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Hyperspectral characterization of freezing injury and its biochemical impacts in oilseed rape leaves

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A B S T R A C T

Automatic detection and monitoring of freezing injury in crops is of vital importance for assessing plant physiological status and yield losses. This study investigates the potential of hyperspectral techniques for detecting leaves at the stages of freezing and post-thawing injury, and for quantifying the impacts of freezing injury on leaf water and pigment contents. Four experiments were carried out to acquire hyperspectral reflectance and biochemical parameters for oilseed rape plants subjected to freezing treatment. Principal component analysis and support vector machines were applied to raw reflectance, first and second derivatives (SDR), and inverse logarithmic reflectance to differentiate freezing and the different stages of post-thawing from the normal leaf state. The impacts on biochemical retrieval using particular spectral domains were also assessed using a multivariate analysis. Results showed that SDR generated the highest classification accuracy (>95.6%) in the detection of post-thawed leaves. The optimal ratio vegetation index (RVI) generated the highest predictive accuracy for changes in leaf water content, with a cross validated coefficient of determination (R^2_{cv}) of 0.85 and a cross validated root mean square error ($RMSE_{cv}$) of 2.4161 mg/cm^2. Derivative spectral indices outperformed multivariate statistical methods for the estimation of changes in pigment contents. The highest accuracy was found between the optimal RVI and the change in carotenoids content ($R^2_{CV} = 0.70$ and $RMSE_{CV} = 0.0015$ mg/cm^2). The spectral domain 400–900 nm outperformed the full spectrum in the estimation of individual pigment contents, and hence this domain can be used to reduce redundancy and increase computational efficiency in future operational scenarios. Our findings indicate that hyperspectral remote sensing has considerable potential for characterizing freezing injury in oilseed rape, and this could form a basis for developing satellite remote sensing products for crop monitoring.

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1. Introduction

Winter oilseed rape (*Brassica napus L.*) is an important oilseed crop in China. This crop is mainly cultivated in the Yangtze River basin. Because of the impact of cold spells, winter oilseed rape in this region is frequently subjected to freezing injury, which can lead to a significant decrease in yield and product quality (She et al., 2015; Zhang et al., 2008). Similar negative impacts of freezing are experienced by many different crop types globally (Cromey et al., 1998; Lardon and TriboiBlondel, 1995; Staggenborg and Vanderlip, 1996). Freezing injury is a common weather-induced agricultural hazard and refers to plants suffering from damage when temperatures drop below 0 °C. When leaves are exposed to freezing temperatures, ice crystals are formed between cells. Cellular dehydration can then occur because of the difference in water potential between the inside and the outside of the cell, which draws cytoplasmic water from the cell to the growing mass of extracellular ice. With the decrease in temperature, more water moves from the cytoplasm to intercellular spaces. Permanent freezing injury is caused when dehydration extends beyond the tolerance of the plant and/or ice produces mechanical pressure.

Traditionally, monitoring of freezing injury relies on visual surveys by technicians in the field. This approach is dependent on having staff with sufficient expertise. It is time consuming and labor-intensive.

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Thus, a more effective alternative approach is required for detecting freezing injury in vegetation. Hyperspectral remote sensing has been widely used as a nondestructive technique to monitor various biotic and abiotic stress factors across different spatial scales (Galvao et al., 2011; Liu et al., 2002; Penuelas et al., 1993; Sankaran et al., 2010; Strachan et al., 2002). As the process of freezing injury tends to be fast (often within a few hours), it means that if hyperspectral remotely-sensed data are to be of value in monitoring the process, they need to be acquired at a high temporal resolution. However, currently available optical satellite data lack the spectral and temporal resolution required for monitoring freezing injury in real time. Several satellite missions have been planned to generate suitable data. These include the Geostationary Coastal and Air Pollution Events (GEO-CAPE) mission from the USA (Board, 2007), the Geostationary Environment Monitoring Spectrometer (GEMS) mission from Korea (Bak et al., 2013), and the Sentinel-4 mission from Europe (Berger et al., 2012), which will provide appropriate datasets multiple times per day. In this study, spectroradiometer data were acquired in a laboratory setting at the leaf scale to demonstrate the capabilities for monitoring freezing injury using hyperspectral data, an approach that would provide a basis for airborne and space-borne monitoring in future when remote sensing data of higher spatial, spectral and temporal resolutions become available.

At the leaf scale, the spectral reflectance characteristics across the visible (400–750 nm), near-infrared (750–1300 nm) and shortwave-infrared (1300–2500 nm) ranges are primarily determined by variations in photosynthetic pigment content, leaf structure and water content (Knipling, 1970; Richardson et al., 2002; Slaton et al., 2001) which can be strongly impacted by freezing injury (Gausman et al., 1984; Wang et al., 2016; Wang et al., 2012). Many studies have been carried out on the potential of using hyperspectral techniques in evaluating the quality and safety attributes of food products (e.g. meat and edible fungi) subjected to freeze damage (Gowen et al., 2009; Thyholt and Isaksson, 1997). Gowen et al. (2009) integrated principal components analysis and linear discriminant analysis to differentiate between undamaged and freeze-damaged mushrooms using hyperspectral imaging. Their results indicated that freeze-damaged mushrooms could be classified with high accuracy (>95%) after only 45 min of thawing. Other studies have used hyperspectral data to estimate the changes in biophysical or biochemical parameters after freezing injury. Nicotra et al. (2003) examined the impact of freezing stress on the distribution of photosynthetic pigments in *Eucalyptus pauciflora* leaves using a CASI high-resolution hyperspectral imaging system. Their results demonstrated a considerable spatial variation of chlorophyll content over the surface of the lamina, with marked decreases in chlorophyll content approaching the margins and tips of the leaves. However, changes in the hyperspectral characteristics of crops such as oilseed rape during the freezing injury process are yet to be investigated, and the potential of using hyperspectral techniques to identify leaf status and monitor biochemical changes remains unknown.

A variety of different analytical techniques have been used to automatically detect and classify plant stress from remotely sensed data. Amongst these techniques, support vector machines (SVMs) are promising machine learning methods which are suitable for remote sensing applications due to their ability to generalize well even with limited training samples (Mantero et al., 2005). SVMs have already been used in land cover classification (Gao et al., 2015; Hong et al., 2015; Zhang et al., 2015), quantifying vegetation stress (Adjorlolo et al., 2015; Behmann et al., 2014) and land cover change detection (Hichri et al., 2013; Hussain et al., 2013; Nemmour and Chibani, 2006). Furthermore, SVMs have been used to estimate plant biophysical and biochemical parameters such as LAI, biomass, pigments and nitrogen contents (Gleason and Im, 2012; Verrelst et al., 2012; Yang et al., 2011; Zhai et al., 2013). Hence, SVMs hold promise as a method for characterizing freezing injury in plants using hyperspectral data.

Multi-collinearity is a common problem within hyperspectral data. It results from a large number of highly correlated wave bands. Some techniques have been proposed to reduce the redundancy of hyperspectral data for vegetation applications. Principal component regression (PCR) and partial least square regression (PLSR) can be employed to solve multi-collinearity problems. Many studies have used these techniques to construct predictive relationships between spectral data and vegetation parameters (Adjorlolo et al., 2015; Gonzalez-Fernandez et al., 2015). In order to reduce redundancy in spectral data, some studies have made a comparison between the full spectrum and specific spectral domains (ranges) for estimating vegetation parameters from remotely sensed hyperspectral data using multivariate models (Gonzalez-Fernandez et al., 2015; Darvishzadeh et al., 2008; Huang and Blackburn, 2011). The results indicate that predictive models based on specific spectral domains are superior to models based on the full spectrum. Hence, there is considerable potential for the use of spectral indices, multivariate regression techniques and optimized spectral domains for assessing freezing injury in vegetation using hyperspectral data, and this warrants further investigation.

The overall aim of this study is to determine the applicability of leaf spectral reflectance data for detecting the freezing and post-thawing states of oilseed rape and quantifying the biochemical impacts of freezing. The objectives are to (1) characterize the spectral reflectance of oilseed rape leaves during freezing and post-thawing; (2) identify appropriate analytical techniques that can be applied to reflectance spectra to differentiate between normal leaves and leaves at freezing and different stages of post-thawing; (3) establish predictive models based on leaf spectral reflectance measurements for quantifying the changes in leaf water content (ΔLWC), chlorophyll *a* (ΔChla), chlorophyll *b* (ΔChlb), and carotenoids (ΔCars) induced by freezing injury.

2. Materials and methods

2.1. Plant culture and experimental design

The experiments were conducted at the Campus Experimental Station of Zhejiang University. The seeds were a local commercial variety of oilseed rape (Zheyou No.50). The soil used for this study was paddy soil. The seeds were sown in black plastic pots (18 cm diameter × 16 cm height) on October 13, 2013 and October 20, 2014, and were located outdoors. The seedlings were thinned to two plants per pot at the 3-leaf stage. Plants were watered as necessary and fertilizer was applied according to local agronomic practices. Treatments were carried out at the 8 leaf-stage during the 2013–2014 growing season, while treatments were carried out at the initial stage of budding during the 2014–2015 growing season. The air temperature profile during the growing period is given in Fig. 1.

Each pot containing two plants was transferred to an Aucma freezer (390 L in volume). The freezing of plants was executed under conditions of darkness. Air temperature decreased from laboratory temperature to the lowest temperature range of −10 ~ −12 °C. As the formation of rime on the leaves could affect hyperspectral measurements in various ways, desiccants were applied to reduce the relative humidity within the freezer during the freezing treatments. Before each measurement, we ensured that there was no rime/frost on the observed leaf surfaces based on visual observation. Leaf temperature was monitored at one second intervals by a digital temperature sensor with a precision of ±0.5 °C(−10 °C ~ +85 °C). The temperature sensor was connected to a computer on which an 8-channel temperature data acquisition software was installed to log data. The time course of leaf temperature during a typical freezing treatment is shown in Fig. 2. After treatment, the plants were transferred to a light incubator to thaw at 22 °C day/18 °C night temperatures with an 11 hour photoperiod (7 am–6 am) and light intensity of 8000 LX.

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