Agricultural and Forest Meteorology 232 (2017) 536–542

Contents lists available at ScienceDirect

Agricultural and Forest Meteorology

journal homepage: www.elsevier.com/locate/agrformet

Potential of near infrared spectroscopy to monitor variations in soluble sugars in Loblolly pine seedlings after cold acclimation

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ARTICLE INFO

Article history:
Received 13 June 2016
Received in revised form
10 September 2016
Accepted 10 October 2016
Available online 19 October 2016

Keywords:
Cold acclimation
Pinus taeda L.
Nir
Seedling
Soluble sugar

ABSTRACT

The freeze tolerance of seedlings can be affected by cold acclimation. This acclimation can in turn alter the seedlings absorbance spectra due to variation of soluble sugar content (SSC). If models developed from the absorbance at wavelengths in the near infrared reflectance (NIR) region could provide information concerning the variation in SSC, nursery managers could assess the freeze tolerance of each seedling before planting. The objective of this research was to investigate the potential to use NIR based analysis to study the variation of SSC in Loblolly pine (*Pinus taeda* L.) seedlings after cold acclimation. Seventy seedlings were frozen using different cold treatments and 10 seedlings were designated as controls. The standard wet chemistry method was employed to collect the original SSC data, including glucose, fructose and galactose. NIR spectroscopy was completed by using a NIR machine, with a range of 10,000–4000 cm^{-1}. The results were analyzed using partial least squares regression in an effort to determine the SSC in the leaves, stems and roots. Results showed that the freeze tolerance of seedlings increased after cold acclimation and was positively related to the presence of SSC. After cold acclimation, leaves had the most SSC, and the galactose content was more than that of glucose or fructose in seedlings. Based on the determination coefficient (R^2) and the residual predictive deviation (RPD), the predicted results of NIR models were evaluated. The model for leaf galactose (R^2 = 0.88 and RPD = 2.17) was the most stable and accurate model. This paper demonstrates that NIR coupled with chemometric modeling can be a useful tool in the monitoring of SSC variation and this information could be useful in the prediction of freeze tolerance.

1. Introduction

Forest restoration has become a global topic given the recent interest in carbon sequestration to offset greenhouse gasses (Bergman et al., 2014). Pointed cultivation is rapidly gaining attention as a more appropriate way to restore degraded forests (Appanah et al., 2016; Saito et al., 2016). Cultural practices must focus on overcoming planting stress on harsh restoration sites by enhancing the ability of seedlings to withstand frost, drought, and nutrient deficits (Bigras et al., 2001a; Grossnickle and South, 2014; Jacobs et al., 2015). The freeze tolerance of seedlings is improved when they are exposed to nonfreezing low temperatures, which is called cold acclimation (Bigras et al., 2001b; Hale and Orcutt, 1987; Sasaki et al., 1998). Low temperatures influence the physiological and biochemical processes that impart freezing tolerance in plants (Alden and Hermann, 1971; Grossnickle and South, 2014). It has been observed that cold acclimation increases sugar content in seedlings (Bertrand et al., 1999; Dalen et al., 2015; Sasaki et al., 1998). Laboratory studies have demonstrated that soluble sugars accumulated in plants can serve as cryoprotectants, which can prevent or decrease the freezing point of water in seedlings (Jones et al., 1999; Karimi and Ershadi, 2015; Morin et al., 2007). The precise function of these sugars is not well known, but sugar content and distribution appear to be essential in the acclimation of seedlings to cold temperatures, and has been shown to be a superior indicator of freeze tolerance (Ershadi et al., 2016; Meng et al., 2015; Welling and Palva, 2006). The cold acclimation phase of plants can be assessed on the basis of the rise in soluble sugar content (SSC) that occurs within them (Ögren, 1999). Wet chemistry analysis is a useful method for an accurate evaluation of the SSC in

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http://dx.doi.org/10.1016/j.agrformet.2016.10.012
0168-1923/

plants (Henderson and Henderson, 1986; Puzic, 1990). However, the disadvantage remains that this method is labor-intensive and partially or completely destructive. There is great demand for data concerning SSC in plants to be used for predicting their freeze tolerance. Near-infrared reflectance (NIR) spectroscopy is considered to be a valuable tool to complement, or even replace, wet chemistry analysis of SSC (Brown and Moore, 1987; Foley et al., 1998).

NIR spectroscopy is a rapid monitoring and non-destructive method that has been used to evaluate freeze tolerance in some species (Castillo et al., 2008; Sundblad et al., 2001). It is clear that freeze tolerance detection with NIR must have some relationship to the NIR signal, which records the interaction of infrared light with matter (Smith, 1998). One possible connection between freeze tolerance and NIR spectra is the change in SSC upon exposure to lower temperatures. In the NIR region (from 12,820–4000 cm^{-1} wavenumber), radiation is absorbed by different chemical bonds (e.g., C—H, C═O, and O—H) of sugars present in samples(Golic et al., 2003). Furthermore, the radiation is absorbed in accordance with the SSC (Giangiacomo, 2006). However, any change in the soluble sugar content cannot be directly interpreted from the spectra because of the physical (e.g., scattering or reflective) properties of the samples (Blanco and Villarroya, 2002; Foley et al., 1998; Shenk et al., 2008). In order to extract this information, multivariate statistical models (such as partial least-squares (PLS) regression) are needed to describe the relationship between the NIR spectral absorbance and SSC assessed by reference methods (Rambla et al., 1997). A final, reliable model can then be used for fast and reliable prediction of SSC in new samples of the same origin, and where only the NIR spectra was examined. Such a predictive model of SSC might also aid in the analysis and predictor of plant freeze tolerance (Lennartsson and Ögren, 2003).

Loblolly pine (*Pinus taeda* L.) is the leading commercial timber species in the southern United States and is also considered to be the favored species for planting throughout much of that area (Borders and Bailey, 2001; Gupta and Durzan, 1991). The increasingly sophisticated techniques available for seedling cultivation have prompted the planting of Loblolly pine throughout the world (Landis et al., 2010). However, the production of pine seedlings in nurseries is dependent on seedling survival particularly during exposure to freezing temperatures (Bigras et al., 2001a). Freeze injuries to the loblolly pine seedling may reduce growth rates or cause mortality (Lantz, 1985; South, 2006). Freeze damage (FD) can also cause leaves to lose water and become cortex, and xylem tissues to turn tan or brown, which is also a basis for the direct visual evaluation of FD in field (Rowan, 1985; South, 2006). However, the visible method is slow and requires large numbers of seedlings to achieve statistically reliable results (Sundblad et al., 2001). As previously stated, cold acclimation is part of the transition of the seedling from a non-hardy to a hardy state (Bigras et al., 2001a; Grossnickle and South, 2014; Thomashow, 2001; Welling and Palva, 2006). During this process, there are organ-related changes in the SSC content and this suggests that freeze tolerance might be predictable if the SSC is known. Therefore, the objective of this paper is to explore the potential of NIR based models to accurately estimate SSC (including glucose, fructose and galactose) in loblolly pine seedlings after cold acclimation. Also, the ability of NIR to assess variation in SSC within the whole seedling is compared with the NIRability to assess SSC in different organs (e.g., root, stem and leaf).

2. Materials and methods

2.1. Plant material and experimental design

Eighty loblolly pine seedlings from a freeze-sensitive family were lifted by hand from the bed at the Georgia Super Tree Nursery near Shellman, GA on November 5, 2015. All bare-root seedlings were placed in a black plastic bag and transported to Auburn University and stored in a cooler. The seedlings were 6 months old with uniform height (23.6 ± 1.4 cm) and diameter (5.1 ± 0.7 mm). All bare-root seedlings were divided into 8 equal groups. Ten seedlings (control group R) were examined using wet chemistry and spectroscopic analysis immediately. Sixty seedlings (group A, B, C, D,E and F) were potted into cylindrical plant nursery pots (12 cm in diameter, 12 cm in height) with potting media (sphagnum peat moss) and maintained simultaneously in two walk-in coolers (4 °Cand 10 °C respectively) and one environmental chamber (20 °C) with different temperature treatments for cold acclimation, respectively (Table 1). The potted seedlings were watered twice a week as needed based on gravimetric techniques (Timmer and Armstrong, 1989). Photoperiod was set at 9 h and did not vary for the potted seedlings (except group D). The remaining ten bare-root seedlings (group G) were moistened with water, placed in a black plastic box, and stored in a walk-in cooler at 4 °C for 4 weeks. After cold acclimation, the seedlings were moved to a Delfield-Alco 6000 series freezer for FD. The temperature of the seedlings was reduced to −15 °C at a rate of 5 °C per hour, and remained at this temperature for 3 h. The seedlings were then removed to a cold chamber to thaw at 4° C for 24 h (Zapata-Valenzuela et al., 2015). After thawing, the seedlings were removed to the environmental chamber and watered twice a week to allow damage symptoms to develop.

Table 1
Cold acclimation treatments for seedlings.

Group	Day temperature/°C	Night temperature/°C	Time
A	20	4	4 weeks
B	10	4	4 weeks
C	4	4	4 weeks
D	10 (full exposure)		4 weeks
E	20/10	10/4	2 weeks/2 weeks
F	−15		3 h
G	4 (full dark)		4 weeks

2.2. FD measurements

FD was assessed on a weekly basis and was subjectively scored on a scale of 0 to 100% in increments of 10%, with 0% indicating little or no browning of the leaves and 100% indicating complete leaves browning (Zapata-Valenzuela et al., 2015).

2.3. Sample preparation

Leaves were pulled off the seedlings. Roots were washed with distilled water and then cut at the root-collar. Leaves, roots and stems were oven dried at 105 °Cfor 30 min and then at 70 °C for 2–3 days, and then were ground using a Wiley Mill. To improve the precision of NIR models, the ground powder samples were passed through 80-mesh (Jiang et al., 2014), and then stored in airtight containers at room temperature, in the dark, until collection of the NIR spectrum.

2.4. NIR measurements

The 80-mesh powder samples were placed on the diamond plate of the NIR machine (PerkinElmer spectrum 400 FT-NIR spectrometer, Waltham, MA, USA). The reflectance spectra were collected in the range of 10,000–4000 cm^{-1} (1000–2500 nm with 1 nm intervals) from an average of 32 scans and no zero filling (Jiang et al., 2014).