is due to the large change in the absorption cross section of the vibration oscillation of the methyl (-CH$_3$) plane [22]. With the same intensity of laser irradiation, between more photons and the methyl group (-CH$_3$) do not happen inelastic collision. The chemical bond force constant of the plane vibration sway changes.

![Figure 4. Comparison of Raman spectra between different positions of leaves of rice blast and healthy leaves. (BECWS, between the edge and center of the white spot; BWS, beyond the white spot; WSE, white spot edge; WSC, white spot center; HL, healthy leaf.)](image)

In the Raman shifts, there are seven wavenumbers and three wavenumbers offsets between the WSC and the BEWSC at the left peak, as also in the comparison of the middle peak and right peak, the rice blast a certain degree of redshifts occurs in three different locations of infected leaves (redshifts represent shifts from high frequencies to low frequencies). At the right peak, the C=C stretching vibration is abnormal, and two wavenumber shifts occur. The main attribution was the lack of moisture filling of the diseased leaves, furthermore, the certain molecular bond breaks in the cell wall of the leaf after fungal infection. With the lack of surrounding coupling force, the chemical bond force constant of C=C stretching vibration decreases and the formation of changes in the characteristic vibration frequency as well. Specific quantitative information is shown in Table 2.

The peak width at half height (PWHH) of the three characteristic peaks is shown in Figure 5. It was found that the widths of the rice blast widened to varying degrees in the comparison of the left peaks. This can be mainly attributed to the significant change in the absorption cross section of the in-plane rocking vibration of -CH$_3$; at the intermediate peak and the study of the relation among the
BWS, the BECWS and the HLWSE, we subsequently found out PWHH decreased, which was mainly attributable to C-C expansion and contraction; However, in the right peak, because the C=C stretching vibration becomes inactive, so, the PWHHs of the WSE and BECWS become decreased.

**Table 2.** Characteristic peak intensity and Raman shifts table for different locations of healthy leaves and rice blast affected leaves.

| Item        | HL               | WSE              | BWS              | BECWS             |
|-------------|------------------|------------------|------------------|-------------------|
| Left peak   | Intensity (a.u.) | 11945 ± 126      | 59340 ± 126      | 7797 ± 126        | 5904 ± 126        |
| Raman Shift (cm⁻¹) | 1002.87 ± 0.6  | 1009.63 ± 0.6   | 1002.45 ± 0.6   | 1006.53 ± 0.6    |
| Middle peak | Intensity (a.u.) | 29934 ± 126      | 11760 ± 126      | 19934 ± 126       | 11868 ± 126       |
| Raman Shift (cm⁻¹) | 1156.5 ± 0.6   | 1154.4 ± 0.6    | 1154.4 ± 0.6    | 1154.4 ± 0.6     |
| Right peak  | Intensity (a.u.) | 27839 ± 126      | 8705 ± 126       | 19555 ± 126       | 11018 ± 126       |
| Raman Shift (cm⁻¹) | 1522.36 ± 0.6  | 1522.18 ± 0.6   | 1519.16 ± 0.6   | 1520.76 ± 0.6    |

**Figure 5.** Comparison of the PWHH between leaves from health and and rice blast affected plants.

4.2.2. *Paddy rice bakanae.* In the comparative Raman spectroscopy of paddy rice bakanae leaves and healthy leaves, we selected the typical rice leaves line diagram in the samples. After denoising or other treatment, we found that the characteristic peak intensity was lower than healthy leaves. As shown in Figure 6, the spectral peak at the left shifts from 1002.87 to 1010.52 cm⁻¹, which was mainly attributed to the infection of the paddy rice seedlings by Fusarium fujikuroi [23]. And the activity of bacterium becomes increased, which results in a vibrational oscillation of the methyl (-CH₃) plane, in addition there was also a change in the characteristic frequency. Finally, the full width at half maximum of the rice bakanae leaves become wide and the peak is broadened. At the 1156.5 cm⁻¹ and 1522.36 cm⁻¹ also exist the same trend.
4.2.3. Paddy rice infested by Chilo suppressalis (Walker). A comparison of Raman spectrums collected from health rice leaves and of rice leaves infested by Chilo suppressalis (Walker) is shown in Figure 7, the relative intensity is one third to half times that of healthy leaves which is due to the strong invasive ability of the aphid in early invasion. It can turn rice plants into hollow seedlings. The Raman spectral intensity is weaker than that of healthy leaves. It was found that the left peak has Raman shift deviation compared to the healthy leaves, which was mainly attributed to the vibration of the methyl (-CH$_3$) plane and infected by the stem borer. Finally, the cell activity has changed [24]. In the middle, the C-C stretching vibration was abnormal, especially the intensity of the leaves of paddy rice leaves infested by Chilo suppressalis (Walker) was very different compared to healthy leaves. In addition, the moisture content of the diseased leaves was very low. The characteristic frequency of the peaks changed and the Raman peaks become very small.

**Figure 6.** Raman spectra of Paddy rice leaves infected with bakanae disease.

**Figure 7.** Raman spectrum of rice leaves infested with Chilo suppressalis (Walker).
5. Conclusions
This work contains Raman spectral data for leaves from healthy rice plants, and rice plants affected by three common pests and diseases. Subsequently, we implemented the work of feature extraction and comparative analysis, it was found that leaves from diseased rice plants have larger Raman shifts compared with the characteristic frequency points of leaves from healthy rice plants. The phenomenon is mainly due to the changes of the chemical and physical properties to the infected paddy rice, which affects the characteristics of Raman peak. This was especially apparent in the rice blast when different locations on the same leaf were compared. We conclude that there is a negative correlation between the severity of the diseased location and the relative Raman peak intensity. The comparison of the left peak is found in paddy rice plants infected by rice blast, and the peak width at half height increased. In the Raman spectroscopy of the paddy rice bakanae, it is found that a typical Raman shift occurs in the left peak. In the study of the rice leaves infested by Chilo suppressalis (Walker), Raman peak is not obvious and Raman line type is broadened. In this work, by the perspective of atomic bonding, it was shown that Raman spectroscopy can be used for early discrimination of the rice plants infected by three common pests and diseases.

References
[1] Mousanejad S, et al. 2010 Assessment of Yield Loss Due to Rice Blast Disease in Iran Journal of Agricultural Science and Technology 12(3) 357-364
[2] Goncalves A, et al. 2019 Pre and Postharvest Strategies to Minimize Mycotoxin Contamination in the Rice Food Chain Comprehensive Reviews in Food Science and Food Safety 18(2) 441-454
[3] Shiba T, et al. 2018 Spread and yield loss mechanisms of rice stripe disease in rice paddies Field C-rops Research 217 211-217
[4] Schulz H, et al. 2007 Identification and quantification of valuable plant substances by IR and Raman spectroscopy Vibrational Spectroscopy 43(1) 13-25
[5] Butler H J, et al. 2016 Using Raman spectroscopy to characterize biological materials Nature Protocols 11 664-687
[6] Littlejohn G R, et al. 2015 In vivo chemical and structural analysis of plant cuticular waxes using stimulated Raman scattering microscopy Plant Physiology 168 18-28
[7] Ellepola S W, et al. 2006 Raman spectroscopic study of rice globulin Journal of Cereal Science 43 85-93
[8] Hwang J, et al. 2012 Enhanced Raman spectroscopic discrimination of the geographical origins of rice samples via transmission spectral collection through packed grains Talanta 101 488-494
[9] Feng X, et al. 2013 Preliminary study on classification of rice and detection of paraffin in the adulterated samples by Raman spectroscopy combined with multivariate analysis Talanta 115 548-555
[10] Guo Y, et al. 2013 Infrared and Raman Spectroscopic Characterization of Structural Changes in Albumin, Globulin, Glutelin, and Prolamin during Rice Aging Journal of Agricultural and Food Chemistry 61 185-192
[11] Xu W, et al. 2014 Comparison of fourier transform Near infrared, Visible near infrared, Mid infrared and Raman spectroscopy as non-invasive tools for transgenic rice discrimination Transactions of the ASABE 57(1) 141-150
[12] Li F, et al. 2018 Rapid Screening of Cadmium in Rice and Identification of Geographical Origins by Spectral Method International Journal of Environmental Research and Public Health 15 312
[13] Weng S, et al. 2018 Fast and Quantitative Analysis of Ediphenphos Residue in Rice Using Surface-Enhanced Raman Spectroscopy Journal of Food Science 83(4) 1179-1185
[14] Hellwarth R W 1963 Theory of Stimulated Raman Physical Review 130(5) 1850
[15] Westad F, Martens H, et al. 1999 Shift and intensity modeling in spectroscopy-general concept
and applications *Chemometrics and intelligent laboratory systems* **45**(1-2) 361-370

[16] Bian H, Gao J, et al. 2018 Error analysis of the spectral shift for partial least squares models in Raman spectroscopy *Optics Express* **26**(7) 8016-8027

[17] Bian H, Zhang Y L, Gao W R, et al. 2019 Fourier based partial least squares algorithm: new insight into influence of spectral shift in “frequency domain” *Optics Express* **27**(3) 2926-2936

[18] Wolthuis R, Tjiang G C H, Puppels G J, et al. 2006 Estimating the influence of experimental parameters on the prediction error of PLS calibration models based on Raman spectra *Journal of Raman Spectroscopy* **37**(1-3) 447-466

[19] Tan F, et al. 2015 Analyzing plant characteristics of rice suffering leaf blast in cold area based on Raman spectrum *Transactions of the Chinese Society of Agricultural Engineering* **31**(4) 191-196

[20] Xu X, et al. 2018 Quantitative proteomics analysis of proteins involved in leaf senescence of rice (Oryza sativa L.) *Plant Growth Regulation* **84**(2) 341-349

[21] Atta B M, et al. 2018 Chlorophyll as a biomarker for early disease diagnosis *Laser Physics* **28**(6) 065607

[22] Goh L P W, et al. 2018 Molecular identification of blast resistance and pathogenesis-related genes in various traditional paddy varieties from different divisions of Sabah, East Malaysia *International Food Research Journal* **25**(2) 626-631

[23] Matic S, et al. 2017 The puzzle of bakanae disease through interactions between Fusarium fujikuroi and rice *Frontiers in Bioscience* **9** 333-344

[24] Xin D, et al. 2017 Morphological and phylogenetic analysis of a microsporidium (Nosema sp.) isolated from rice stem borer, Chilo suppressalis (Walker) (Lepidoptera: Pyralidae) *Parasitology Research* **116**(10) 2741-2746