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Quantitative dynamics of stem water soluble carbohydrates in wheat can be monitored in the field using hyperspectral reflectance

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ABSTRACT

The capacity of wheat to store water soluble carbohydrates (WSC) in the stem is regarded as a promising trait to buffer yield in environments with limited water availability. A high throughput, field-applicable, phenotyping technique would not only benefit agronomy/physiology applications but also help its quantification in wheat breeding programmes. The aim of this study was to evaluate if it was possible to estimate the concentration (WSCc, mg g^{-1}) and amount (WSCa, g m^{-2}) of stem WSC non-destructively and in situ using hyperspectral data obtained in wheat canopies, as opposed to currently available labour intensive laboratory methods. Hyperspectral reflectance data were obtained proximally at varying developmental stages from the canopy of wheat trials with a limited number of related genotypes growing under a range of management treatments, in two successive years. Data were calibrated, firstly independently for each year and then jointly, to provide a measure of stem WSC using partial least squares regression on wavelengths in the range of 350–1290 nm. Pre-treated spectra (second derivative) enabled calibrations for the combined years with concentration (WSCc, mg g^{-1}) ($r^2 = 0.90$) and amount (WSCa, g m^{-2}) ($r^2 = 0.88$) of water soluble carbohydrate in the stems. In addition, from the same measurement, other canopy properties, leaf area index and canopy water content, could be simultaneously predicted. This study has shown that calibration models from canopy level data can robustly predict the dynamics of stem WSC throughout crop stages and treatments, while at the same time including variation in indices diagnostic of crop water and cover status, such as the Water Index and Enhanced Vegetation Index. Promising WSC prediction using spectral data below 1000 nm needs to be investigated further, in order to harness the potential for impact using low cost silicon detectors.

1. Introduction

Economically important grasses from temperate areas, such as wheat, barley and oats, store water soluble carbohydrates (WSC) in the stems (Vijn and Smeekens, 1999; Halford et al., 2011). These carbohydrates, upon mobilisation to the grains, sustain the grain filling rate when photosynthesis declines. WSC can make up for a significant proportion of yield, from 10 to 20% under non-stressed conditions (Gebbing and Schnyder, 1999; Shearman et al., 2005; Dreccer et al., 2009) to up to 50% under severe stress, such as heat or terminal drought (Blum, 1998 and references therein; van Herwaarden et al., 1998a, 1998b) and possibly disease (Blum, 1998). In wheat, the main fraction of the WSC pool is constituted by fructans, which are linear and branched fructose polymers synthesised from sucrose (Kuhbauch and Thome, 1989; Vijn and Smeekens, 1999). Fructans are produced and stored in parenchyma cells encircling the vascular bundles of stem internodes in wheat and, unlike starch, are water soluble. Accumulation starts when internodes are still elongating and continues during grain filling, but total amounts and extent depend on environmental conditions (Bonnett and Incoll, 1993; Goggin and Setter, 2004; Dreccer et al., 2009). The two last internodes below the spike, i.e. peduncle and penultimate node, have the higher WSC levels (Wardlaw and Willenbrink, 1994; Gebbing, 2003). Traditionally, WSC levels are determined after destructive harvest and fine particle tissue grinding by a wet chemistry method (Yemm and Willis, 1954) but can also be evaluated in similarly ground samples using near infrared reflectance (NIR) spectroscopy calibrated against the wet chemistry method (Smith et al., 1998; Wang et al., 2011).

The ability to store and remobilise large amounts of stem WSC is a desirable trait to incorporate in wheat germplasm during the breeding process (Rebetzke et al., 2008a) for environments where terminal water stress occurs frequently, such as the Western or

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Northern growing regions of the Australian wheat-belt (Asseng and van Herwaarden, 2003). In a study involving three wheat mapping populations, Rebetzke et al. (2008b) showed a large range of WSC among the progeny, measured as concentration (WSCc, mg g^{-1}) or amounts (WSCa, g m^{-2}). Once a set of lines has been scored for WSCc or WSCa, the rankings are moderately to highly repeatable across environments (see also Dreccer et al., 2009). Consistency of rankings is lower for WSCa than WSCc, as its calculation involves the additional variation in biomass production (Rebetzke et al., 2008b). More importantly, while data support a role for selection for WSCa as a trait that can ensure stable yield and large grain size, the complex genetic control (many independent quantitative trait loci of individual small effect) limits the direct use of marker assisted selection in breeding programmes (Rebetzke et al., 2008b). This signals the need for a high throughput, remote sensing based phenotyping methodology that can be applied in early generation selection, where the number of lines under evaluation is high, and estimates of line rankings can assist in moving forward in the programme a selected group for further testing across environments. A similar concept is currently applied successfully in tree breeding, where complex wood quality and yield traits are evaluated in-forest using a portable NIR system and help in selection or association mapping (Meder et al., 2011; Dillon et al., 2012). Other potential uses of the remotely sensed estimates of stem WSC in wheat would be at the paddock level, to use as an input to a crop simulation model to estimate the buffer capacity of the crop and add accuracy to yield predictions supporting growers' decisions regarding advanced sales of grains under a range of likely seasonal scenarios.

The aim of this study was to evaluate if it was possible to estimate the concentration and amount of stem WSC non-destructively and in situ using a proximal (or near field) hyperspectral sensor to obtain spectral data from the canopy (and not the stem directly), as opposed to currently available labour intensive methods involving harvesting, processing, grinding and laboratory protocols (wet chemistry and/or NIR spectroscopy). Our assumption was that once stems are exposed they contribute to light scattering and canopy reflectance directly, whereas in plants starting stem elongation, differences in stem WSC would be related to differences in leaf composition. This would allow for indirect measurement of the stem WSC form spectral data obtained on the canopy, thereby enabling proximal (or even remote) sensing of stem WSC level. In this initial investigation, the focus was on capturing the seasonal dynamics, under a range of treatments. Therefore the method was developed collecting data throughout the season, using wheat lines from the same population, i.e. recombinant inbred lines, contrasting for WSC level but similar for height and phenology, under a range of management conditions (Dreccer et al., 2009, 2013). Additionally, the relationships between WSC level, canopy water content and leaf N content, as well as reflectance indices such as the Water Index (WI) (Penuelas et al., 1993) and the Enhanced Vegetation Index (EVI) (Huete et al., 1994) were explored. These associations were investigated among other reasons because stems with high WSC have been associated with higher dry matter content (Xue et al., 2009) and high WSC levels have sometimes (e.g. McIntyre et al., 2011) but not always (e.g. Dreccer et al., 2013) been negatively related to the assimilation of nitrogen.

2. Materials and methods

2.1. Trials

Ten field and rainout shelter trials were conducted during 2006 and 2007 growing seasons (Table 1). Treatments were aimed at expanding the WSC range and exploring a wide spectrum of agronomic conditions. Management treatments consisted of manipulating plant density, sowing dates, nitrogen and/or water availability. The genotypic component consisted of 2–4 recombinant inbred lines from the Seri/Babax population (Olivares-Villegas et al., 2007) contrasting for WSC accumulation (Dreccer et al., 2009), plus the parental lines included in some of the trials. The experimental site was at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) field station in Gatton (Queensland, Australia, 27.55° S lat, 152.33° E long). The soils were Black Vertosols which can hold up to 250 mm of plant available water over the maximum root depth of 180 cm. Plots were maintained weed, pest and disease free and nutrients were non-limiting unless otherwise indicated. Weather data were downloaded from the local meteorological stations (Australian Bureau of Meteorology, SILO patch-point dataset, www.bom.gov.au/silo) or measured at the trial. Trials were either randomised block designs with a single factor (genotype) or factorials (e.g. genotype × density) with a row-column design, all with 3 or 4 replicates. Experimental plots were seven rows wide, at 0.22 m inter row distance (or 0.20 m in 2006) and 8 m long except in the rainout shelter trials, where plots were 5 m long.

2.2. Measurements

2.2.1. Canopy reflectance

Canopy reflectance was measured in the 350–2500 nm range with a hyperspectral radiometer (FieldSpec® 3, Analytical Spectral Devices, Boulder, CO), which has a spectral resolution of 3–10 nm and a sampling interval of 1.4 nm in the 350–1000 nm region and 2 nm for the region between 1000 and 2500 nm. Readings were taken on days with clear sky around solar noon, in reflectance mode, after standardisation with a 99% Spectralon panel (Spectralon, Labsphere Inc., North Sutton, NH), a polytetrafluoroethylene surface of near perfect diffuse reflectance and relatively flat spectral distribution in the UV–VIS–NIR region of the spectrum. The spectroradiometer was mounted on a 4-wheel drive motorbike fitted with an extensible boom (following Rodriguez et al., 2005) that held the unfocussed fibre optic (FOV = 25°) perpendicular to the ground and over the plots, with the fibre head centred on row 4 of the plot, at a nominal height of 1.35 m above the soil resulting in a nominal sampling spot of 60 cm diameter at soil level (Fig. 1a and b). Two recordings consisting of averages of 50 scans each were taken from each plot at each sampling. In this manner we distinguish between remote sensing data acquisition using satellite or air-borne sensors and the near-field or proximal sensor used in this study.

2.2.2. Crop measurements

A bordered biomass cut totalling 0.5 m row was taken on the spot where the spectroradiometer sampled, immediately after the measurement. Stems and spikes were counted, plants separated in organs (stem + sheaths, spike, leaf, senesced (≥50% yellow leaf) and fresh weight recorded). Green leaf area was measured (for calculation of Leaf Area Index, LAI) and organs dried at 70 °C for three days and weighed. Crop phenological stages were recorded using the decimal code (DC) of Zadoks et al. (1974), with anthesis diagnosed when 50% of the spikes had anthers extruded (DC65). Across trials, samples were taken at one or more of the following stages (1) from start of stem elongation till the swelling of the second node was detectable (DC30-32), (2) from early to full boot, i.e. when the spike is enclosed in the sheath of the flag leaf (DC40-45), (3) once spike emergence was complete, from the beginning of flowering to the beginning of grain growth (DC60-70), (4) during the "milk" stage or active grain filling (DC70-80) and (5) during the "dough" stage of grain filling, when grains have increasingly higher content of solids (DC80+). On occasions, biomass cuts at key stages, e.g. DC60-70 in