Chapter 16: Water soluble carbohydrate content

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Water soluble carbohydrates (WSC) are sugars such as fructans, sucrose, glucose and fructose which are accumulated in the stem as reserves. WSC accumulate up to and around anthesis and are partitioned to the stem, from where they are later available as a reservoir for remobilization to the developing grains. These reserves are an important source of carbon for grain-filling as grain demand frequently exceeds current assimilation, potentially contributing 10-20% of the grain yield under favorable conditions. In particular, this trait has been shown to be adaptive for drought, heat and/or disease stress tolerance when the supply of carbohydrates from photosynthesis during grain-filling is inhibited/limited and stored WSC may contribute up to 50% of the grain yield. For instance, under terminal drought stress (e.g., in Australian environments where deep soil water is not available), WSC have been shown to buffer biomass production, grain yield and harvest index (HI), associated with increased water uptake (WU) and water-use efficiency (WUE). Trait-based breeding for genotypes with greater stem storage and remobilization of WSC may result in improved grain-filling and increased yields.

Accumulation of WSC is a function of genetic characteristics –specifically the stem's storage capacity- as well as environment which will influence the former as well as the subsequent availability of assimilates for storage. The total amount of WSC may be 40% or more of the total stem dry mass when WSC levels peak in early grain filling (Kiniry, 1993; Reynolds *et al.*, 2009). WSC storage may show trade-off with investment in other sinks such as deeper root growth (Lopes and Reynolds, 2010), tiller survival or developing spikes. The major proportion of WSC are located in the peduncle and penultimate internode, so taller lines with long peduncles tend to have a larger capacity. WSC may be expressed as a concentration in dry mass (either as a percentage (%WSC) or as mg g^{-1}) to demonstrate the potential stem storage capacity of the genotype; or as the content per stem (g $stem^{-1}$) or per unit area (g m^{-2}) to give an absolute measurement of the carbohydrates available to the grain.

Site and environmental conditions

Samples can be taken under most environmental conditions. However, it is important that the plant surfaces are not wet from dew, irrigation or rain.

Time of day

Samples should be taken in the morning –as this is coolest time of the day– to reduce carbohydrate losses from respiration, and allows time for same-day processing.

Plant developmental stage

Measurements can be taken at any developmental stage from the end of stem elongation, and/or at regular intervals from mid anthesis to physiological maturity, depending on the experimental objectives/ timing of peak stress:

- For peak WSC: take samples at anthesis +7 (for drought) to 14 days (for favorable conditions). Note that in severely stressed conditions the peak WSC may occur before anthesis.
- For measurement of changes in WSC accumulation and remobilization: take sequential samples from anthesis to physiological maturity; every 7-14 days.

Number of samples per plot

Take one sample of 20 culms per plot.

Procedure

The following procedure describes the determination of WSC concentration from randomly selected fertile main culms, alternatively culms can be selected from the in-season biomass samples taken at anthesis +7days (see this volume, Chapter 15). See Schematic 16.1.

Take the following equipment to the field:

- Pre-labeled paper bags
- Secateurs/knife

Advice on taking measurements

Collect the stem samples in paper bags which have adequate ventilation to allow uniform drying (e.g., with holes punched in the bag). It is important that samples are kept cool and processed, and dried, as quickly as possible to reduce respiratory losses of carbohydrates – typically within 2 hours of cutting.

Sampling for WSC is often combined with in-season biomass sampling and partitioning (see this volume, Chapter 15). Ensure to plan sampling approach carefully to allow for maximal data collection/economy of sampling (e.g., data on partitioning weights can be collected on the same 20 culm sample). The leaf lamina and/or leaf sheath may also be analyzed for WSC separately, or not removed from the stem for 'whole stem' analysis.

Preparations

1. Prepare labeled paper bags for oven drying: use medium-sized bags with holes punch in them to increase oven drying efficiency (use a hole-punch, and ensure you have a similar hole pattern in each bag).

Field measurements

2. Randomly select 20 fertile main culms from each plot, ensuring that all culms have a well formed spike (Figure 16.1A).
3. Place into a pre-labeled paper bag.

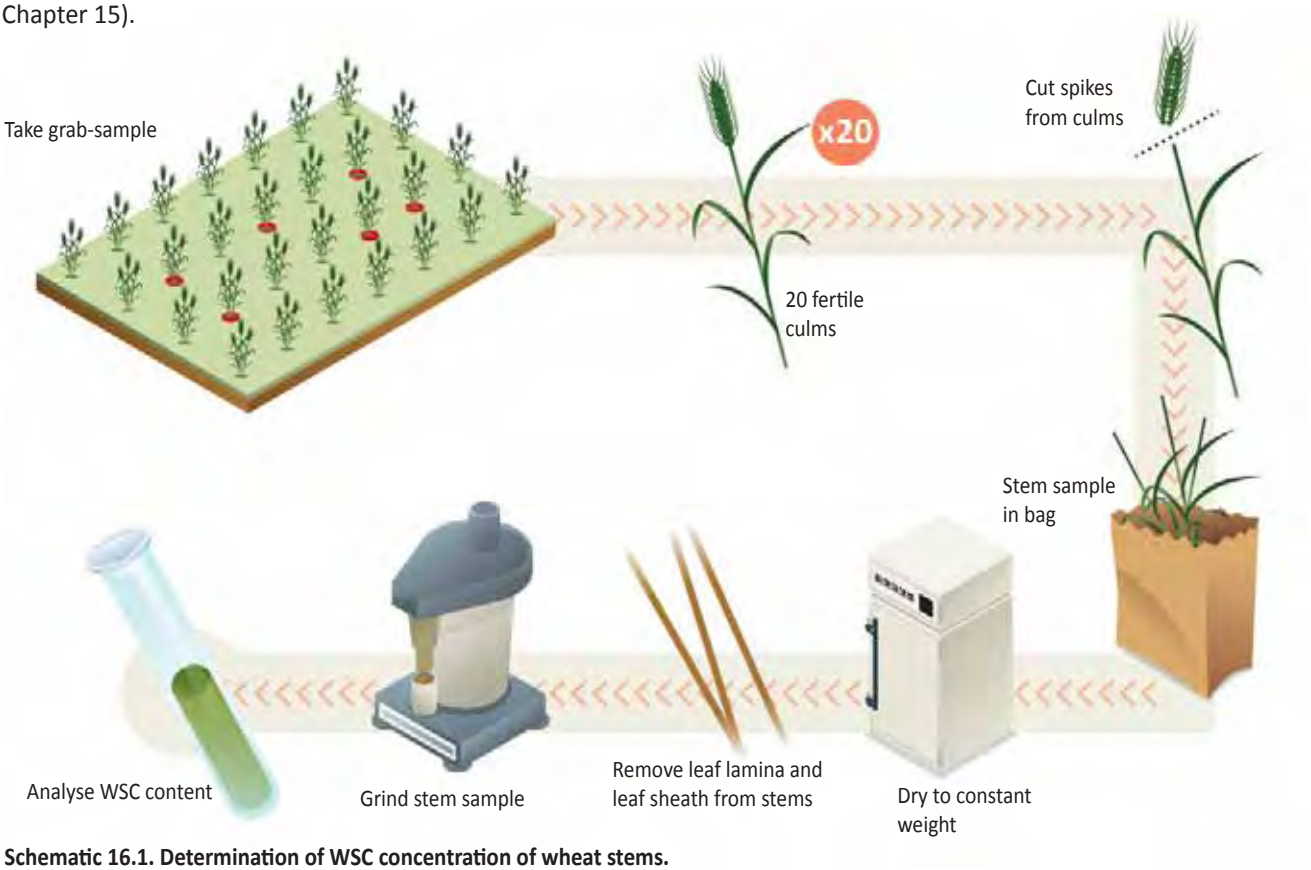
Or, randomly select a 20 culm sub-sample from the in-season biomass sample (as detailed in this volume, Chapter 15).

Laboratory measurements

4. Cut the spike from the stem at the spike collar.
5. Oven dry whole culm samples at 60-75°C until they reach constant weight (i.e., for at least 48h).
6. Remove the leaf lamina and leaf sheath from the stems (Figure 16.1B).
7. Weigh the dry stem sample (for calculation of WSC content per stem or unit area) (DW_20stems).
8. Grind the stem sample (e.g., using a mill with a 0.5 mm screen). Ensure to clean the mill carefully between samples (Figure 16.1C).
9. Place ground sample into a labeled envelope.

Analysis

Analysis of prepared samples is typically outsourced to a specialist laboratory: (A) by the Anthrone method (cost US$ 5.00 per sample), or (B) or scanned by near infrared reflectance spectroscopy (NIRS) using a calibration curve (cost US$ 0.50 per sample). NIRS is an indirect method, but has the advantage of also giving %N values when using a %N calibration curve. (see Figure 16.2).



Schematic 16.1. Determination of WSC concentration of wheat stems.

Anthrone method for WSC concentration
This is a quantitative colorimetric estimation for the carbohydrate content of a solution. A green color is produced when carbohydrates are heated with anthrone in acid solution (for details see Yemm and Willis, 1954).

Near infrared reflectance spectroscopy using calibration curves
Near infrared reflectance spectroscopy (NIRS) can be used to estimate WSC concentration using predictive equations developed and cross-validated using the results of chemical analyses by the Anthrone method. Samples are scanned at 1585-1595 and 1900-2498 nm. A different calibration curve is required for different developmental stages and environments. Note that when NIRS is used, it is recommended to replicate 5% of samples analyzed by the Anthrone method to check the calibration (see Figure 16.2).

Figure 16.1. Sampling for WSC content: (A) taking 20 stems in-field; (B) removing by hand the leaf lamina and leaf sheaves from dry stems; and, (C) grinding dry stem sample using a cyclone mill.

Data and calculations

Data is typically given as %WSC in dry matter. This can be used to calculate the WSC content per stem (g stem^{-1}) or per unit area (g m^{-2}):

WSC (g stem^{-1}) = %WSC × ((DW_20stems) / 20)
Equation 16.1

WSC (g m^{-2}) = WSC (g stem^{-1}) × stems m^{-2}
Equation 16.2

In optimal conditions, peak WSC concentration ranges between 10-25%; WSC content per 2 g stem is 0.2-0.5 g stem^{-1}; and, WSC content per m^{-2} at a stem density of 300 m^{-2} is 60-100 g m^{-2}.

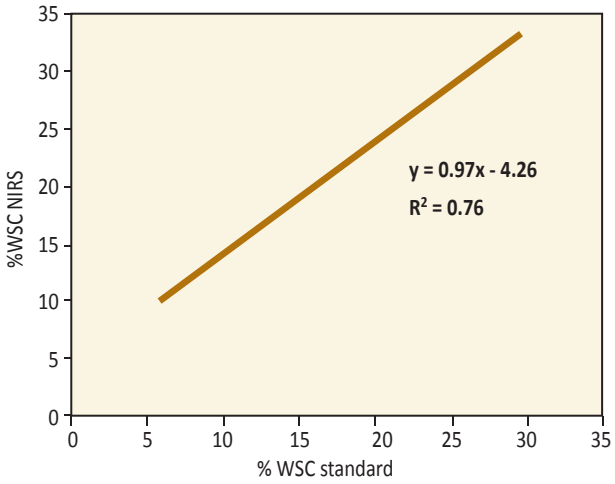


Figure 16.2. Calibration curve to estimate WSC concentration (%) from near infrared reflectance spectroscopy values at anthesis (adapted from Pinto *et al.*, 2006).

Troubleshooting

Problem	Solution
Large error variance in data.	Check that the mill is consistently grinding to 0.5 mm and sieve carefully to ensure good particle distribution within sample. When grinding samples, it is important that the mill is thoroughly cleaned between samples to avoid cross contamination. Ensure to re-dry samples before NIRS analysis to removed any reabsorbed moisture which may affect readings.

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