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Spectral Identification of Stress Types for Maize Seedlings Under Single and Combined Stresses

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ABSTRACT Plants frequently suffer from different types of stress and their combination. Timely and effective monitoring of plant stresses is necessary for the precision management of crops and environmental protection. Hyperspectral remote sensing may help monitoring demand based on spectral feature analysis. However, existing studies are still insufficient for the spectral identification of plant stress types, including the combined stress type. In this paper, drought, copper, and their combined stresses on maize seedlings were designed to analyze differences of plant parameters and spectral indices by comparing with a control group. The experimental results indicate that: 1) chlorophyll content, leaf area, and relative water content could be used as key parameters to express the inter-type stress differences, and in particular, chlorophyll content was the most important bio-parameter due to its unique characteristic to distinguish a combined stress from drought stress; 2) red-edge position, the first derivative at the red edge and shortwave infrared water stress index was found to be effective for characterizing the three plant parameters under plant stresses because they could minimize the effect of variations of stress types on the prediction of these parameters; and 3) the three spectral indices might be used to identify the three stress types of maize seedlings by a decision tree analysis. The results may be useful for the precision management of crops and for environmental protection and monitoring as well.

INDEX TERMS Hyperspectral, stress type, maize seedlings, plant parameters, spectral indices.

I. INTRODUCTION

The normal growth of plants requires a specific environment and condition. Any unfavorable conditions or substances that affect or block a plant's metabolism, growth or development are to be regarded as stress [1]. Drought and heavy metal pollution are two typical types of stress. Drought is one of the major disasters that threaten human survival and economic development, and it is on the rise in terms of its frequency, intensity and duration. As global warming continues, the long-term drying trend in most areas of the world leads to frequent drought disasters. For example, 41% of the world's land and more than two billion people are exposed by drought [2]. Additionally, there are some direct human-induced factors explaining the drought, such as the over-exploitation of groundwater and aquifer disturbance by underground mining. For example, approximately 30% of water loss occurred in areas with stable and thick aquifers after mining in the Shendong mining area in western China [3].

At the same time, with the development of the economy and the improvement of people's standard of living, the scale and pace of development of mining, smelting, and processing manufacturing production activities are at a high level. Due to poor management and monitoring of the activities, they have resulted in a widespread land contamination and river pollution with heavy metals in some areas, such as industrial areas [4], mining areas [5], [6], city suburbs [7], rural areas [8], and sewage irrigation areas [9]. For example, copper contamination is a big issue of environmental concern because of fungicide and pesticide that are rich in copper [10]. The pollution negatively impacts the growth of plants and poses a major threat to the safety of grain yield and quality. For instance, the maximum concentration of Cu reached 16491 ug/L in a river water surrounding the Dexing mining area, which is located in northeastern Jiangxi province, China [11]. The pollution resulted in a serious degeneration of the local environmental quality [12].

In general, droughts are extensive in terms of space and occur frequently, while pollution with heavy metals is easily diffused [2], [13], [14]. Therefore, the two types of stress may occur simultaneously in a region. Crops are likely to suffer from drought stress, heavy metal stress, or even both types of stress, which is called combined stress [15]. If we can determine the type of stress applied to plants, the corresponding measures, such as water and fertilizer management, could be carried out to relieve or eliminate stress to protect the normal growth of plants.

Hyperspectral remote sensing is an important technology because of its rapid, nondestructive and macro-scale detection ability [16]. Based on the spectral characteristics of plants, the remote sensing may provide an effective means for the stress monitoring of crops [17]. Successful stress monitoring by remote sensing may help answer the following three questions: (1) Whether a plant is under stress? (2) What is the type of the stress? And (3) what is the extent of the stress? At present, there are various studies on examining spectral characteristics of plants under stress and on hyperspectral remote sensing monitoring of plant growth states [18], [19]. There are various types of stress, including drought stress[20], heavy metal stress [21], salt stress [22], [23], and diseases and insect pests [24]–[26], etc. Overall, most of the research focuses the physiological changes induced by a single stress, such as drought or heavy metals. A few researchers have paid attention to the difference of spectral characteristics of plants under different stress types. For example, Yuan *et al.* [27] investigated the difference of reflectance spectrum of wheat leaves under different disease conditions at a leaf level, suggesting the potential use of hyperspectral data in discriminating yellow rust, powdery mildew and wheat aphid infestation in winter wheat. de Jong *et al.* [28] conducted a laboratory experiment to measure the spectral response of *Buxus sempervirens* to different types of environmental stress, and they found that the spectral response did not show differences for different stress types. However, combined stress of different single stresses may be related to characteristics of individual stresses. But the combined stress may be more complicated than single stresses in the physiological status and spectral response of plants [29]. Therefore, it is necessary to study the spectral characteristic differences under different stress types and their combined stresses [15].

Maize is one of the most widely planted crops in the world. It is a critically important source of food, feed, energy and forage [30]. At the early growth stages, leaf growth is one of the most sensitive processes to stressors [31]. In this study, we take maize seedlings as an example to identify drought stress, copper stress and combined stress of drought and copper for the maize seedling by analyzing plant parameters and spectral indices (SIs) derived from hyperspectral measurements. Therefore, the typical plant parameters (including bio-physical and bio-chemical parameters) of seedlings and selected SIs were measured and extracted from the maize seedlings and their spectral measurements. The following three research questions are addressed in this study: (1) What are the differences of the plant parameters when maize seedlings suffer from different stress types (including combined stress)? (2) How do spectra respond to different stress types? And (3) how are the stress types identified by using SIs?

II. MATERIAL AND METHODS

A. DESIGN OF STRESS EXPERIMENT

The experiment was performed outside the laboratory. Air-dried garden soil was put into each plastic pot of 20 cm height and 20 cm diameter. Then, "Xianyu-335" maize seeds were grown in the soil, with one seed in one pot. At the beginning, all the seedlings were provided with sufficient water. When seedlings grew to the point of having five leaves, three types of stresses were applied for the seedlings compared with a control group. Each group included four repeats. After two weeks of stress, the plant parameters and spectra were measured for data collection.

(1) Control group (CK). The seedlings grew normally without any stress in this group. The maximum water holding capacity of the soil is approximately 25% according to our test. The soil water content was maintained at approximately 15%, which was 60% of the maximum field water holding capacity. The soil water content was controlled by weighing the total weight of the soil and pot.

(2) Drought stress group (D). The soil water content was maintained at approximately 7.5%, which was 30% of the maximum field water holding capacity, representing severe drought [32].

(3) Copper stress group (C). The samples were spiked using $CuSO_4.5H_2O$ solution. The copper concentration in the soil was controlled at 500 ppm, which exceeds the Grade 3 limit value of GB15618(i.e., 400 ppm) [33]. According to previous research, a small amount of copper in soil would promote the growth of maize, but it would be destructive to the growth of plants when the copper concentration exceeded a certain limit [34]. The concentration level of 500 ppm exceeded the safety limit of copper in the soil. Therefore, it would be expected to have a destructive effect on the growth of maize.

(4) Combined stress group (DC). This group was controlled by both drought and copper stresses. On the one hand, the copper concentration in soil was controlled at 500 ppm by making use of chemical analysis of $CuSO_4.5H_2O$. On the other hand, the soil water content was maintained at approximately 7.5% using the weighing method.

B. MEASUREMENTS OF PLANT PARAMETERS

The difference of maize growth between different stress types became remarkable after a two-week stress experiment. The control group had grown to 8 stretched leaves; the copper stress group had grown to 7 leaves; and both the drought stress and the combined stress groups had grown to 6 leaves. To ensure that the data are comparable, the three top stretched leaves of each plant were measured for the plant parameters' data collection. Because these stretched leaves could represent the canopy, the spectrum was measured at the canopy level.

(1) Chlorophyll content. The chlorophyll content was measured by SPAD-502, which is broadly used in forestry and agriculture because of its convenience and nondestructive properties. However, it is worth noting that the chlorophyll content is different from what is measured by SPAD-502, but the SPAD-502 readings are closely correlated with the chlorophyll content [35]. Therefore, to be convenient, the SPAD-502 readings are hereafter referred to as the chlorophyll content. The chlorophyll content was measured seven times at seven points with equal intervals for each leaf. These measured points should not be located at a vein. The average value of the seven chlorophyll reading data was set as the final chlorophyll content of each leaf.

(2) Leaf area. The leaf length and width were measured for one leaf area multiplied by the experience coefficient 0.75. The sum value of the three top leaves was set as the final leaf area of each plant.

(3) Biomass. The dry weight of the plant was set as its biomass.

(4) Relative water content (RWC, %). First, the fresh weight of the plant leaves was measured as the wet weight. Then, the plant leaves were put into an oven at 80 degrees Celsius to dry to a constant weight, which was set as the dry weight. The water content of the leaf was calculated as follows:

$$\mathrm{RWC} = (\mathrm{W_f} - \mathrm{W_d})/\mathrm{W_f} * 100\% \tag{1}$$

where $\mathrm{W_f}$ is the fresh weight, and $\mathrm{W_d}$ is the dry weight.

(5) Plant height. The vertical distance from the soil surface to the leaf top was measured as the height of the plant.

(6) Stretch ratio of leaf blade. It is calculated by leaf blade width in the natural state and flat state.

$$\mathrm{R_s} = \mathrm{L_n}/\mathrm{L_f} \tag{2}$$

where $\mathrm{R_s}$ is the stretch ratio of leaf blade, $\mathrm{L_n}$ is the leaf blade width in the natural state, and $\mathrm{L_f}$ is the leaf blade width in the flat state.

C. MEASUREMENTS OF SPECTRUM

The spectrum was measured prior to the plant parameters' measurement because the parameters' measurement would destroy the plant. Before measuring, to eliminate the influence of background on a target spectrum, a black plastic sheet was utilized to cover the soil part of the pot. An HR-1024 spectrometer, made by American Spectra Vista Corporation (SVC), was used for spectral measurement. The spectral range is 350 − 2500 nm. The spectral resolution is 3.5 nm from 350 nm to 1000 nm, 9.5 nm from 1000 nm to 1890 nm, and 6.5 nm from 1890 nm to 2500 nm. The measurement was implemented indoors. A halogen lamp was selected as the light source, with an incidence angle of 45 degrees and a distance of 75 cm to the target. The observation time was 2 s. Each plant pot was measured 4 times, with a rotation of 90 degrees. The average value of the four spectral curves was set as the spectral curve of each plant.

D. SELECTION OF SPECTRAL INDICES

There are many studies focusing on using various SIs to evaluate vegetation characteristics. In this study, it is not necessary for us to test all SIs. The SIs would be selected based on its inherent relationship with the three key parameters: chlorophyll content, leaf area and RWC, especially for abroad-leaf plant. As a result, after a literature review, a set of SIs potentially selected as source indices in this study is listed in Table 1.

E. DATA ANALYSIS

SVC software was used to remove the overlap of spectral data near 1000 nm and 1900 nm. The derivative spectra of the spectral curves were calculated by MATLAB. The correlation analysis between plant parameters and statistical test for significant differences between the stress types were performed by using EXCEL software.

(1) A statistical test for significant differences between stress types was made for each selected plant parameter.

(2) A correlation analysis between plant parameters was performed to select key plant parameters, which could be useful for a discrimination of different types of stresses.

(3) Selected SIs would be calculated from measured spectra to indicate the spectral variations of the stress groups and control group.

(4) Based on relationships between the different stress types, the key plant parameters and effective SIs would be identified for the discrimination of different types of stresses.

III. RESULTS AND ANALYSIS

A. DIFFERENCE OF PLANT PARAMETERS

(1) Chlorophyll content. D$\gg$CK$\approx$DC$\approx$C and CK$\gg$C, in which "$\gg$" means "greater than" with a statistically significant difference ($p<0.05$), while "$\approx$" means no statistically significant difference ($p\geqslant0.05$) in a two-sample difference of means t-test. It was found that the chlorophyll content increased under drought stress in this experiment, with the SPAD reading value being 5.9 higher compared with CK. A possible explanation is that an increase of the chlorophyll content is to maintain the photosynthesis rate in the context of the reduced leaf area under drought stress. Under copper stress, excessive copper would destroy the synthesis of chlorophyll and result in decreased chlorophyll content, a decline in the SPAD reading value of 7.4 compared with CK. Under the combined stress, the chlorophyll content of the leaves was slightly higher than CK, but the difference was not statistically significant. Therefore, the combined stress may not obviously affect the chlorophyll

TABLE 1. Summary of a set of spectral indices (SIs) selected in this study, with their references. The name of the index and its formula are given, together with the parameters (chlorophyll, leaf area, or RWC) to which they were constructed for.

SIs	Formula	Corresponding Parameter	Reference
REP	The position of the inflexion point in the red edge region (680 to 780 nm)	Chlorophyll	[48]
mND_{705}	$\frac{R_{750} - R_{705}}{R_{750} + R_{705} - 2 * R_{445}}$		[43, 49]
mSR_{705}	$\frac{R_{750} - R_{445}}{R_{705} - R_{445}}$		[49]
TCARI/OSAVI	$\frac{3 * [(R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550}) * (R_{700}/R_{670})]}{(1 + 0.16) * (R_{800} - R_{670})/(R_{800} + R_{670} + 0.16)}$		[46]
TCARI	$3 * [(R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550}) * (R_{700}/R_{670})]$		[46]
DDn	$2 * R_{730} - R_{680} - R_{780}$		[47]
ND_{chl}	$(R_{925} - R_{710})/(R_{925} + R_{710})$		[47]
dRE	Sum of first derivative reflectance spectra between 680 and 780nm	Leaf area	[50]
MCARI2	$\frac{1.5[2.5(R_{800} - R_{670}) - 1.3(R_{800} - R_{550})]}{\sqrt{(2R_{800} + 1)^2 - (6R_{800} - 5\sqrt{R_{670}}) - 0.5}}$		[51]
$WDRVI_{Red\text{-}edge}$	$\frac{\alpha * R_{NIR} - R_{Red\ Edge}}{\alpha * R_{NIR} + R_{Red\ Edge}} + \frac{1 - \alpha}{1 + \alpha}$		[52]
D_{LAI}	$R_{1725} - R_{970}$		[47]
$CI_{Red\text{-}edge}$	$\frac{R_{NIR}}{R_{Red-edge}} - 1$		[53]
dND	$\frac{dR_{1415} - dR_{1530}}{dR_{1415} + dR_{1530}}$	Water content	[54]
SR1640	$\frac{R_{1060}}{R_{1640}}$		[55]
NDII	$\frac{R_{860} - R_{1650}}{R_{860} + R_{1650}}$		[56]
SIWSI	$\frac{R_{858.5} - R_{1640}}{R_{858.5} + R_{1640}}$		[57]
SR2	$\frac{R_{1070}}{R_{1340}}$		[55]

content because the combined stress resulted in an integrative effect of both drought and copper stresses on plants (Fig. 1a).

(2) Leaf area. CK≫C≫DC≈D. Under drought stress, the plant reduced the exposure of its leaf area in order to reduce transpiration. The drought stress led to the decline in the leaf area, losing approximately 58 cm^2 compared with CK. Under the copper stress, the growth of the leaves was affected by copper, which also led to a decline in the leaf area. The 16 cm^2 of decline in the area was far below the drought-induced result. Under the combined stress, the leaf area was slightly lower than the drought stress, but the difference was not statistically significant. This result meant that the combined stress had not worsened the leaf area compared with single-factor stress, drought or copper (Fig. 1b).

(3) Biomass. CK≫C≫DC≈D. Biomass is an important index for monitoring of crop growth and yield estimation. Copper and drought stresses would inhibit the normal growth of a plant. The biomass of maize seedlings was smallest under drought stress, and it was reduced by 2.1 g compared with CK. The biomass of the plants was reduced by 0.4g and 1.7g under copper and combined stress, respectively (Fig. 1c).

(4) Relative water content (RWC). CK≫C≈D≫DC. The drought stress led to a decrease of the moisture in the soil, so the water supply to the plant decreased greatly. As a result, the RWC of the plant dropped by 8% compared with CK. The copper stress reduced the water potential of the soil and influenced the water supply for the plant. Therefore, the RWC of the plant decreased slightly, by 2%, under the copper stress. Under the combined stress, the RWC of the plant decreased sharply by a combined influence of the water shortage and the reduced water potential of the soil. The RWC was lowered by 16% compared with CK (Fig. 1d).

(5) Plant height. CK≈C≫D≈DC. Copper stress affected the plant growth, and the plant height declined approximately 7 cm compared with CK. Furthermore, the drought stress had more influence on the plant height than done by the other

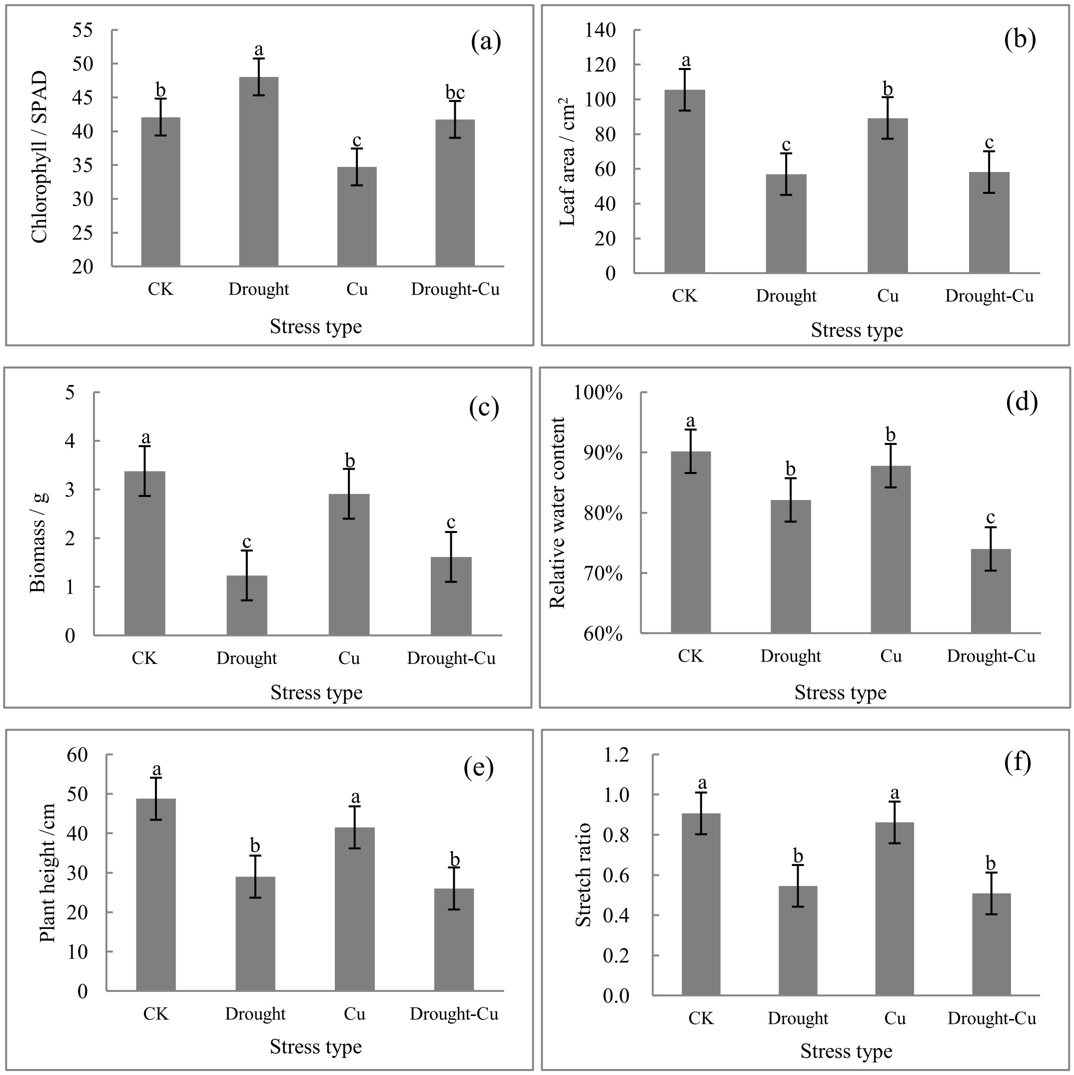

FIGURE 1. Plant parameters of maize seedlings for different stress types: (a) Chlorophyll/SPAD readings, (b) Leaf area, (c) Biomass, (d) Relative water content, (e) Plant height, and (f) Stretch ratio. Values represent the mean ± SD of the repetitions. Values followed by the same letters are not significantly different from each other ($P < 0.05$).

stress types, declining approximately 20 cm. The greatest decrease of 22 cm in plant height occurred under the combined stress (Fig. 1e).

(6) Stretch ratio of leaf blade. CK≈C≫D≈DC. Leaves would curl in arid conditions to reduce water loss. Experimental results showed that the stretch ratio under the copper stress was slightly lower than the CK group, but the difference was not statistically significant. The drought stress made the stretch ratio of the leaves decline greatly, till 0.546. The lowest stretch ratio was 0.508, induced under the combined stress (Fig. 1f).

These plant parameters have a certain correlation between them. The pairwise correlation coefficients between the parameters are shown in Table 2. The best correlation was found between the RWC and stretch ratio. Moreover, good correlations also appeared in most other pairwise comparisons. The chlorophyll content was an exception. It was negatively correlated with all other parameters, and the correlation was weak. In other words, only the chlorophyll content showed a unique difference.

TABLE 2. Correlation analysis results between any two plant parameters ($n=16$, $*$ means $p<0.05$, $**$ means $p<0.01$).

Correlation	Leaf area	Chlorophyll content	Water content	Plant height	Stretch ratio
Biomass	0.96**	-0.55*	0.77**	0.93**	0.85**
Leaf area		-0.44	0.80**	0.94**	0.82**
Chlorophyll content			-0.34	-0.41	-0.56*
Water content				0.88**	0.89**
Plant height					0.84**

Considering inter-parameter correlations and inter-type (stress) differences, three parameters were identified as the key parameters to identify stress types. Chlorophyll was selected as one of the key parameters due to its unique characteristics for stress detection. Compared with the stretch ratio, the RWC could significantly affect the spectral reflectance at some bands and could be retrieved from the canopy spectrum more simply. Therefore, the RWC was selected as a second key parameter. In addition, because the leaf area had a direct effect on the plant's canopy spectrum, it was selected as the third key parameter instead of biomass. As a result, the chlorophyll content, RWC and leaf area were selected as the three key parameters for stress detection.

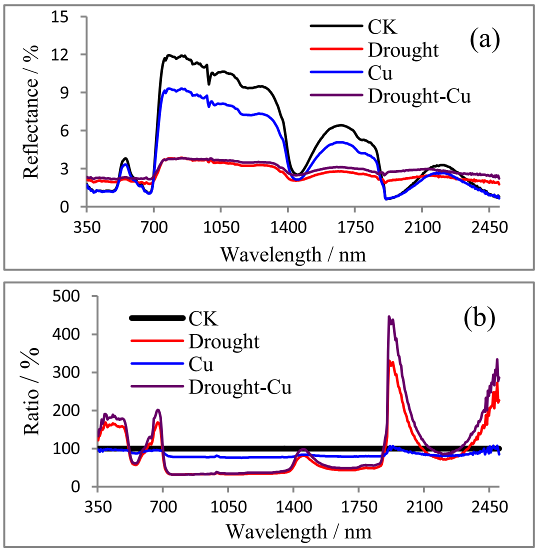

FIGURE 2. **Spectral differences of different stress types: (a) Reflectance of the four types of stresses, and (b) Ratio of reflectance of each stress to CK (reference).**

B. DIFFERENCE OF SPECTRAL RESPONSES

The canopy spectra of three types of stresses were different from the CK group (Fig. 2). In general, from 760 nm to 1400 nm, the drought and combined stress groups led to the lowest reflectance; the copper stress group led to high reflectance; and the CK group led to the highest reflectance. This difference was mainly related to the leaf area of the plant. The greater leaf area would lead to greater reflectance in this spectral region. In the visible spectral range, "peak" and "valley" spectral characteristics on the green peak (560 nm) and the red valley (680 nm) under drought stress and combined stress were not obvious compared to the CK and copper stress. The spectral characteristics may be mainly controlled by leaf area, chlorophyll content and RWC, etc. Therefore, "peak" and "valley" spectral characteristics under the drought stress were slightly sharper than those under the combined stress because the drought stress group had the highest chlorophyll content. At 1400 nm and 1900 nm bands, reflectance from high to low was the combined stress, drought stress, CK and copper stress. The reflectance was mainly controlled by the RWC. The higher the RWC was, the greater the absorptions at 1400 nm and 1900 nm were,

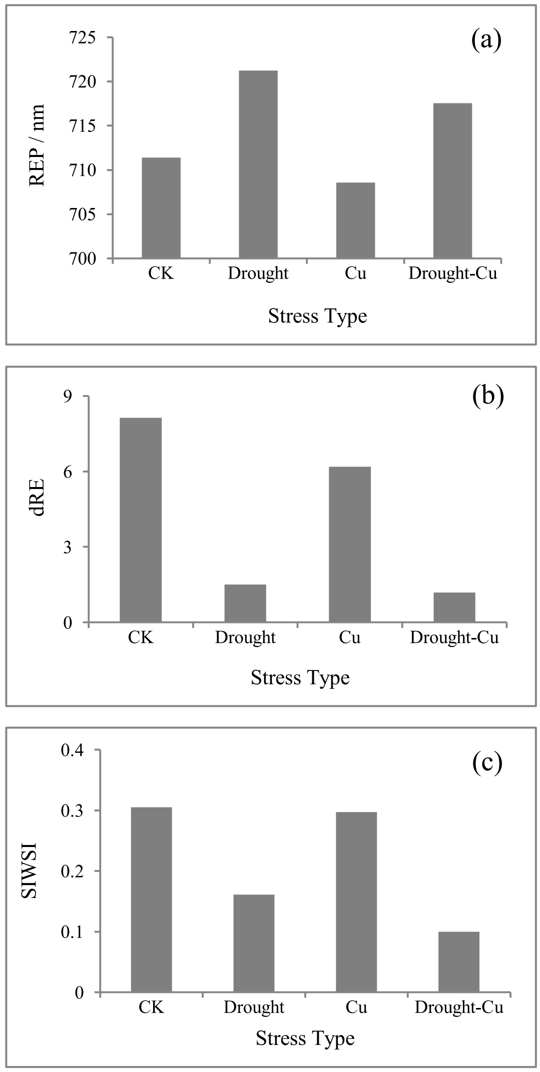

FIGURE 3. **Differences of SIs for different stress types: (a) REP (red-edge position) vs. different stress types, (b) dRE (The first derivative at the red edge) vs. different stress types, and (c) SIWSI (Shortwave Infrared Water Stress Index) vs. different stress types.**

and the lower the reflectance at the two bands. This result implied that plant reflectance might play a limited role in direct parameter inversion for multi-stress plants.

C. SPECTRAL INDICES OF PLANTS

The correlation coefficient for each SI and each corresponding parameter was obtained (Table 3). For chlorophyll, REP was the most closely related index, followed by mND705 and mSR705. Inversely, other indices were weakly related to chlorophyll. For leaf area, most of the selected indices had a close relationship with the parameter, in which dRE was the most closely related index. For RWC, all the selected indices were highly related to it, and SIWSI had the best performance.

As a result, REP, dRE and SIWSI were confirmed as the better SIs for identifying stress types. Their values for corresponding stress types are presented in Fig. 3. A test was

TABLE 3. Correlation analyses between SIs and plant parameters ($n=16$, $*p<0.05$, $**p<0.01$).

SIs	Correlation Coefficient	Corresponding Parameter
ND_{chl}	-0.330	
DDn	-0.369	
TCARI/OSAVI	-0.396	
TCARI	-0.398	Chlorophyll
mSR_{705}	0.512*	
mND_{705}	0.572*	
REP	**0.795****	
$WDRVI_{Red\text{-}edge}$	0.706**	
MCARI2	0.717**	
$CI_{Red\text{-}edge}$	0.758**	Leaf area
D_{LAI}	0.862**	
dRE	**0.864****	
dND	0.851**	
SR2	0.893**	
SR1640	0.901**	Water content
NDII	0.925**	
SIWSI	**0.929****	

performed for testing the statistical significance of differences of the three SIs between different stress types. The results are summarized as follows.

(1) REP. D≫DC≫CK≈C. This result was basically consistent with chlorophyll except for the relationship between CK and DC. The REP of the DC group was greater than that of the CK group with a statistical significance, while the difference in the chlorophyll between the CK and DC groups was not statistically significant (Fig. 3a).

(2) dRE. CK≫C≫DC≈D. This result was completely consistent with the leaf area (Fig. 3b).

(3) SIWSI. CK≫C≫D≈DC. This result was almost consistent with the RWC, except for the relationship between DC and D. The difference was not statistically significant for SIWSI between D and DC, while the RWC of the DC group was significantly lower than that of the D group (Fig. 3c).

D. IDENTIFICATION OF STRESS TYPES BASED ON SIS

There were significant differences in the SIs between the CK group and the other stressed groups. Therefore, the CK group was set as the general reference for comparison with others, and stress types could be identified according to key SIs based on the decision tree method. There were three steps for the identification (Fig. 4) as follows.

(1) Identification of stress and no stress. The dRE or SIWSI of unknown types of stress was compared to the CK; if they were lower than the CK, then it meant that the maize seedlings were in the stressed condition. Otherwise, it meant that the maize seedlings did not suffer from stress.

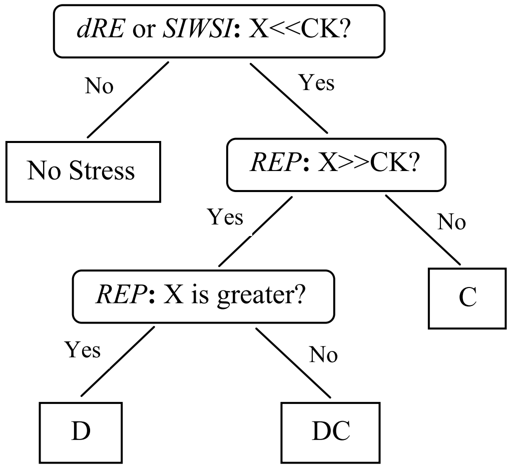

FIGURE 4. Decision tree for identification of stress types(where X means unknown type of stress, "≫" means "greater than" with a significant difference ($p<0.05$), and "≪" means "lower than" with a significant difference ($p<0.05$)).

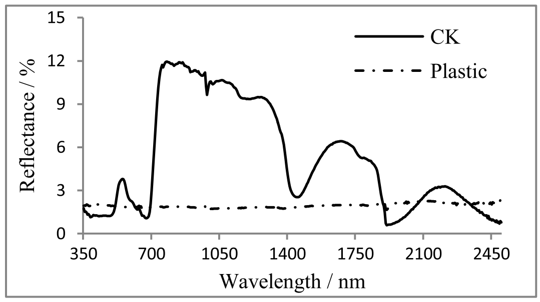

FIGURE 5. A plastic spectrum compared to a plant canopy spectrum.

(2) Identification of copper stress. The REP of unknown types of stress was compared to the CK. If it was not greater than the CK, then it meant that maize seedlings were suffering from the copper stress. Otherwise, it meant that they were in the other two types of stressed conditions.

(3) Identification of drought and combined stresses. The REP of the two types of stress were compared to each other. The type with the greater REP would be identified as the drought stress and otherwise as the combined stress.

IV. DISCUSSION

In this experiment, black plastic was used to cover the soil to eliminate the influence of soil background on the target (plant canopy) spectrum. Its reflectance is less than 2.4%, and the reflectance at the majority of the wavelength range is less than 2% (Fig. 5). The reflectance was very low relative to the whole plant spectrum. Moreover, we used the same background to take spectral measurements from all plant samples. Therefore, the background impacts could be minimized. However, in the field, the canopy spectra of the seedlings of maize would be greatly affected by the soil background. How to eliminate the influence of the soil background when estimating plant parameters is critical to remote sensing applications. Therefore, the soil factor should be considered in future experiments.

The chlorophyll content and REP played a critical role for identifying stress types. Under drought stress, the chlorophyll content of the leaf may increase or decrease, which is related to the plant species and stress time [36]–[39]. In our experiment, an increase of the chlorophyll content was noted under the drought stress. Two causes might explain the phenomena of chlorophyll increase, including a loss of turgor or a reduction of leaf growth, leading to increasing chlorophyll content [40]. The chlorophyll content is the only bio-parameter that increased when the pant suffered from the drought stress compared to the control group. Therefore, the chlorophyll content might be used to identify the drought stress and combined stress due to its unique characteristics. In considering the different abilities of SIs to retrieve the three bio-parameters (i.e., chlorophyll content, leaf area, and RWC) and to identify the three stress types, REP was the most important index for identification of the three stress types among the three key spectral indices. However, dRE and SIWSI were also important for distinguishing the three stress types because they also had a good performance in identifying the stress types.

The combined stress was controlled by both copper and drought stresses. The bio-parameters of the combined-type stressed plants were derived from both individual type stressed plants. The chlorophyll content of the combined-type stressed plants was greater than that the copper stressed plants but lower than the drought stress. Therefore, the result looked like a combination of the two single stresses. However, this phenomenon was not observed in the other two parameters (i.e., leaf area and RWC). The leaf area and RWC of the combined-type stressed plants were roughly equal to the drought stress and lower than that induced by the copper stress. Therefore, the values of the stress influencing on the different bio-parameters and their corresponding SIs were different.

Various SIs could be used to evaluate different plant parameters. Therefore, the chlorophyll content, leaf area and RWC could be estimated from hyperspectral data. Such conclusions have been proven by evaluating different tree species (coniferous, broadleaf) [41], [42], different scales(canopy, leaf) [43], [44] and different growth stages (seedling stage, and heading stage, etc.) [45] using different SIs. In this study, spectra were collected at the canopy scale of maize at the seedling stage. Generally, the leaf area and RWC could correlate significantly with their corresponding SIs. However, some SIs for estimating the chlorophyll content could not repeat their previous performance in this study. For example, although mND705 and mSR705 were very effective for chlorophyll estimation across a wide range of species and leaf structures [43], it was found that they were not highly related to the chlorophyll in this study at the canopy level. In addition, TCARI/OSAVI and TCARI were validated to be sensitive to the chlorophyll content variations and very resistant to the variations of LAI [46], but their correlation coefficients with the chlorophyll content in our study were not positive. This difference reflected the poor robustness of TCARI/OSAVI and TCARI in early growth conditions when LAI is relatively low. Although DDn and NDchl were determined as the best indices for canopy chlorophyll estimation in work by le Maire *et al.* [47], both were not correlated with chlorophyll in this study. This result might be due to the difference between forests and crop. In other words, these SIs might be sensitive to stress-type variations, but they could not perform well when estimating chlorophyll content under some types of stress.

Fortunately, the REP index was found to be the best index for chlorophyll estimation. Because a linear extrapolation method could be used to determine the REP index in the case of maize leaves at different developmental stages [48], we tested it and proved it to be workable in this study. The maximum correlation coefficient with the REP index also indicated that the REP could minimize the effect of variations in the stress type on the prediction of chlorophyll content. However, chlorophyll was overestimated by REP for both copper and combined stresses (Fig. 2a vs. Fig. 4a). This result could be explained by the measuring procedure of chlorophyll as follows. In this study, the chlorophyll of each seedling sample was calculated by averaging three leaves. However, upper leaves of the canopy, which usually contain more chlorophyll than the lower ones in the two types of stress, would contribute more to the spectral estimation of the chlorophyll content.

V. CONCLUSIONS

In this experiment, three stress types (drought stress, copper stress and combined (drought and copper) stress) plus one control were designed and studied with maize seedlings. Different plant parameters (bio-parameters) and hyperspectral measurements were measured and taken from the different types of stressed maize seedlings. After carrying out statistical and correlation analyses between bio-parameters and spectral indices associated with the different stress types, several conclusions were derived from the experiment as follows:

(1) Differences in the plant parameters between different stress types were statistically significant, and three key parameters (chlorophyll content, leaf area and RWC) were selected to express such differences. In particular, the chlorophyll content was the most important bio-parameter because of its unique ability to distinguish the combined stress from the drought stress.

(2) Three spectral indices (SIs) (REP, dRE, and SIWSI), which could minimize the effect of variations of stress types on the prediction of the key bio-parameters, were sensitive to changes in the three plant parameters, and thus they might potentially be used to estimate the bio-parameters. However, there were differences in the SIs between the three stress types in terms of the plant parameters (chlorophyll content, leaf area and RWC).

(3) Based on a decision tree analysis, the three SIs (i.e., REP, dRE and SIWSI) might be used to identify the three stress types of maize seedlings. Thus, it would be beneficial

to remote sensing applications in a precision management of crops and environmental monitoring.

Limited by the experimental condition, we conducted the stress experiment of maize only at the seedling stage. All results derived from the data measured/extracted from the experiment may not be applicable for other growth stages. Actually, different stress types may occur at different growth stages. Therefore, different experiments should be designed for different growth stages in the future. Furthermore, the soil background effect always exists in practice when monitoring crops in the field. Therefore, the effect of the soil background on plant spectra should be considered when inversing plant parameters from remotely sensed data in future studies.

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