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MONITORING THE CHLOROPHYLL FLUORESCENCE PARAMETERS IN RICE UNDER FLOODING AND WATERLOGGING STRESS BASED ON REMOTE SENSING

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ABSTRACT—Flood and waterlog disaster is one of the most serious catastrophes for rice in China. Timely and accurately monitoring waterlogging damage can provide quantitative damage assessment and support for after-flood field management. Chlorophyll fluorescence (CF) is directly related to the waterlogging stress. This paper aims to establish models to monitor the change of chlorophyll fluorescence parameters (FPs) at different growth stages under waterlogging stress based on hyperspectral data. Waterlogging stress was simulated in experimental environment. Back Propagation Neural Network (BPNN) model were proposed by analyzing the relationship between chlorophyll fluorescence parameters (FPs) and spectra absorption feature parameters, which were extracted from continuum removal spectra (550nm-750nm) to represent absorption features. The experimental results indicated that absorption feature parameters and BPNN can improve the estimation accuracy of FPs under flooding and waterlogging stress.

Key Words: Rice, Flood and waterlogging stress, Chlorophyll fluorescence parameters, Neural network

1. INTRODUCTION

Rice is one of the most important food crops in China. It is a swamp crop and has strong waterlogging tolerance ability. However, submerged by flooding for a long time, rice will also has lower yield. Considering the sudden and destructive characteristics of flood, it's significant to explore the effects of waterlogging on rice (Li et al., 2004).

The method of chlorophyll fluorescence (CF) has been used to detect physiological status of plants under different abiotic stress widely (Maxwell et al., 2000). Changes in chlorophyll function frequently precede in chlorophyll content, and can be observed long before leaves become chlorotic under stress (Zarco-Tejada et al., 2000). Therefore, CF is a good probe to detect the change of physiology in adversity stress, which makes non-destructive monitoring of chlorophyll fluorescence parameters (FPs) to be significance.

With the development of remote sensing, lots of researches have been focused on monitoring chlorophyll fluorescence with remote data. The primary method for FPs monitoring is to build spectral indices with single-band reflectance spectra and investigating the relationship between FPs and reflectance spectra, and many remarkable results have been achieved. For example, the relationship between non-photochemical quenching of chlorophyll fluorescence (NPQ) and Physiological Reflectance Index (PRI) [PRI= (R531-R570)/ (R531+R570)] in grapevine (*Vitis vinifera* L.) was studied, and the result showed that PRI could monitor the chlorophyll fluorescence parameters efficiently under water stress (Evain et al., 2004). In particular, fluorescence ratio indices (FRI)[FRI=R690/R600] showed a strong positive curvilinear relationship with steady-state fluorescence (Fs), which the R^2 was 0.75 (Dobrowski et al., 2005). There was a positive relationship between PRI measured at the canopy-level and light-adapted fluorescence ($\Delta F/F'm$; R^2=0.69) under salinity stress (Naumann et al., 2008). And the feasibility of monitoring the chlorophyll fluorescence parameter Fv/Fm in compact corn by hyperspectral data was also studied (Tan et al., 2011).

Using remote sensing data to monitor the change of FPs in different abiotic stress is an effective method from the present study. While monitoring the FPs for rice under waterlogging stress is also an important branch. In this paper, the FPs values were evaluated by continuum-removal method. Four absorption feature parameters were acquired by the continuum-removed treatment of canopy spectra ranging from 550nm to 750nm. Also, a Back Propagation Neural Network (BPNN) model was displayed. The results indicated that absorption feature parameters and BPNN model could precisely monitor the change of chlorophyll fluorescence parameters for rice under flooding and waterlogging stress.

2. MATERIALS AND METHODS

2.1 Experimental Design

The experiments were conducted at the water-saving experiment base, Beijing Academy of Agriculture and Forestry Sciences (39.93°N, 116.27°E), China. "Yue Fu" selected as rice varieties has a long cultivation history in the Sujiatuo rice cultivation base, Beijing. Flooding stress intensity was simulated by three waterlogging depths and three duration at 4 growth stages, i.e. tillering stage, jointing stage, heading stage, filling stage, and each stress intensity had 3 replicates. There are also 3 groups were treated as control (CK). The two waterlogging depths was half-submergence (HS), complete submergence (CS) respectively, the former refers to the middle of the rice plants from the root were submerged with water, the latter refers to all the plants were submerged. Three submerged duration was 3days, 6days, 9days respectively. The rice sample in one growth stage were submerged at the same time, and drained after 3days, 6days, 9days respectively, then canopy spectra and growth vigor data were measured with all rice plants under the normal water environment. Table 1 showed the date of collecting data. In particular, experimental rice were all managed to field cultivation way except submergence treatment.

2.2 Measurement of Hyperspectral Reflectance

All canopy hyperspectral measurements were taken using the FieldSpec Pro FR2500 field spectrometer (Analytical Spectral Device Inc., USA). This spectrometer operating in the 350~2,500 nm hyperspectral region, while the optical fiber probe with 25°field of view. The measurements were carried out from a height of 0.4 m above the canopy under clear sky conditions between 1,000 and 1,400 h (Beijing local time). Measurements of vegetation radiance were made at 10 sample sites in each plot, and the mean of the measurement was taken as the final canopy spectral reflectance value. A panel radiance measurement was conducted before and after the vegetation measurement by two scans each time.

Table 1. Date of Data Collection

Growth Stages	Date Month-Day
Tillering	07-03 07-06 07-09 07-12
Jointing	07-26 07-29 08-01 08-04
Heading	08-17 08-20 08-23 08-26
Filling	09-07 09-10 09-13 09-16

2.3 Measurements of Chlorophyll Fluorescence

Leaf chlorophyll fluorescence measurements were conducted on the leaf of each plant, which is the same parts of reflectance spectra measured, and the instrument is a pulse-amplitude-modulation fluorometer (PAM 2100, Walz, Effeltrich, Germany). Before measuring FPs, leaves were put in dark-adapted state for 20 min using light exclusion clips, then the minimum chlorophyll in the dark-adapted state (Fo) and maximum chlorophyll in the dark-adapted state (Fm) were measured, each treatment was measured 5 times and the average value was taken as the final value. Using these parameters, the maximum PSII photochemical efficiency, Fv/Fm = (Fm-Fo)/Fm were calculated.

2.4 Continuum Removal Method

Continuum removal method can sort out the useful information from the background noise, and important absorption characteristics could be enhanced. The definition of continuum is point-by-point connecting those protruding "peak" points, and the exterior angle of polyline on the "peak" points is greater than 180°. Relative reflectance of continuum (R′) is the actual spectral band value divided by the corresponding band value on continuum. After using continuum removal to normalize reflectance spectra, the continuum-removed data are equal to 1.0 on “peak” points, while the others are less than 1.0 (Wang et al., 2008). Figure 1 show the canopy spectral reflectance in rice and its continuum.

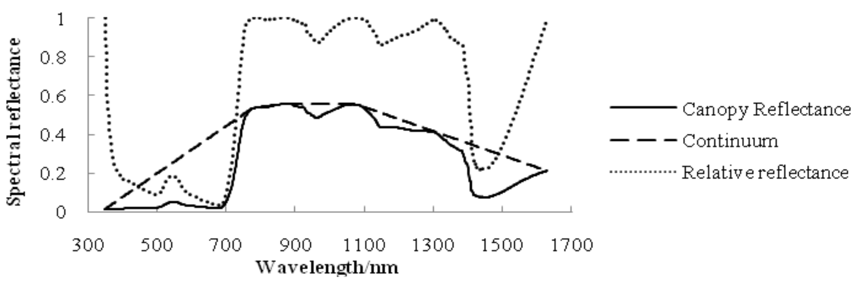


Figure 1. Canopy spectral reflectance of rice and its continuum (Jointing)

Four absorption features could be acquired by the continuum-removed treatment of canopy spectra reflectance curve: 1) The total area of absorption peak (A), which is the integration area of absorption peak; 2) The left area of absorption peak (A_1). Take the minimum reflection wavelength of absorption peak as the critical point, and the left integration area of absorption peak is A_1; 3) The right area of absorption peak (A_2), which is the remaining area of A except A_1 (Zhang et al., 2010); 4) Absorption depth (Dh), Dh=1-R′, and R′ is the relative reflectance of continuum at the max absorption position. Figure 2 show the absorption peaks and their associated areas.

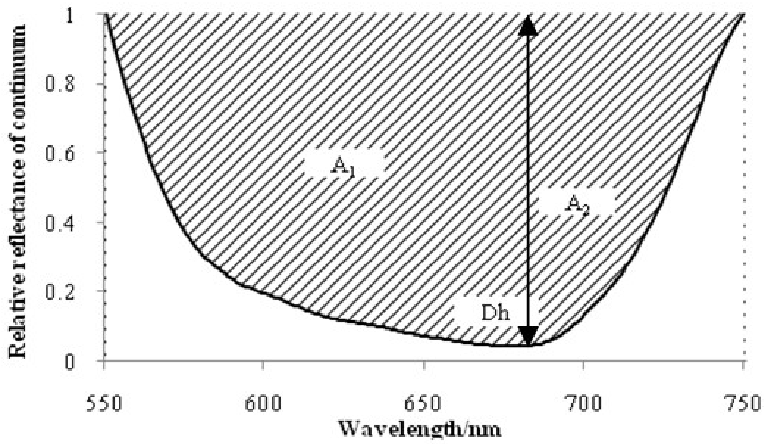


Figure 2. Absorption features of continuum-removed spectra reflectance

In order to contrast with other spectrum parameters, the Structural Independent Pigment Index (SIPI) and Physiological Reflectance Index (PRI) were selected to make correlation analysis with chlorophyll fluorescence parameters (FPs).

3 DATA ANALYSIS AND RESULTS

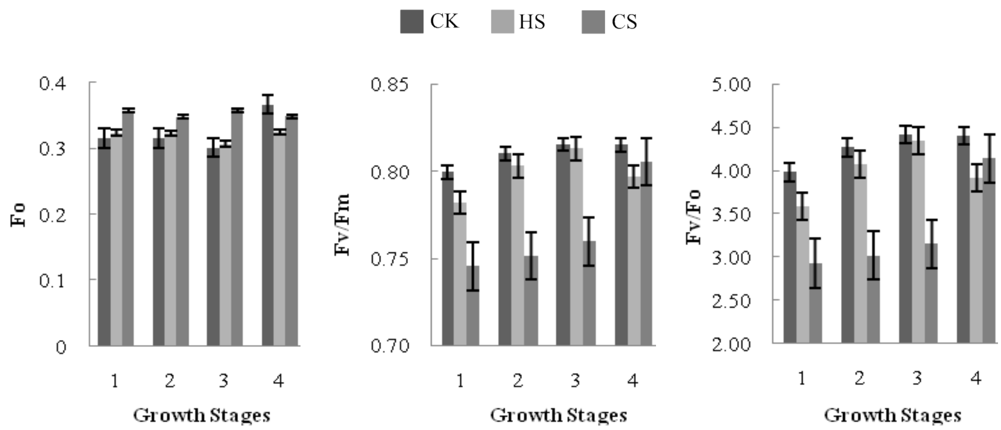
3.1 Change of FPs

There are several chlorophyll fluorescence parameters related to the stress closely: 1)Fo. Fo is the fluorescent yield while PSII reaction center open fully, which is related to the chlorophyll concentration. The value of Fo will increase while the PSII reaction center of plant leaves occurs reversible inactivation or appears unable reversed damage (Chen et al., 2006). 2) Fv/Fm. Fv/Fm is the maximum PSII photochemical efficiency. In normal conditions, regardless of species and growth environment, it changes small extremely. But the value of Fv/Fm will reduce dramatically under abiotic stress (Li et al., 2011). 3) Fv/Fo. Fv/Fo is the potential photochemical activity of PSII. Figure 3 showed the change of three FPs (Fo, Fv/Fm, Fv/Fo) at different growth stages under different water depths.

As Figure 3 showed, with the increase of water depth, the value of Fo increased at tillering stage, jointing stage and heading stage. Compared with CK, the Fo of CS increased by 13.32%, 10.25% and 18.81% at tillering, jointing, heading respectively, which showed that the increase of stress intensity had affected the physiological function of rice leaf. The Fo of CK is 0.367, which was the maximum value at filling stage, while Fo of HS and CS decreased by 11.54% , 4.99% than CK respectively. The order of Fo at filling stage was CK>CS>HS.

Fv/Fm and Fv/Fo decreased as water depth increased at tillering, jointing and heading stage. Compared with the CK, the two FPs (Fv/Fm , Fv/Fo) of HS and CS decreased by 6.73% and 26.44%, 7.28% and 29.25%, 6.87% and 28.56% at tillering, jointing and heading respectively.

This indicated that the submergence stress damaged PSII and reduced the conversion efficiency of solar energy. Fv/Fm, Fv/Fo at filling stage show the similar trends with Fo (Figure 3).



1 Tillering, 2 Jointing, 3 Heading, 4 Filling

Figure 3. Change of FPs for rice under different water depth at different growth stages (6d)

3.2 Continuum-removed Spectra Absorption Feature

Red band displayed a good spectral response in physiological change of rice under flooding and waterlogging stress. Based on continuum removal treatment, this study analyzed the variation of spectra absorption feature parameters under different stress intensity. Canopy spectra ranging from 550nm to 750nm were treated by continuum removal, and extracted spectra absorption feature parameters (Table 2).

Table 2. Continuum-removed absorption feature parameters of 550-570nm

Stages	Tillering			Jointing			Heading			Filling		
Stress	CK	HS	CS	CK	HS	CS	CK	HS	CS	CK	HS	CS
MAP/nm	677	677	676	678	678	679	678	678	678	678	678	678
Dh	0.926	0.917	0.839	0.960	0.954	0.843	0.953	0.921	0.904	0.884	0.896	0.873
A	132.60	130.06	109.42	146.81	144.24	109.29	142.24	129.02	121.10	113.68	118.89	111.32
A_1	90.34	89.46	76.76	100.40	99.69	76.97	99.18	88.74	84.99	80.59	84.12	78.89
A_2	42.26	40.60	32.66	46.41	44.54	32.32	43.06	40.27	36.10	33.10	34.76	32.43

All of the max absorption position (MAP) were about 678nm at four growth stages, and the maximum change rate was only 2nm, which had no significant relationship with stress degree as well. This showed that the max absorption position is only determined by the characteristic of absorption material, and vegetation biochemical changes will not affect the location. Dh, A, A_1,A_2 of tillering, jointing, heading stages decrease with the raise of the stress intensity, and the main reason was that more deeper the water was, more seriously the physiological processes inhibited, which would make the plant unable to conduct normal photosynthesis. Consequently, chlorophyll content decreased, red-band absorption became less narrow, and the reflectance increased.

3.3 Correlation Between FPs and Continuum-removed Parameters

Fo and absorption feature parameters were negative correlation at four growth stages, while Fv/Fm and Fv/Fo displayed a positive correlation except filling stage (Table III). The correlation coefficient between FPs (Fo, Fv/Fm, Fv/Fo) and absorption feature parameters all passed the 0.01 significant level test at tillering, jointing, heading stages. In particular, the value of correlation coefficient between Fv/Fm and A_1 was 0.747 and 0.831 at tillering, jointing stage respectively.

By analyzing the absorption feature parameters, correlation of A_1 was higher than others at tillering and jointing stages, while A_2 was highest at heading and filling stages. Correlation between the absorption feature parameters and FPs before heading was higher than heading and filling stages in general. This was largely because after heading, the effect of rice panicle weakened the role of leaves on canopy spectral reflectance, and led to the correlation between FPs and feature parameters becoming lower.

As Table 3 showed, the correlation coefficient between SIPI and Fo, Fv/Fm, Fv/Fo all passed the 0.01 significant level test at tillering, jointing and heading stages, while no significant correlation at filling stage. PRI and FPs reached significant level only at jointing stage.

In general, compared with SIPI and PRI, the correlation between continuum-removed absorption feature parameters and FPs were higher, which indicated the possibility of using these parameters to monitor FPs for rice under flooding and waterlogging stress. Especially A_1 and A_2 presented greater possibility.

Table 3. Correlation coefficient R between FPs and absorption feature parameters

Growth Stages	FPs	Dh	A	A_1	A_2	SIPI	PRI
	Fo	-0.554**	-0.634**	-0.665**	-0.588**	-0.626**	0.184
Tillering	Fv/Fm	0.711**	0.738**	0.747**	0.707**	0.720**	0.008
	Fv/Fo	0.588**	0.636**	0.657**	0.584**	0.611**	0.079
	Fo	-0.798**	-0.787**	-0.811**	-0.727**	-0.799**	-0.721**
Jointing	Fv/Fm	0.821**	0.801**	0.831**	0.732**	0.829**	0.682**
	Fv/Fo	0.753**	0.747**	0.778**	0.678**	0.769**	0.624**
	Fo	-0.539**	-0.531**	-0.493**	-0.569**	-0.486**	-0.309
Heading	Fv/Fm	0.556**	0.559**	0.516**	0.590**	0.487**	0.337
	Fv/Fo	0.576**	0.580**	0.537**	0.593**	0.503**	0.363*
	Fo	-0.389*	-0.402*	-0.371*	-0.450*	-0.391*	-0.257
Filling	Fv/Fm	-0.198	-0.236	-0.211	-0.276	-0.176	-0.224
	Fv/Fo	-0.186	-0.234	-0.209	-0.275	-0.164	-0.189

** Significance of mean difference at 0.01 probability levels;
* Significance of mean difference at 0.05 probability levels;

3.4 Monitoring Models of FPs

By the above analysis, FPs and continuum-removed absorption feature parameters had significant correlation coefficient from tillering stage to heading stage, so the absorption feature parameters Dh, A, A_1, A_2 could be used to monitor the change of chlorophyll fluorescence parameters before filling. The optimal parameter of each growth stage was selected for modeling based on linear, power and exponential regression method. The optimal absorption feature parameters were A_1, A_1, A_2 at tillering, jointing, heading stage respectively, and the optimal regression equation of FPs at each growth stage were showed in Table 4.

As Table 4 showed, the regression models were established using the spectral absorption feature parameters, and these models presented good performance at tillering, jointing and heading stage. However, because the single variable models are easy to treat the effects of other factors completely as univariate results, it tended to be instability. The relationship between chlorophyll fluorescence and spectral feature parameters cannot be explained by correlativity completely, where the correlation of best model tend to be unsatisfactory (R^2=0.70). In order to monitor FPs more accurately, by applying the 4 absorption feature parameters before filling as the inputs, and Fo, Fv/Fm and Fv/Fo as the target output, BP neural network (BPNN) monitoring model of FPs was established. It's worth noting that three FPs were calculated by BPNN respectively in this paper, so the nodes of input layer was four, while the nodes of output layer was one.

Table 4. The optimal regression equation of FPs at each growth stage

Growth Stages	Regression Equation	R^2	F	sig.
Tillering	$\mathrm{Fo} = -0.001 + 0.421A_1$	0.564	35.496	0.000
	$\mathrm{Fv/Fm} = 0.387e^{0.007A_1}$	0.573	37.567	0.000
	$\mathrm{Fv/Fo} = 0.33e^{0.025A_1}$	0.532	31.821	0.000
Jointing	$\mathrm{Fo} = -0.002A_1 + 0.51$	0.658	53.780	0.000
	$\mathrm{Fv/Fm} = 0.104A_1^{0.444}$	0.700	65.400	0.000
	$\mathrm{Fv/Fo} = 0.51e^{0.021A_1}$	0.670	56.724	0.000
Heading	$\mathrm{Fo} = 0.652\ln(A_2) - 0.088$	0.424	27.654	0.000
	$\mathrm{Fv/Fm} = 0.673A_2 + 0.003$	0.448	28.383	0.000
	$\mathrm{Fv/Fo} = 0.767A_2 + 0.079$	0.452	30.602	0.000

According to the Kolmogorov theorem, a three-layer neural network is sufficient to accomplish any mapping from n-dimensional to m-dimensional (Lu et al., 1994), so one hidden layer is enough. Therefore, network structure consists of one input layer, one hidden layer and one output layer in this paper. By adjusting the number of nodes in hidden layer, network structure could be optimized. Because the number of nodes in hidden layer must be little, basic principle for determining the number of hidden nodes is taking as far as possible compact structure with satisfying accuracy. The empirical equation 1 was used to calculate the number of nodes.

$$i = \sqrt{n+m} + a \tag{1}$$

where *i, n, m* meaned the number of nodes in hidden, input, output layer respectively. *a* was a constant ranging from 1 to 10.

Thus, the number of nodes in hidden layer could be ranging from 4 to 13.

Number of sample amounts to 90 before filling in this study. Based on 60 as a training sample set, 30 as a validation sample set, BP neural network model was established, and the data were selected randomly. After training the network repeatedly with the nodes of hidden layer ranging from 4 to 13, the optimal model was obtained. The best monitoring model of Fv/Fm, Fv/Fo was established when the nodes of hidden layer reached 7, and R^2 was 0.873 and 0.851 respectively. For Fo, the nodes of hidden layer should reached 9 (R^2=0.819).The scatterplot of predicted value and measured value were showed in Figure 4.

From Figure 4, we could see that monitoring accuracy of FPs was significantly improved by BPNN, and R^2 of measured value and predicted value obtained by BPNN model for Fo, Fv/Fm, Fv/Fo were all greater than 0.75. This result indicated that the BPNN model had significant advantages for non-linear mapping relation, and this model could be used to monitor the chlorophyll fluorescence parameters under flooding and waterlogging stress.

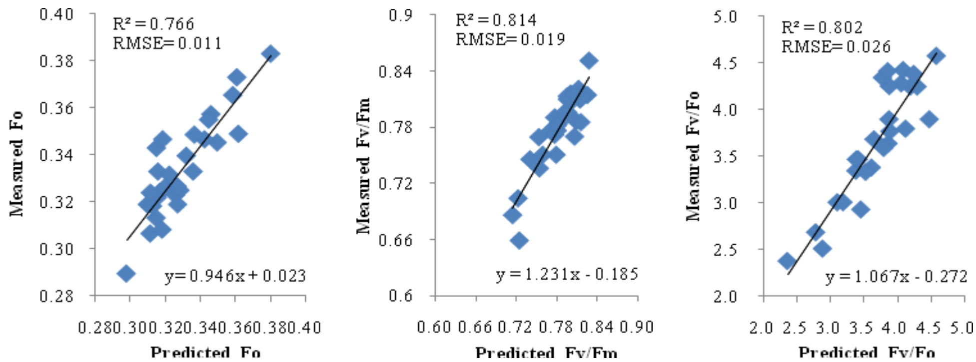


Figure 4. Scatterplot of measured and predicted FPs values

4 DISCUSSION AND CONCLUSION

Based on the flood simulation experiment, the change of chlorophyll fluorescence parameters at different growth stages under flooding and waterlogging stress was studied in this paper. The continuum removal treatment was applied to red band (550-750nm), and the maximum absorption position and absorption feature parameters were extracted in this “red band” region. Then the monitoring model of chlorophyll fluorescence parameters was established by BPNN.

The chlorophyll fluorescence parameters (Fo, Fv/Fm, Fv/Fo) presented effective response to the waterlogging stress intensity. The increasing water depth influenced the physiological processes, and made the PSII reaction center occur unable reversed damage, which led to the increasing of Fo and decrease in Fv/Fm, Fv/Fo with the water increasing depth. Four absorption feature parameters extracted by continuum removal treatment showed a consistent regularity with the change of waterlogging stress intensity. Before filling stage, these parameters displayed significant correlation with chlorophyll fluorescence parameters, the best correlation was obtained between A_1 and Fv/Fm at jointing stage.

Depends on the absorption feature parameters, the regression model was established to monitor the chlorophyll fluorescence parameters. The area of absorption peak contains more energy, thus the excellent anti-jamming capability was obtained by the estimation model of Fv/Fm, which was based on the left area of absorption peak A_1 (R^2=0.7). This model was simple and practical but low accuracy and poor physical theory basis.

Red-band (550-750nm) was the plant chlorophyll fluorescence emission region, chlorophyll fluorescence had certain contribution to its spectral reflectance (Zhang et al., 2009). Spectral curves of continuum removal enlarged the red band absorption valley, and different intensity of stress in rice refers to different depth of band variable. As a comprehensive index of red band absorption depth and width, absorption area had a good response to different stress intensity. So quantitative analysis of the variation characteristics of chlorophyll fluorescence parameters under different stress intensity could be made by taking advantage of 550-750 nm continuum-removed spectral absorption feature parameters.

It had been proved that three layer BP neural networks could approximate any nonlinear continuous function with arbitrary precision, which made it particularly suitable for solving problems with complex internal mechanism. And, the results showed that BPNN model had strong ability for nonlinear approach which could actually reflect the nonlinear relationship between FPs and continuum-removed absorption feature parameters. In this paper, the best models were single-hidden layer BPNN for Fo, Fv/Fm, and Fv/Fo, which greatly improved the accuracy of FPs change monitoring.

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