Non-structural carbohydrate partitioning in grass stems: a target to increase yield stability, stress tolerance, and biofuel production

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Abstract

A dramatic change in agricultural crops is needed in order to keep pace with the demands of an increasing human population, exponential need for renewable fuels, and uncertain climatic changes. Grasses make up the vast majority of agricultural commodities. How these grasses capture, transport, and store carbohydrates underpins all aspects of crop productivity. Sink–source dynamics within the plant direct how much, where, and when carbohydrates are allocated, as well as determine the harvestable tissue. Carbohydrate partitioning can limit the yield capacity of these plants, thus offering a potential target for crop improvement. Grasses have the ability to buffer this sink–source interaction by transiently storing carbohydrates in stem tissue when production from the source is greater than whole-plant demand. These reserves improve yield stability in grain crops by providing an alternative source when photosynthetic capacity is reduced during the later phases of grain filling, or during periods of environmental and biotic stresses. Domesticated grasses such as sugarcane and sweet sorghum have undergone selection for high accumulation of stem carbohydrates, which serve as the primary sources of sugars for human and animal consumption, as well as ethanol production for fuel. With the enormous expectations placed on agricultural production in the near future, research into carbohydrate partitioning in grasses is essential for maintaining and increasing yields in grass crops. This review highlights the current knowledge of non-structural carbohydrate dynamics in grass stems and discusses the impacts of stem reserves in essential agronomic grasses.

Key words: Carbohydrate partitioning, grasses, non-structural carbohydrates, parenchyma cells, sink–source buffering, stem, yield stability.

Introduction

Grasses have co-evolved with humans since the dawn of agriculture ∼10 000 years ago. They represent the most productive and widely grown crop family and provide a foundation for human life across the globe. Grain-producing grasses such as rice (Oryza spp.), maize (Zea mays), sorghum (Sorghum bicolor), wheat (Triticum aestivum), barley (Hordeum vulgare), and oats (Avena sativa) provide the majority of calories consumed by people worldwide (FAO, 2009). Sugarcane (Saccharum officinarum) and sweet sorghum produce the bulk of water-soluble carbohydrates, mainly sucrose, for use in human and animal foods, along with directly fermentable substrate for ethanol production (Waclawowsky et al., 2010). Forage grasses such as ryegrass (Lolium perenne), switchgrass (Panicum virgatum), and miscanthus (Miscanthus×giganteus) provide much of the biomass consumed by ruminant agricultural and wild pastoral animals as well as lignocellulose feedstocks for second-generation biofuel production (Mitchell et al., 2008; Somerville et al., 2010).

The process by which grasses produce, transport, and store carbohydrates underpins all aspects of yield traits. Despite our current knowledge of the location and
harvestable amount of these important carbohydrates, we are only beginning to understand the molecular, genetic, and physiological mechanisms that regulate their transport and allocation preceding harvest. Expanding such knowledge is vital to creating and implementing rational strategies to optimize whole-plant carbohydrate partitioning for increased productivity.

For many of the grasses, rate of gain in yield potential has been slowing while overall grain demands continue to increase. Yields from primary crops such as maize, rice, and wheat are predicted to decline over the next two decades in semi-arid regions of the world due to climate change (Lobell et al., 2008). In Africa, modern agricultural intensification has not yet been achieved and per capita cereal production has been declining since the 1960s (St.Clair and Lynch, 2010). Additionally, the merger of food and energy markets, induced by increased demand for biofuels, now puts an enormous strain on the supply and demand dynamics of agricultural commodities—greatly impacting the availability of grain-based foods worldwide (Oosterveer and Mol, 2010). When all of these factors are put into the same equation to predict the critical variable in the future of agricultural production, the physiology of the plant is the factor that is under the most pressure to change and will have the greatest impact compared with other agronomic factors (Rosenzweig and Parry, 1994; Gregory and George, 2011). Most importantly, many of the targeted traits relate directly to the physiology of sink–source dynamics and whole-plant carbohydrate partitioning (Fischer and Edmeades, 2010).

When we view carbohydrate partitioning on a whole-plant level, we find something interesting about the grasses—their stems. Many grasses, along with other monocots, such as pineapple and agave, store excess carbohydrates in the form of soluble sugars or sugar polymers within the vegetative tissues (Antony et al., 2008; Davis et al., 2011). Grass stems are capable of storing substantial amounts of soluble carbohydrates in the storage parenchyma cells that surround the vascular bundles located within internode tissues (Hoffmann-Thoma et al., 1996; Rae et al., 2005a).

The Green Revolution of the 1960s resulted in high-yielding wheat and rice varieties with altered morphology compared with the traditional land races, and resistance to devastating pathogens such as wheat rust (Borlaug, 2007). This dramatic increase in yield was largely due to a shift in the way grasses partition structural carbohydrates in the stem (Evenson and Gollin, 2003). Short statured, ‘semi-dwarf’, genotypes were selected: these varieties redirected more carbohydrate (Fedoroff et al., 2010). Another aim must be to decrease CO₂ emissions and aid in carbon sequestration by a reduction in tilling, which would also protect top soil degradation and erosion (Beddington, 2010). Manipulating stem non-structural carbohydrates (NSCs) provides one avenue to stabilize and increase productivity in the face of rapidly changing demand in the next century.

This review focuses on the roles of NSCs in grass stems and their impacts on sink–source dynamics, overall productivity, and implications for future uses in agriculturally and economically critical grass species. I will begin the discussion with an overview of the roles of NSCs in grass stems, then move to mechanistic models of radial transport, current knowledge of stem NSCs in agricultural grasses, and end with prospects and implications for stem NSC research.

NSCs in grass stems

What are the roles of stem carbohydrates in the grasses? The most widely accepted hypothesis is that grasses store excess carbohydrates in stem tissues to buffer source–sink interactions during different stages of growth and in varying environmental conditions (Schnyder, 1993; Asseng and Herwaarden, 2003; Slafer, 2003; Ruuska et al., 2006). This represents long-term buffering, unlike the short-term buffering role for transient starch and sugar reserves in the leaves, which vary on a diurnal basis. Stem storage parenchyma cells, that encircle the vascular bundle, are considered an in-route storage compartment, which theoretically could be a competing sink along the path to terminal sinks such as the roots and seeds (Wardlaw, 1990; Sadras and Denison, 2009). Such a dynamic has been suggested for the internodes of sugarcane and sweet sorghum which retain large amounts of carbohydrate after flowering (McBee and Miller, 1982; Walsh et al., 2005; Garside and Bell, 2009). However, in general, these stem storage carbohydrate pools do not compete with other sink tissues in the plant; rather, they store excess photosynthate during periods of low sink strength (Slafer, 2003). There are periods in the plant life cycle when carbohydrate production in source tissues exceeds the demands of sink tissues, and vice versa. This is in agreement with a more integrative view of sink–source interactions which emphasizes a co-limitation, rather than strictly sink or source limitation (Slafer, 2003; Álvaro et al., 2008; Acree and Slafer, 2011). For example in wheat, stem carbohydrates progressively accumulate in the stem until the stage of rapid grain filling when stem carbohydrates reserves become mobile and partition to the developing grain (Ruuska et al., 2006). Additionally, buffering confers an ability to tolerate catastrophes, such as pathogen or herbivore attack, and rapidly re-grow after such events.

Buffering either directly or indirectly increases the fecundity of the plant by increasing the ability to produce viable seeds. Additionally, placing the buffering storage pool away from the photosynthetically mature tissues may reduce feedback inhibition of photosynthesis due to excessive carbohydrate (Pollock et al., 2003). This phenomenon has
been documented in sugarcane where cultivars and species that do not accumulate high levels of sucrose in the stem have higher photosynthetic rates than the high-sucrose-accumulating cultivars (Irvine, 1975). Thus, a robust buffering capacity of the stem may be one reason why grasses seem to have considerable plasticity in sink–source dynamics, allowing them to be more readily modified for uses in high-production agriculture.

The nature of the plant life cycle also has a dramatic impact on whole-plant carbohydrate partitioning (Thompson and Stewart, 1981). Most of the grain-producing grasses are monocarpic annuals with only one chance at producing offspring before the life cycle ends. Thus, carbohydrate reserves buffer against extended periods of low light or drought, allowing plants to produce some viable seed. Perennials go one step further by storing carbohydrates in stems and roots, which may include rhizomes, to fuel re-growth of new tissues in the spring. Sugarcane is a perennial grass that stores carbohydrate in the stems and rhizomes (Matsuoka and Garcia, 2011). These reserves peak at the end of the vegetative cycle, and are used during flowering and seed production. After the sexual reproductive phase is complete, remaining stem NSC reserves are mobilized to produce new vegetative structures. This is why sugarcane is harvested before the reproductive phase begins (Komor, 2000). One way in which breeders have increased stem carbohydrate reserves is to delay reproduction and seed set. Due to this strong selection, a majority of sugarcane cultivars now are either sterile or the reproductive cycle has been dramatically delayed, sometimes for years (Rae et al., 2005a).

Radial transfer between the phloem and stem storage parenchyma

Storage and remobilization strategies depend to a large degree on the form in which carbohydrates are stored. Depending on the species, stem carbohydrates can be stored as soluble sugars, such as sucrose, fructans (soluble polymers of fructose), or starch (insoluble polymers of glucose) (Halford et al., 2011). The amount of stored carbohydrate in the stem also varies with the species and cultivar of grass and is dependent on the stage of development and growing conditions. Some grasses that have a central lacuna can also store carbohydrate in the chloroanchyma cells along the periphery of the stem.

Sucrose is stored in the stems of the Andropogoneae tribe, which includes C₄ grasses such as maize, sorghum, and sugarcane (Dillon et al., 2007; Glassop et al., 2010). In the mature internodes, sucrose degradation and synthesis are minimal. Since sucrose is also the only carbohydrate in the phloem sap of these species, direct transport mechanisms are hypothesized to be the primary driving forces for storage and remobilization. However, the minimal sucrose ‘futile cycling’ may play important roles in sugar signalling, sugar accumulation, and allocation of carbon to biological processes that may regulate transport (Rossouw et al., 2010). Temperate C₃ grasses, as well as rice, which grows in hotter climates, convert imported sucrose into carbohydrate polymers for sequestration, then convert them back into sucrose for translocation out of the stem (Halford et al., 2011). Thus, in these grasses, storage and remobilization is dependent on both transport mechanisms and metabolic processes within the stems.

The symplasmic path

A brief introduction to the models of transport between storage parenchyma cells and the transport systems is necessary for understanding carbohydrate partitioning processes in grass stems. Symplasmic transport is cell-to-cell movement of molecules through plasmodesmata (Fig. 1A).

**Fig. 1.** Putative models for radial transport in grass stems. Pathways for carbohydrate import into stem storage parenchyma cells are presented on the left. Pathways for re-mobilization and phloem loading from storage parenchyma cells are on the right. SE/CCC, sieve element–companion cell complex.
The driving forces behind symplasmic transport are mass flow and diffusion, driven by pressure and chemical gradients as well as respiration (Patrick, 1997; Slewinski and Braun, 2010). Downhill gradients are generated by metabolizing or converting sugars within the cell, or transporting them out of the cytosol into membrane-bound compartments such as the vacuole (Patrick et al., 2001).

Initial phloem unloading into developing and elongating grass internodes appears to be symplasmic, similar to the unloading mechanism in most rapidly growing vegetative sink tissues (Aoki et al., 2004). However, there is usually a dramatic change in metabolic and transport reprogramming during the switch from utilization sink to storage sink in the mature stem (Hoffmann-Thoma et al., 1996). At this transition, when sucrose is no longer used for tissue construction, some grasses may switch to an apoplastic transport mechanism (below) when high concentrations of sucrose begin to accumulate (Tarpley and Vietor, 2007).

The main storage compartment for soluble sugars within the storage parenchyma cells is the vacuole (Pollock and Kingston-Smith, 1997; Rae et al., 2009). In most plants, sucrose enters the vacuole and is cleaved by invertases, producing glucose and fructose (Koch, 2004). However, some grasses suppress invertase activity in the vacuolar lumen, as well as sucrose synthase activity in the cytosol, and store only sucrose in the vacuoles of the mature stem storage parenchyma cells (Tarpley et al., 1996; Tarpley and Vietor, 2007). Storage of sucrose in these cell types involves active sucrose transporters embedded within the tonoplast membrane (Rae et al., 2005a). For example, in rice, H⁺–sucrose symport from the vacuole lumen to the cytosol across the tonoplast membrane in most vegetative tissues was recently found to be mediated by the SUCROSE TRANSPORTER 2 protein (OsSUT2) (Eom et al., 2011). Plants that are homozygous for a null mutation in the OsSUT2 gene accumulate excessive sucrose in vacuoles and are impaired in whole-plant carbohydrate partitioning. Presumably the expression and function of OsSUT2 extends to the stem storage parenchyma cells, but has yet to be experimentally confirmed. Orthologues of the Arabidopsis tonoplastic monosaccharide transporters, AtTMT1 and AtTMT2, may mediate sucrose–H⁺ antiporter activity that shuttles sucrose from the vacuole across the monolayer membrane into the cytosol (Schulz et al., 2011). More work is needed to elucidate the functions of the tonoplastic sugar transporters in grasses, which may perform different functions in different species (Braun and Slewinski, 2009; Slewinski and Braun, 2010).

The apoplastic path

Cells that do not have sufficient plasmodesmal connections, or have connections that are blocked, use apoplastic transport to shuttle carbohydrates from cell to cell (Fig. 1B) (Rennie and Turgeon, 2009). Apoplastic transfer between cells is composed of both a linear efflux phase driven by passive movement though a membrane into the surrounding cell wall, and an energy-dependent transport component, necessary for retrieval from the apoplast (Patrick, 1997). The recently described SWEET family of passive sugar effluxors is most probably responsible for the linear phase of sucrose export from cells (Chen et al., 2011). The ‘active’ phase, governed by Michaelis–Menten kinetics, results from energy-dependent transporters that span the membranes (Patrick, 1997). Active transport of sucrose and monosaccharides is mediated by sucrose and monosaccharide/proton symporters or antiporters. Apoplastic transport, both the passive linear component and the active component, are presumed to function at most cell wall interfaces. However there are some interfaces where cell walls are highly suberized or lignified, and thus dramatically restrict apoplastic movement (Rae et al., 2005a). Symplasmic transport though plasmodesmata would be the most likely path of flux from cell to cell in this case.

Sequestration by polymer synthesis

Some grasses store NSCs in the form of polymers in the storage parenchyma cells, either as starch or as fructans (Fig. 1C) (Morvan-Bertrand et al., 2001; Xue et al., 2008). Most tissues that produce carbohydrate polymers have high symplasmic connectivity to surrounding cells. Polymerizing carbohydrates within the cells reduces the osmotic strength of the sugars, thus plants are able to store more reduced carbon per unit volume (Spoelen and Nelson, 1988). Polymer synthesis, which is mostly restricted to plastids or vacuoles, also produces a chemical gradient necessary to drive passive flux in the direction of the storage cell when polymers are formed, or in the direction of export when polymers are degraded and converted back to sucrose (Schnyder et al., 1988; Patrick, 1997).

Grass species that are indigenous to temperate climates and that regularly experience periods of drought and frost typically utilize fructans as a carbohydrate storage pool (Halford et al., 2011). Fructans are synthesized in the vacuolar lumen from imported sucrose (Pollock et al., 2003). Either sucrose:sucrose 1-fructosyltransferase (1-SST) or sucrose:fructan 6-fructosyltransferase (6-SFT) adds a fructose moiety to the fructose on a sucrose molecule to initiate fructan synthesis. 6-SFT then adds consecutive fructose moieties to the previously attached fructose moiety to produce an elongating fructose polymer anchored on a sucrose base (Chalmers et al., 2005). The length and branching of the fructan polymer vary widely between tissues, species, and environmental conditions. Usually a higher concentration of fructans is associated with a higher degree of polymerization (Ruuska et al., 2006, 2008). When reserves are mobilized, fructans are hydrolysed in the vacuole by fructan exohydrolases and invertases into free fructose. Fructose is then exported from the vacuole, re-synthesized into sucrose in the cytosol, and exported into the phloem by the apoplastic mechanism (Berthier et al., 2009). (For further discussion and review of fructan metabolism, see Pollock and Cairns, 1991; Cairns, 1992;
Schnyder, 1993; Vijn and Smeekens, 1999; Van den Ende et al., 2004; Chalmers et al., 2005).

Only a few grasses use starch as a primary reserve in the stems (discussed below). Unlike fructans, starch is formed in the plastids/amyloplasts by metabolic events both in the plastid and in the cytosol. Remobilization requires the breakdown of starch in the plastids, then sucrose re-synthesis in the cytosol before export from the cell. For further discussions on starch metabolism, see Zeeman et al. (2010) and Weise et al. (2011).

**Mechanisms of partitioning and impacts in the agricultural grasses**

**Maize**

Sucrose in the maize stem is considered to be the major component of the ‘buffering system’ in the sink–source relationship (Daynard et al., 1969). Indeed, one hypothesis to explain the origins and domestication of maize is that teosinte, the ancestor of maize, was originally cultivated for the soluble sugars in the stems (Willaman et al., 1924; Singleton, 1948; Smallley and Blake, 2003). Stem sucrose was most probably extracted by chewing or sucking the stalks, then fermenting the extract into a ‘stem sugar beer’. Subsequent development as a grain crop may have occurred when favourable mutations made the teosinte seeds more palatable and abundant, and thus more amenable for human consumption. Subsequently, seeds, not stem sugar, were selected.

Stem sugar in maize is now considered a secondary trait and has received minimal attention in the last few decades, mainly due to the focus on primary traits such as yield, lodging resistance, drought tolerance, and nutritional content. However, with the revitalization of liquid biofuels, maize stem sugar has, again, become a central target for breeding and biotechnology as a source for renewable energy (White et al., 2011). Previous interest in the production of ethanol from maize stems peaked in the late 1970s and early 1980s, when oil prices rose and prompted interest in alternative energy sources, in a trend similar to that of recent years (Widstrom et al., 1987).

Sucrose intercellular transport in maize stems is presumed to be apoplastic (Setter and Flannigan, 1986). In the storage parenchyma cells, sucrose is transported in, stored, and effluxed unaltered. Reducing sugars and starch contribute minimally to total stem carbohydrate reserves. Hexoses usually do not fluctuate with change in overall stem dry matter accumulation/depletion or by alterations in the overall source–sink balance of the plant (Sayre et al., 1931; Setter and Flannigan, 1986). Thus partitioning in the stem is presumably due to the activity of sucrose transporters located in the plasma membranes of the companion cell–sieve element complex and the adjacent parenchyma cells, as well as in the membranes of the tonoplasts. To date, only one member of the sucrose transporter family of maize, ZmSUT1, has been characterized. ZmSUT1 is expressed in photosynthetic leaves and functions in phloem loading in source leaves (Slewinski et al., 2009, 2010). The mechanism of re-mobilization and phloem loading in the stem remains unclear. It is possible that phloem loading also occurs via an apoplastic mechanism that involves ZmSUT1, which has the possibility of mediating both phloem loading and unloading depending on both the chemical and electrochemical gradients that are present (Carpaneto et al., 2005). Characterization of the remaining family members will hopefully reveal which sucrose transporters are involved in stem carbohydrate partitioning (Braun and Slewinski, 2009). It will be interesting to see if the sucrose transporter transcript level or protein abundance in both the vascular and storage parenchyma cells is correlated with increased phloem unloading and storage capacity in stems, thus providing a target to manipulate stem NSC reserves in maize.

Sink–source manipulations also provide some insight into the buffering capacity of the maize stem. For example, when leaves above and below the ear are removed at the time of silking, plants accumulate less sucrose in the stems compared with unaltered control plants (Jones and Simmons, 1983). When ears are removed, sucrose accumulates to a much higher concentration relative to the defoliated and control plants (Fig. 2) (Van Reen and Singleton, 1952; Hume and Campbell, 1972). Major contributions of the stem NSC reserves can also be seen when plants in the mid to late stages of grain filling are defoliated by herbicides (Donaghy et al., 2008). When photosynthesis is compromised, maize stems enhance re-mobilization of stem NSCs, while accelerating the kernel filling and maturation process (Allison and Watson, 1966; Rajcan and Tollenaar, 1999). This suggests that leveraging stem reserves may be one way to increase yield stability in environments where either photosynthesis, or the photosynthetic structures, are damaged in the later phase of kernel maturation.

![Dry matter partitioning in maize](http://jxb.oxfordjournals.org/)

**Fig. 2.** Dry matter partitioning in the vegetative tissues and the ear of maize over the life of the plant. Adapted from Sayre et al. [Journal of the American Society of Agronomy 23, 751–753 (1931), with permission, copyright American Society of Agronomy]; Allison JCS and Watson DJ. 1996. The production and distribution of dry matter in maize after flowering. Annals of Botany 30, 365–381, with permission from Oxford University Press; and Hume and Campbell (1972) with permission from the author.
This conclusion is further supported by studies using radioactive carbon tracers that show that stem NSC reserves contribute little to final grain fill under normal field conditions (Clique et al., 1990), but play a significant role under suboptimal conditions (Jurgens et al., 1978; Setter et al., 2001). For example, when $^{14}C$-labelled sucrose is injected into the apoplastic of the stems of leafless explants of maize, 30% of the radioactivity is recovered in the kernels 2 d later (Setter and Meller, 1984). This suggests that although stem carbohydrates do not directly increase yield potential in maize under high input agricultural practices, they greatly contribute to yield stability when plants are challenged by environmental and biotic stressors, or are grown on marginal and rain-fed lands.

Photosynthesis in maize leaves is at a maximum during silking, then steadily declines during the phase of rapid grain filling (Ying et al., 2000). Stem sucrose, along with declining current photosynthate, provides the steady stream of carbohydrates needed for efficient grain filling. Under normal field conditions, stem carbohydrates accumulate until the mid-phase of grain filling, when concentrations begin to plummet (Fig. 2) (Sayre et al., 1931; Hume and Campbell, 1972). This coincides with the start of rapid starch storage in the endosperm (Setter and Flannigan, 1989). High-grain-yielding varieties tend to have low amounts of stem carbohydrate that steadily decrease over time. In contrast, lower yielding varieties tend to have high concentrations of stem sugars that gradually increase during the grain-filling period (Van Reen and Singleton, 1952). Interestingly, when the ears of either the high- or low-yielding varieties are removed, stem carbohydrates reach equivalent amounts in both varieties (Daynard et al., 1969).

This suggests there are no differences in the transport or storage capacities in the two varieties; it is utilization by the developing ear that impacts stem carbohydrate reserves.

Stem NSC reserves may also be essential to stabilize carbohydrate availability during the early phases of ear development when sink strength capacity of the grain is determined (Borrás et al., 2004). The number and size of cells in the endosperm, which regulates the entire carbohydrate storage capacity by determining the total number of starch grains, are established before the phase of rapid biomass accumulation (Borrás and Westgate, 2006; Gambin et al., 2007). These early events determine the final yield potential for the kernel. Disruptions in water or carbohydrate availability during this time greatly reduce the sink potential of the kernel, leading to smaller kernels even if excess carbohydrate is available during the later phase of rapid grain fill (Borrás et al., 2004). Additionally, disruptions in water and carbohydrate supply can lead to floret abortion in the ear, which dramatically decreases the yield capacity of the plant (Setter and Flannigan, 2001; Wang et al., 2002; Yu and Setter, 2003). However, some of these detrimental effects can be reduced by injecting sucrose directly into the stems of stressed plants, reducing floret abortion and maintaining grain filling (Hiyane et al., 2010).

Standability is a critically important trait for mass production agriculture (Betrán et al., 2003; Flint-Garcia et al., 2003). Plants must remain erect for harvesting, and to ensure proper grain quality by keeping grains away from the soil surface. Unfortunately, many hybrids with high yield potential suffer from lodging susceptibility due to weak stem architecture or late season stem rot (Mortimore and Ward, 1964). However, it was noticed early in hybrid development that concentrations of stem soluble sugars have a positive correlation with standability in maize (Campbell, 1963; Mortimore and Ward, 1964). Cultivars and hybrids that have high concentrations of stem carbohydrates have greater standability. How are stem sugars and lodging resistance connected? One possibility is that high sugar concentrations within the stem may keep the cells alive longer, presumably due to the active component of carbohydrate remobilization (Campbell, 1963). This may prevent degradation from opportunistic necrotrophs which degrade dead tissues and compromise the structural integrity of the stalk before the grain is harvested. Stem sugar storage may be an essential component of the ‘functional staygreen’ trait, the delay of senescence at the end of the season or during periods of environmental stress (Thomas and Howarth, 2000), providing an alternative transient sink for photosynthate, while also prolonging standability and photosynthesis for grain fill.

The diversity of maize genetic resources along with the tools currently available for breeding suggests that leveraging stem NSC buffering capacity is an achievable objective over the short term. Carbohydrate concentrations and volumes of maize stems appear to have high heritabilities (Widstrom et al., 1984). Additionally, maize displays a vast range of phenotypic and genetic variability for the trait (Daynard et al., 1969; White et al., 2011). Thus, there is considerable potential to breed high-yielding maize lines for direct stem sucrose production, as well as to breed it as a secondary trait to increase standability, drought tolerance, and yield stability. Several lines of high sugar ‘Sweet Maize’ have already been developed and marketed as forage crops. These include the Cargill High-Sugar 50 line (Marten and Westerberg, 1972) and the Connecticut 103 inbred line (Singleton, 1948; Van Reen and Singleton, 1952). The strategy behind the Cargill High-Sugar 50 line is to prevent or delay the production of fertile ears, therefore saturating the stem with sucrose that would have been partitioned to the grain. Unfortunately, storage in the stem cannot fully compensate for the loss of storage in the grains, resulting in a net reduction of carbohydrate accumulation in this line compared with normal grain maize when used as silage. Many farmers prefer to grow normal grain maize, giving them the option to use it for grain or forage, rather than only having a forage crop (Marten and Westerberg, 1972). In the case of the Connecticut 103 inbred line, internodes were found to have significantly more vascular bundles, little to no pith, and high juice volumes when compared with other inbred lines (Singleton, 1948). The stem anatomy and physiology of Connecticut 103 resembles more those of high-yielding cultivars of sugarcane and sweet sorghum, rather than those of traditional maize stems.
Can breeders develop maize that has both high stem sucrose and grain yields? Is it possible to overlay the grain productivity of the temperate grain maize-producing regions with the productivity of sugarcane fields of the tropics? Sweet corn production could provide a model for such a system (Willaman et al., 1924). Sweet corn is harvested when plants are still photosynthetically active. The ear has been filled to the desired capacity of carbohydrates and removed for human consumption, but the plant is programmed to continue production and allocation of carbohydrates until the genetic termination point has been reached. The green stalks, left standing without the strong sink of the ear, fill with sugars derived from residual photosynthesis in the leaves. These stalks have reached the peak of sugar storage capacity and can be harvested and treated in the same way as a sweet sorghum crop for processing (Singleton, 1948). The next challenge for such a maize plant, as well as high-grain-yielding sweet sorghum varieties, will be to extract the grain and sucrose-rich stem juice during harvest in the field, while leaving the vegetative biomass to replenish the soil organic matter.

There is considerable potential to exploit stem NSCs in maize. Controlling NSC reserves in maize stalks has been proposed as one way to mitigate the deleterious effects of seasonal instability on maize yields in environmentally unpredictable regions (Shiferaw et al., 2011). For example, leveraging the stem NSC buffering system may be crucial in new varieties produced for developing countries, such as sub-Saharan Africa, Asia, and Latin America, where maize is grown as a rain-fed crop, and thus more prone to experience dramatic shifts in water availability. To be most effective in maize, NSC reserves must be balanced with remobilization to ensure the highest obtainable yields, without yield penalties caused by excessive sucrose storage in the stem (Monneveux et al., 2008).

**Sorghum**

Although sorghum only plays a modest role in highly industrialized agriculture, it has been, and will continue to be, an essential crop for many subsistence farmers in developing countries. Due to sorghum’s drought tolerance, it has become an important staple food for humans and animals in many arid regions of the world (Garrity et al., 2010). Sweet sorghum varieties are also gaining prominence as a temperate substitute for tropical sugarcane to produce sucrose for food and ethanol production. Thus, there is considerable interest in developing sweet sorghum varieties to fuel next-generation biofuels in non-tropical and marginal areas.

Sorghum presents an interesting model to study stem NSC partitioning and its effects on other agronomic traits due to the diverse varieties that are widely grown. The main classes of sorghum are fibre, forage, grain, and sweet (Dolciotti et al., 1998). The two most commonly grown types are grain sorghum, grown for seed, and sweet sorghum which is grown for the sucrose-filled stalks similar to sugarcane. The primary difference between the two lines is that one partitions carbohydrate mostly to one sink, the grains, and the other to two sinks (grains plus stem). In the case of sweet sorghum, stem storage parenchyma cells have been bred to be terminal sucrose sinks, rather than transient storage compartments. Thus selection has produced a dominant stem sink that appears to have higher priority for sucrose storage than growing vegetative tissues or reproductive structures (McBee and Miller, 1982). Grain sorghums usually contain less carbohydrate in the upper internodes compared with sweet sorghum varieties, presumable due to increased partitioning to the grains in the apical panicle.

Sorghum is similar to maize in that it employs the apoplastic mechanism of importing, storing, and remobilizing sucrose in mature internodes of the stem (Tarpley and Vietor, 2007). Sucrose is neither converted to other substrates nor metabolized in the process. For example, when internode tissues are infused with sucrose carrying a tritium label on the fructose moiety, 81% of the sucrose is recovered unchanged 1 d after infusion, suggesting that most of the sucrose translocated into the stem storage parenchyma is not hydrolysed or re-synthesized. In addition, sucrose synthase and sucrose phosphate synthase activities are not correlated with sucrose carbohydrate accumulation within the internode (Hoffmann-Thoma et al., 1996). Thus, the majority of sucrose transport is most probably mediated by the sugar transporter proteins, for which expression patterns and functions remain unknown (Braun and Slewinski, 2009).

Sugar accumulation in internodes rapidly increases once internode elongation has ceased (Hoffmann-Thoma et al., 1996). It is still unclear when the internodes switch from being symplasmic utilization sinks to apoplastic storage sinks. A decline in sucrose cleavage, presumably by reducing the activities of sucrose synthase and invertase activity, has been associated with this possible transition point (Tarpley et al., 1994, 1996). There are, however, notable exceptions. Some sorghum cultivars such as ‘Tracy’ have been bred to store hexoses at concentrations similar to those of sucrose in the stem tissues. This line is particularly useful in producing commercial syrups (Hoffmann-Thoma et al., 1996). Increased hexose concentration could not be attributed to elevated or prolonged invertase activity; therefore, it is still unknown how these lines accumulate high levels of hexoses.

Quantitative trait locus (QTL) analyses on populations generated from crosses between grain and sweet sorghum varieties reveal that the there are few large-effect loci that condition the ‘pithy’ stalks of grain sorghum or ‘juicy’ stalks of sweet sorghum (Ritter et al., 2008; Murray et al., 2009). ‘Pithy’ stems are characterized by an abundance of dead-air-filled cells in the centre of the stem with vascular bundles concentrated around the periphery of the stem (Fig. 3). ‘Juicy’ stems have little to no dead-air-filled cells, and usually have abundant vascular bundles across the diameter of the stem. One QTL on chromosome 3 has been shown in three independent analyses to control between 18% and 25% of the phenotypic variation for these traits (Natoli et al., 2002; Ritter et al., 2008; Murray et al., 2009).
Sucrose-yield QTLs in sweet sorghum varieties are usually pleiotropic and co-localize with other traits such as stem diameter, plant height, flowering time, and tillering. Sucrose yield per unit area is usually highly correlated with stem density in the field (Vietor et al., 1990). In contrast, the sucrose concentration trait in the stem appears to be quantitative, resulting from many small-effect QTLs widely distributed across the genome (Shiringani et al., 2010). Stem sugar concentration is an additive trait, but is not noticeably increased in hybrids. Presumably, this lack of heterosis for the trait is due to repeated selection for high juice sucrose concentrations, which may now be at a physiological ceiling for sucrose concentration (Pfeiffer et al., 2010). If sugar yields per plant are to increase rapidly, breeders may need to select for juice volume, a trait that is correlated with stem volume and overall plant biomass. Storage of soluble carbohydrates in the stem may be similar to storage capacity of starch in the endosperm, which is determined by the number of starch grains. The number of vacuoles, determined by the number of storage parenchyma cells in the internode, is thus a general indicator of sink capacity in sorghum stems.

Light interception and the staygreen trait greatly affect the concentrations of carbohydrates in the internodes (Cook and Evans, 1983). An increase in sucrose yields in sweet sorghum varieties may be achieved by genetic adjustment of leaf angles to optimize light interception. Additionally, staygreen varieties of sweet sorghum usually have higher stem sugar concentrations than senescing lines (Borrell and Hammer, 2000). This may be due to the reduced need for re-mobilizing stem sucrose in addition to prolonged photosynthetic capacity (Duncan et al., 1981). Thus the staygreen trait could be a significant trait in sweet sorghum varieties in which stem sugar concentrations have not reached the physiological maximum.

In warmer climates, sorghum fields can be ratooned (Byrt et al., 2011); that is, harvested multiple times from one initial planting. The effectiveness of ratooning depends on which cultivars of sorghum are selected and how much of the vegetative tissue is left for the regenerative process to occur. When the plants are harvested, sugars in the stem stubble left on the base are mobilized and transported to new tillers (Fig. 4). Thus the root systems have already been ‘paid for’ in the carbon economy of the first cropping cycle. Now the plants can more rapidly regenerate vegetatively. This works well with highly tillered cultivars of either sweet or grain-type sorghums that produce many stalks. The rotation speed and quality of the ratoon crop may depend on the carbohydrates left in the stubble after harvest which can support initial growth before photosynthetic capacity is regained (Dohleman and Long, 2009; Turgeon, 2010). Ratooning also depends on the precise timing of senescence of the floral structures to ensure proper dry-down of the grains, the last step in seed maturation, while initiating new vegetative growth at the base of the plant. Much more work is needed to determine the impact these carbohydrates have on the ratooned harvest and the development of agronomic practices which would optimize the successive harvests.

Many grain sorghum varieties also have the ability to remain highly productive during drought stress (Massacci et al., 1996). Although the mechanisms that underlie this trait are currently unknown, it is reasonable to hypothesize that soluble carbohydrates within the vegetative structures play a role. Soluble sugars in sorghum stems may be

**Fig. 3.** Grain sorghum cultivar showing thin (A) and pithy (C) stem. Sweet sorghum cultivar with a thick (B) and juicy (D) stem. Stem tissue is composed of vascular bundles with surrounding parenchyma cells and also dead-air-filled parenchyma cells (white arrowheads) which are more prevalent in C than in D. The white dashed line in A and C represents the regions where sections in C and D were taken. Bar, 5 mm.

**Fig. 4.** Sorghum tiller re-growth after the stem has been harvested.
functionally similar to those recently described for trees (Q. Fu et al., 2011). It was proposed that in trees, high concentrations of soluble sugars, and other osmotically active compounds in the leaves, help to draw water into the canopy to offset low hydrolytic conductance values of the xylem transport stream. Thus, soluble sugars in the vegetative tissues function in whole-plant water relations, as well as in water stress tolerance.

**Sugarcane**

Almost 70% of commercially produced sucrose is derived from sugarcane stems (Carson and Botha, 2002). Additionally, sugarcane has become a primary feedstock for the production of ethanol in tropical countries such as Brazil, supplying a large portion of liquid fuel. For the rest of the world, it is estimated that by 2030 oil demand will be 50–60% higher than current production. Reserves of easily extractable crude oil for better referred to as ‘one-time, incredibly concentrated reserve of solar energy’ (Pelletier, 2010) are arguably declining. Therefore alternative, liquid, renewable fuels such as sugarcane ethanol will become even more critical for sustaining energy demands in the near future while advanced generation biofuels are brought online at practical, ecologically and economically feasible scales (Hotta et al., 2010; Buckeridge et al., 2011).

Sugarcane accumulates large amounts of highly concentrated sucrose in the stem storage parenchyma cells. Sucrose concentrations within stems can reach up to 0.7 M (~Brix of 20) or ~50% of the stem dry weight (Moore, 1995; Inman-Bamber et al., 2009). Unlike maize and sorghum, sugarcane appears to employ a symplastic transport mechanism of phloem unloading into the storage parenchyma cells (Fig. 1) (Welbaum et al., 1992; Walsh et al., 2005). Suberization and lignification of the cell walls that surround the phloem block most, if not all, apoplastic sucrose movement from cell to cell. Additionally, asymmetrically labelled sucrose fed into the internodes remains intact, suggesting there is little breakdown and re-synthesis in the mature internode. This correlates with earlier studies that suggest that futile cycling of carbohydrates also decreases as the internode matures (Uys et al., 2007). Therefore, sucrose influx to the storage parenchyma is primarily conducted through the abundant plasmodesmata that provide cytosolic continuity between cells which are divided by highly suberized and lignified walls.

Presumably, the primary mechanism for sucrose storage and mobilization is derived from the activity of sucrose transporters on the tonoplast membranes of the storage parenchyma cells (Rae et al., 2009). Recent evidence from modelling studies further supports the idea that sucrose movement into the vacuole is against a concentration gradient, driven by an active transport mechanism (Moore, 2005). The turgor and sink strength in the storage parenchyma cells may also be balanced by increasing the sucrose concentration in the apoplast that surrounds these cells (Walsh et al., 2005). The highly suberized and lignified barrier limits the spread of the sucrose to non-storage cells, which prevents sucrose from leaking into the xylem stream. This also prevents water movement into the concentrated apoplastic sucrose pool (Jacobsen et al., 1992; Welbaum et al., 1992). It has been proposed that ShSUT1, a plasma membrane-localized sucrose transporter expressed in the storage parenchyma cells, most probably functions to retrieve sucrose from the apoplastic space, indirectly regulating sucrose flux into the parenchyma cell (Rae et al., 2005b). Interestingly, there is significant expression of hexose transporters in the storage parenchyma tissues, but their roles are still unclear (Casu et al., 2003). It is likely that orthologues of AtSUC4, AtTMT1, and AtTMT2 in sugarcane also mediate sugar flux across the tonoplast membrane, regulated by both sucrose and hexose concentrations in the cells (Schulz et al., 2011).

Evidence suggests that once these cells have been filled to capacity, plasmodesmata in the storage parenchyma cells become blocked, which serves to prevent backflow (Rae et al., 2005a). Due to the lignified and suberized cell walls surrounding the storage parenchyma cells, export to the vascular parenchyma cells during the remobilization process also appears to be symplasmic, driven by osmotic potential between storage parenchyma cells and the phloem when plasmodesmata are re-opened for transport (Rae et al., 2005a). However, it is still unclear whether phloem loading in the stems is apoplastic or symplasmic during remobilization. It is possible that once sucrose reaches the vascular parenchyma cells, it is effluxed into the apoplast directly adjacent to the sieve elements and companion cells, and retrieved by sucrose transporters. Extracellular invertase activity could also play a role in the reversal of sucrose accumulation to export remobilization as suggested by the expression of hexose transporter in the stem phloem tissue (Casu et al., 2003). However, more work to support such a mechanism is needed.

Improvement of yields in sugarcane by conventional breeding is reaching a plateau, mostly due to the narrow gene pool available in the commercial hybrid genetic stock (Grof and Campbell, 2001). It is also possible that sugarcane has reached a physiological limit with sucrose concentrations at ~17–20% (w/v) (Jackson, 2005). Integration of other secondary traits such as disease resistance, cold tolerance, and ratoon performance may have slowed the direct improvement of sugar concentration in the stems. Similarly to sweet sorghum, higher yields of sugar have been obtained by breeding for stem volume and stem juice yield, while maintaining high sucrose concentrations. The final volume of the internode space capable of storing sucrose is determined during the phase of internode elongation, well before sucrose accumulation begins (Glassop et al., 2007). Once cell volume is established, dry matter, mostly sucrose, progressively replaces the water until maximum physiological concentrations are reached. Thus further sucrose yields attained from breeding may come from morphological, not physiological, selection.

Sucrose concentration is greatly affected by agronomic practices such as planting density. Higher stalk density increases sucrose partitioning to the stems in sugarcane. In
contrast, wider spacing increases assimilate supply in the source leaves, but results in decreased partitioning to stored sucrose in the stem, thus lowering yields (Pammenter and Allison, 2002). However, shading leaves of more widely spaced plants results in increased partitioning of sucrose to the stem, even though overall biomass accumulation is lower. A similar trend was reported for sucrose concentrations in maize stems (Hume and Campbell, 1972). These data imply that shading impacts partitioning within grass stems, suggesting signalling mechanisms that operate through assimilates themselves, or hormones associated with shade avoidance or vegetative development. Cooler temperatures are also known to induce higher sucrose accumulation in sugarcane stems, presumably due to decreased growth in vegetative structures, which are competing sinks for sucrose (Lingle et al., 2002). It will be interesting to investigate the mechanism that these hybrids use to transport and store sucrose in the internodes, given that each parent uses a different mechanism.

Raising the concentration of sucrose alone in sugarcane stems by the use of biotechnology has been difficult. Only a handful of the specific transporters, enzymes, or other proteins that regulate sucrose accumulation are currently known (Casu et al., 2005). Sugarcane is also prone to transgene silencing, which limits the successes of many transgenic approaches (Mudge et al., 2009). Microarray analysis on sugarcane genotypes that vary in sucrose content revealed that many of the genes associated with high sucrose content show overlap with drought data sets, but appear to be mostly independent from abscisic acid signalling (Papin-Terzi et al., 2009). In spite of the difficulty, there have been reports of transgenic sugarcane plants that show increased sucrose accumulation. Down-regulation of pyrophosphate-fructose-6-phosphate 1-phosphotransferase activity leads to a 3- to 6-fold increase in sucrose in immature stems, presumably by limiting the conversion of hexose phosphates to triose phosphates (van der Merwe et al., 2010). Additionally, down-regulation of neutral invertase results in an increased sucrose to hexose ratio by decreasing respiration and sucrose cycling in the mature stems (Rossouw et al., 2010).

Is the plateau in sucrose accumulation due to osmotic limitations? Production of sucrose isomers in sugarcane suggests not. Transgenic sugarcane plants expressing a -vacuolar-localized sucrose isomerase that produces the disaccharide isomaltose accumulate double the amount of soluble carbon in the stem storage parenchyma (Wu and Birch, 2007). Isomaltose is biologically active in plant cells, but may not trigger the same sugar signalling mechanism as sucrose or hexoses. In the transgenic lines that produced high concentrations of isomaltose there was also an increase in photosynthetic rates in the leaves. This suggests that the plants are capable of producing and storing more carbohydrates, but may be limited by a feedback mechanism linked to sucrose or hexose concentrations and not total osmotic potential. These data also correlate to physiological evidence that suggest a more sink-limiting model in sugarcane,
suggesting that photosynthetic rates in the leaves are regulated by the downstream capacity of storage in the stems (McCormick et al., 2008a, b, c). As seen with the production of isomaltose, the source tissues will adapt to changes in the metabolism and compartmentalization in sink tissues.

Symplasmic transport through the storage parenchyma cells has been suggested to be a control point for sucrose flux into the storage cells (Walsh et al., 2005). Thus manipulation of plasmodesmata may be one method to alter sucrose partitioning within the stem. Flux through plasmodesmata is altered by the presence of viral movement proteins, thereby facilitating cell-to-cell trafficking of viruses (Ueki and Citovsky, 2011). Strategic expression of these proteins in stem storage parenchyma cells has been proposed as one avenue to manipulate symplasmic flux and sucrose accumulation rates in sugarcane.

The reduced-growth phenomenon documented in sugarcane is presumably caused by feedback inhibition when stem sucrose becomes elevated, which also increases foliar carbohydrate concentrations (van Heerden et al., 2010). Foliar hexose pools, especially glucose, have been suggested to inhibit photosynthesis as a mechanism of feedback regulation in sugarcane (McCormick et al., 2008c). The majority of the sugar signalling mechanisms, such as the hexokinase-dependent mechanism, are located in the cytosol (Granot, 2008; Slewinski, 2011). One way to defeat such feedback inhibition would be to overexpress the tonoplast-localized glucose/sucrose transporters to reduce the cytosolic pool of glucose, regardless of the sink strength of the stalk (Wingenter et al., 2010). Proof of concept for this mechanism was recently shown in Arabidopsis where increased activity of AtTMT1 apparently ‘hides’ glucose from cytosol-localized sensing mechanisms without disrupting the carbohydrate dynamics of the plant (Wingenter et al., 2010; Slewinski, 2011). Overexpression of AtTMT1 in Arabidopsis also leads to an increased rate of photosynthesis, carbohydrate partitioning, and reduced whole-plant respiration. Perhaps the same process of overexpressing TMTs could be engineered into sugarcane to reduce feedback inhibition and mitigate the reduced growth phenomenon.

Rice

Rice is the most important crop on the earth, providing 35–60% of the calories consumed by almost half of humanity (Fageria et al., 2003). It is estimated that 30–40% of the grain mass can be comprised of previously stored stem reserves when plants are grown under normal conditions (Yoshida, 1981). Carbohydrate partitioning in rice plants is also interesting because plants store carbohydrate reserves as sucrose in the leaves and both stalk and sucrose in the stems—a pattern that diverges from many of the grasses (Scofield et al., 2009).

Phloem unloading into stem storage parenchyma cells is presumably symplasmic (Scofield et al., 2007). Chemical and osmotic gradients necessary for influx into the cells are generated by rapid conversion of sucrose to starch which is stored in the amyloplasts (Patrick, 1997). It is unclear how sucrose is exported from the stem storage parenchyma cells back to the phloem during the re-mobilization process. It is possible that during export, cells block plasmodesmata and switch to an apoplastic mechanism which utilizes sucrose transporters, such as OsSU1, to pump sucrose actively against a concentration gradient into the phloem (Scofield et al., 2007).

Crop management practices can also have a dramatic effect on carbohydrate partitioning in rice (Fageria et al., 2003). One of the largest concerns is the use of fresh water for rice production. Taking into consideration that rice production consumes almost 80% of the fresh water in Asia, practices that reduce water consumption, such as partial drying during the crop cycle, may become more common as the abundance of fresh water declines (Yang and Zhang, 2010a). Fortuitously, partial drying of the soil enhances the mobilization of carbohydrates from the stems and expedites the grain filling process, leading to high grain yield and harvest index. However, this only works if the drying is controlled in a manner that does not severely impact current photosynthesis and induce a drought response. For a more in-depth discussion of water management practices and rice yields, I refer readers to Yang and Zhang (2010a).

Quantifying NSCs in stems at harvest is one way to detect sink–source imbalances in grain-producing grasses. A recent survey of major domestic cultivars of rice grown under normal agricultural conditions in Japan found that all varieties have significant NSC left in the straw (Park et al., 2011). Other reports indicate that if too much nitrogen is applied to fields, or plants express the staygreen condition longer than is needed for grain fill, stems become saturated with starch (Pan et al., 2011). This starch is then disposed of in the chaff during harvest, which decreases the harvest index. Interestingly, hybrid rice varieties also have reduced harvest index, presumably due to the same phenomenon. These data suggest that rice exhibits more sink than source limitation imposed by constraints in the transport or grain-filling processes.

This raises some important questions. Will increasing carbohydrate source strength be enough to increase yield? Does increased and sustained photosynthesis correlate with increased grain fill and harvest index? Carbohydrate partitioning involves not only the production, storage, and utilization of carbohydrates, but is also critically dependent on transport. Transport is determined by both the physiology and vascular architecture of the transport system (Dinan and Lemoine, 2010). Rice panicles that produce more spikelets per panicle, so-called ‘Super Rice’, have problems filling grains produced on the inferior spikelets (Yang and Zhang, 2010b). These plants become more ‘source limited’ as spikelet density increases, but the grain-filling problem may result from inadequate vascular connections to the inferior spikelet. This results in normal grain filling in the superior spikelet, but reduced in the inferior spikelets, throughout the panicle (Yang et al., 2002). Such morphogenetic constraints in the vascular
system can confound investigations into sink–source dynamics (Watson and Casper, 1984). Furthermore, grass inflorescences do not function as a homogeneous sink tissue, but function sectorially based on architecture (Cook and Evans, 1983). This raises great concern about increasing the productivity of rice, as well as other grain crops by increasing photosynthetic capacity, carbon storage, or spikelet density. Research is now directed towards installing the high capacity C₄ photosynthetic system into rice (Sage and Zhu, 2011). Such questions need to be addressed in the near future to optimize the efficacy of such strategies. New limiting factors to yield enhancement appear as other factors become non-limiting. Increasing productivity will most probably require an extensive redesign of the plant, including the physical architecture of the vascular system and buffering capabilities in the stem, not simply the introduction of the C₄ photosynthetic syndrome.

It is also important to understand that, as noted above for maize, NSCs in the stems during the pre-anthesis stages of development may dramatically affect final sink strength by increasing spikelet survival during the exceptionally sensitive early development phase of the spike. Higher reserves of NSCs may also impact cell size and starch granule number in the spikelets early in development, which are the primary indicators of sink potential during grain fill (J. Fu et al., 2011). Total above-ground biomass has a positive correlation with sink strength in varieties that are proportionally higher in inferior spikelet density.

In climates that have extended growing seasons, rice can also be ratooned (Turner and Jund, 1993). The second harvest only yields about one-third of the first harvest (Harrell et al., 2009). However, increasing the total yield from one planting without significant inputs can drastically improve grain production on a per-land and fertilizer basis (Santos et al., 2003). The speed of vegetative re-growth, flowering, and grain filling depends on the initial reserves that remain after the first harvest. For rice, this is correlated with the size of the stem tissue that remains (Jones, 1993). Starch in the remaining stem is quickly hydrolysed and transported to new tillers at the base. Alternatively, if the stem is long enough, carbohydrates will be partitioned to nodes on the stem to produce new floral structures immediately. Regeneration of floral structures is usually more rapid than in the first crop. Grain set and filling are also significantly accelerated (Harrell et al., 2009). As with most plants, the early investment in photosynthetic organs has the most impact on overall plant growth and development (Turgeon, 2010).

Is it possible to optimize the sugar transport stream further in order to convert more of the NSCs in the stem into grain mass? As the data highlighted above suggest, there is still much that remains unknown about carbohydrate partitioning in rice, and how entire life cycle dynamics impact yield. Even though many aspects of carbohydrate partitioning remain elusive, there is potential for improvement in the way currently grown cultivars convert carbohydrate stores into harvested grains.

**Wheat, barley, and oat**

Wheat, barley, and oat collectively provide most of the grain calories in the temperate and cooler regions of the world. The roles and mechanisms of storing and remobilizing stem carbohydrates in wheat have been more intensely studied than in other grain-bearing grasses (Schnyder, 1993; Blum et al., 1994; Wardlaw and Willenbrink, 2000). This is because early researchers discovered a direct correlation between carbohydrate storage in the stems and the ability of the plants to maintain high yield stability under drought or other environmentally deleterious conditions (Binding et al., 1977; Asseng and Herwaarden, 2003). Mobilized reserves from the stem tissues can contribute ~20–40% of the final grain weight under normal stress-free growing conditions (Dreccer et al., 2009). The greater contribution of stem reserves comes into play under stressful conditions during grain fill, such as end-of-season drought, when stem carbohydrates can contribute to ~70% of the final grain mass (Goggin and Setter, 2004; Ehdaie et al., 2006; Rebetzke et al., 2008). High NSC wheat genotypes are usually shorter, produce fewer but more fertile tillers, and flower earlier. Overall biomass of the high NSC lines is low, but a higher proportion is partitioned to the grain, leading to a higher harvest index (Rebetzke et al., 2008).

During the grain-filling process, current photosynthesis usually provides more than enough carbohydrates for the developing grains (Slazer and Andrade, 1991). Only when current photosynthesis drops below the maximum fill rate of the kernel does mobilization from the stem begin. These data imply that grain filling is a rate-limiting process that is driven at maximum capacity during the entire filling process (Bingham et al., 2007). In barley, carbohydrates derived from stem reserves may be preferred to prolonged photosynthesis during the later phases of grain fill. Cultivars with high NSCs in the stems usually produce grains with higher dry mass. However, there does seem to be a trade-off in grain number. Low NSC lines usually produce more grain per unit area, but produce grains with lower individual mass (Rebetzke et al., 2008). Increased grain mass in the high NSC lines appears to compensate for the lower grain number because these lines are overall higher yielding and produce higher quality grain.

Increases in wheat yields over the past few decades were due to producing more grains per unit area rather than increasing individual grain mass (Sadr nas, 2007; Sadr nas and Egli, 2008). Thus, reducing yield losses from floret abortion and sterility, due to carbohydrate starvation, are still critical targets for cultivar improvement (González et al., 2011). However, grain filling in wheat may be similar to that in rice in that the vascular system itself is insufficient to transport needed sugars to the developing spike. For example, the distal florets are connected to the primary vascular bundles in the spike stem through the subvascular system—a transport route that lacks direct connections to the primary phloem (Hanif and Langer, 1972). This raises major concerns for yield improvement in wheat and barley by increasing spikelet number per panicle. As breeders and
basic scientists raise both source and sink potentials, the physical architecture of the vascular system itself may become the new limiting factor in the carbon economy of the plant (Asli and Houshandifar, 2011). Buffering the supply with stem carbohydrate reserves may be one way to maximize transport through a constrained vascular route. However, eventually the vascular system itself will have to be altered to accommodate the high rates of transport to the developing florets and grains. More research is needed in this area to identify substantial bottlenecks in transport and grain-filling processes.

Presumably, influx into storage parenchyma cells of these grasses is symplasmic, as it is in many tissues that accumulate sugar polymers (Aoki et al., 2004). Stem NSC in wheat, barley, and oat is mostly in the form of fructan stored in the vacuoles of the stem storage parenchyma cells. Other major carbohydrates do not significantly contribute to the NSC reserves, or show little variance between high and low stem NSC lines (Bancal and Tribó, 1993; Ruuska et al., 2006). Starch is present in the stem and leaves of wheat, but has been suggested to function as a source for vegetative growth rather than for mobilization to the grain (Scofield et al., 2009). Fructan synthesis may depend more on sucrose availability, as seen when fructans become rapidly labelled when $^{13}$C-sucrose is injected into wheat stems (Hogan and Hendrix, 1986). However, fructan concentrations in wheat are not directly correlated with fructosyltransferase activity (Goggin and Setter, 2004).

The average degree of fructan polymerization in leaves varies depending on cultivar, stage of plant growth, and environmental conditions (Wardlaw and Willenbrink, 1994). NSC accumulation usually occurs between anthesis and completion of endosperm cell division (Wardlaw and Porter, 1967). There also appears to be minimal costs to storing carbohydrates in the wheat stem as there is minimal energy lost to respiration. Thus, the storage and remobilization process appears to be metabolically efficient (Bell and Incoll, 1990; Gebbing and Schnyder, 1999; Xue et al., 2008).

In barley, the most promising potential to increase radiation use efficiency may come from alterations in sink–source dynamics (Bingham et al., 2007; Newton et al., 2011). Barley appears to be much more sink than source limited. Presumably, the low sink capacity of barley leads to a reduction in photosynthetic productivity due to feedback inhibition. In most genotypes and environments, stem carbohydrate reserves build up and are not remobilized to the grain, resulting in a decreased harvest index. Improved partitioning to the ear when the spikelets are forming during the stem elongation phase may help to increase spikelet survival, leading to more grains per plant, thus increasing sink strength and yields (Borrás-Gelonch et al., 2010). For such a scenario to be feasible, stem elongation will have to start earlier in the plant life cycle and near completion before spikelet development progresses, all without disrupting the time to anthesis (Borrás et al., 2009). Because oat employs the same mechanism of fructan storage in stems, progress made in wheat and barley may be transferred into oat (Livingston et al., 1993, 1994).

Improving stress tolerance in wheat by manipulating NSCs in stems has already been put into practice in areas where environmental conditions are suboptimal (Ehdaie et al., 2006). Such is the case in Australia where severe and terminal droughts are common (Bell et al., 2010). Wheat cultivars that have high NSCs in stems show improved floret fertility, grain filling, and yield stability when grown under rain-fed or drought conditions. When these lines are grown under irrigated and non-stress conditions, yields from high stem NSC lines are comparable with those of low NSC lines, suggesting there are no yield penalties associated with high NSCs if the plant does not experience stress (Foulkes et al., 2007).

One QTL study for stem NSCs in wheat identified two regions located on chromosomes 1b and 2A (Foulkes et al., 2001). In another QTL study it was proposed that there are between eight and 16 QTLs for stem NSC concentration, and between four and eight QTLs for total NSC yield (Rebetzke et al., 2008). Many of the genes that encode enzymes that function in fructan degradation and remobilization, such as fructan exohydrolase, also co-localize with QTLs for stem NSC concentration, grain-filling rate, and grain weight (Yang et al., 2004). Similar to the grasses mentioned above, the NSC trait in wheat is linked to morphological characteristics. Because stem length, diameter, and solidness all have high heritability values ranging between 0.42 and 0.84 (Ehdaie et al., 2006), breeding programmes could be highly effective at optimizing and combining the traits to increase stem NSCs in wheat varieties designed for more arid and drought-prone areas. There is also high variability in stem elongation rates in wheat and barley. Therefore, the ability to modify the timing of spikelet development and onset of NSC accumulation genetically also shows potential (Slaper and Araus, 2007).

Modulating the transcription of fructan-synthesizing and degradation genes could also be employed for controlling NSC partitioning in wheat and barley stems. Recently, the wheat transcription factor TaMYB13 was found to be a transcriptional activator of Ta1-SST and Ta6SFT, suggesting that TaMYB13 and its orthologues are involved in the regulation of the fructan synthetic pathway (Xue et al., 2011). Perhaps similar MYB-type transcription factors also control carbohydrate partitioning genes in other grasses. Manipulation of such transcriptional modifiers, through either introgression of natural variants or transgenic approaches, could offer a new route to control carbohydrate distribution in grasses.

**Forage and energy grasses**

Mixed-pasture grasslands cover almost a quarter of the total ice-free land area on earth—roughly 30 million km$^2$ (Monfreda et al., 2008; Lee et al., 2010). Most of these grasslands are essential for livestock feed, which dramatically impacts global food production and economies, and supports the indigenous wildlife that inhabits those areas (Lee et al., 2010). Knowledge of the growth and re-growth cycles of grassland species is critical for determining the
stocking density of the pasture and overall grazing management practices (McNaughton, 1979; Lemaire et al., 2009). Overgrazing can lead to pasture collapse, and undergrazing can lead to unproductive land use and deterioration of nutrient value of the fodder. In addition, the stage of regrowth at which the grasses are consumed is important for animal nutrition. Such vast grasslands also act as a carbon sink which can buffer the effects of increased CO₂ emissions. Unfortunately, improper management practices along with recent environmental instability have severely degraded large portions of essential pastures and grasslands, thus exacerbating the effects of climate change, CO₂ sequestration, and pasture-based food production (FAO, 2009).

Most of the economically important forage and energy grasses are perennials, which store moderate levels of carbon reserves in stems and roots to fuel re-growth from year to year, and also after grazing or cropping (reviewed in Fulkerson and Donaghy, 2001). Grasses in pasture lands lose much of their photosynthetic leaf area during grazing; therefore, subsequent photosynthetic capacity is not sufficient to drive re-growth of the photosynthetic tissues (Turner et al., 2006; Lee et al., 2010). Mobilized reserves from stems, pedicels, and roots are utilized until leaves are about three-quarters their final size—the stage when leaves transition into productive source tissues. Presumably, grasses that have evolved in areas where grazing is common have a reduced specific leaf weight, thereby reducing construction costs of a new photosynthetic area after defoliation (Cullen et al., 2006).

In Lolium perenne (ryegrass), fructans in the stem and leaf sheath are used for both energy storage and stress protection (Griffith, 1992; Hisano et al., 2004). After repetitive carbon depletions, photosynthetic and fructan synthesis genes become up-regulated while fructan degradation genes are down-regulated (Lee et al., 2010). This ensures that long-term stores are recovered before the next defoliation event. Fructans also play an important role in freezing tolerance in Lolium. Lolium perenne plants that express both 6-SFT and 1-SST under the 35S promoter have increased tolerance to freezing. Presumably, high levels of fructans help to stabilize membranes under cold stress and support winter hardiness (Hisano et al., 2004). Re-mobilization of carbohydrate reserves in Lolium uses the apoplastic mechanism. Expression of LpSUT1 is strongly induced in the lamina within 24 h of blade detachment, suggesting that fructans stored in the cells are converted into sucrose and pumped into the phloem against a concentration gradient (Berthier et al., 2009).

Energy grasses, such as miscanthus or switchgrass, are grown for their cellulose-rich stems and leaves. Along with stem carbohydrates, these grasses also utilize underground rhizomes to store energy for the subsequent year’s growth (Glowacka, 2011). Switchgrass is similar to rice in that the main store of NSCs in the stems is starch (Smith, 1975). Starch accumulation peaks at the beginning and end of the growing season, before and after flushes of rapid growth. Upon defoliation, starch levels plummet, then rapidly rise once a new photosynthetic area has been produced. Switch-grass plants that overexpress the Corgrass/miR156 transcript, which alters the developmental progression from juvenile to reproductive phase, hyperaccumulate starch in the internode tissue (Chuck et al., 2011). These lines also show a dramatic increase in tillering and biomass accumulation when compared with controls. The hyperaccumulation of starch is presumably due to the absence of flowering in the transgenic lines which partitions more carbon into vegetative storage, similar to the sucrose accumulation patterns in maize and sugarcane cultivars that have a delayed or suppressed reproductive phase. Starch hyperaccumulation in grass biofuels feedstocks may be favourable because starch can be stored as dry matter for long periods. Thus, starch in stems may be easier and more cost-effective to convert into ethanol when compared with soluble sugar or pure cellulose feedstocks (Chuck et al., 2011).

Miscanthus offers the most efficient model for carbon allocation in next-generation biofuel crops. Miscanthus appears to be most similar to sugarcane in that it stores NSCs as sucrose in the stems, although the mechanism for radial sucrose transport into the storage parenchyma cells remains to be elucidated (Burner et al., 2009). The above-ground biomass of miscanthus is harvested after the plant has fully senesced (Glowacka, 2011). Thus, any remaining NSCs, minerals, and high molecular weight compounds that are not incorporated into structural tissues are mobilized and partitioned to rhizomes to increase winter survival and fuel the next year’s growth. Unlike annual grasses, excess NSCs in the stems at the end of the season remains part of the productive carbon economy of the plant. As mentioned above, crosses with sugarcane will potentially result in new high-yielding hybrids that produce both celluloseic biomass and sucrose in more temperate regions of the world.

Prospects for NSC partitioning in grass stems

One of the most disturbing trends in agriculture is the increasing yield gap in the grass crops (Godfray et al., 2010). Yield potentials in many of the crops are already high, but actual yields rarely reach the upper limits. The yield gap results from the accumulation of unfavourable events during the life cycle that lead to a decrease of carbohydrate production or partitioning to the harvested tissue, in most cases the grains (Willenbrink et al., 1998). Closing the yield gap will require drastic exploitation of the sink–source relationship in the grass crops. However, most sink or source components that directly affect yield appear to be negatively correlated with each other (Slafer et al., 1996). The nature of these negative relationships remains unknown. Therefore, new descriptive models are needed that diverge from simple sink- or source-limited systems, to whole-plant entire life cycle systems to understand these relationships fully. This will require deeper investigations into the underlying physiological phenomenon at the ‘crop level of organization’ (Slafer and Savin, 1994; Boote et al.,
to synthesize fructans, as well as the entire process being confined to the vacuole (Pollock and Kingston-Smith, 1997; Cairns, 2003; Rae et al., 2009).

Ratooning can be used as an already existing agricultural model of how to develop and manage perennial grain and biomass crops (DeHaan et al., 2005). More in-depth investigations into current successes and pitfalls will help breeders and biotechnologists select and design essential traits early in development of the perennial counterparts to our current annual crops (Wagoner and Schaeffer, 1990; Cox et al., 2006). Important traits for the perennial conditions will most probably involve vegetative carbohydrate storage organs such as stems, roots, and tubers that fuel rapid re-growth for successive cropings or seasons as well as prolonged resistance to disease and insects (Glover et al., 2010). Prospects to reduce tilling and inputs in such perennial systems are substantial. One of the major problems with prolonged or permanent cropping systems is the infestation of weeds, which consume resources and compete with the crops (Clements and Ditommaso, 2011). Aggressive weeds can destroy up to 100% of a harvest in some annual crops. If such devastation is possible in annual crops, what will happen in the perennial cropping system where weeds will have more opportunities to establish larger reserves of seed banks in the soils, as well as underground structures that will re-fuel their own re-growth year after year? One strategy to help control weed devastation is to increase the growth rate of the crop, thus outcompeting the weeds. As seen with the difference in growth rates between miscanthus and maize (Dohlenman and Long, 2009), initial investments into productive photosynthetic area dramatically alters the plant’s ability to produce biomass and compete with other plants. Proper management of stem carbohydrate reserves, by strategic harvesting practices to maximize reserves that remain in the vegetative structures, may increase the resistance to weed damage in ratooned and perennial crops. Thus, utilizing ratooned crops as a model to optimize and troubleshoot potential problems is essential for the early developmental stages of perennial cropping systems.

New strategies for complex genetic traits

Although there is great potential for the applications of stem NSC buffering, phenotyping stem carbohydrates is usually destructive and time consuming. This makes it difficult for conventional breeding practices, or for agricultural biotechnology companies, to produce products in an inexpensive and timely manner. Indirect phenotyping, by measuring the dry matter content of the stem, is used as an indicator of NSC in wheat stems (Ford et al., 1979; Xue et al., 2009; Saint Pierre et al., 2010). This bypasses the need to extract and quantify fructans and soluble sugars. Methodologies used for sugarcane and sweet sorghum rely on brix, the refractive index of juice that gives an estimate of soluble contents in a liquid, to phenotype stems sugars rapidly (Ritter et al., 2008). New methods of stem near-infrared reflectance spectroscopy are now being developed.
to measure sugar concentration in vivo, eliminating the need for destructive sampling (Wang et al., 2011).

Stem carbohydrate concentrations and total yields appear to be complex genetic traits in the grasses. Very few large-effect QTLs that account for significant variation have been found, or are effective in increasing carbohydrates when introgressed into elite lines (Aitken et al., 2006). Thus stem carbohydrates must be approached using quantitative trait methodologies, similar to grain yield, which result from the additive effects of many small-effect genes dispersed throughout the genome. Many secondary traits that control NSCs in stems also display transgressive phenotypic variance, meaning offspring display phenotypes that are beyond the range of the parents (Ming et al., 2002; Rebetzke et al., 2008). Therefore, hybrids that leverage heterosis are theoretically possible. A wide variation of stem carbohydrate traits already exists within the germplasm used for hybrid production (Ruuksa et al., 2006). Trait-based physiological breeding has already been implemented in wheat in Australia, and CIMMYT includes higher transpiration efficiency, reduced tillering, and dehydration avoidance (Foulkes et al., 2011). Similar approaches can be used for many stem NSC traits in many of the grass species.

Although standard QTL identification and introgression may not be a possibility for stem carbohydrate traits, these traits are excellent candidates for genomic selection which can rapidly compile additive small-effect loci into individual plants. For crops such as sugarcane, genomic selection may be one of the only methods that could be employed to improve sugar traits rapidly due to the genomic complexity, long reproductive cycle, and heterogeneity of the breeding stock. Elite sugarcane parents still have many untapped small- to moderate-effect QTLs that, when collectively utilized on a whole-genome scale, could lead to steady incremental increases in volume and concentration of sucrose (Aitken et al., 2006). Genomic selection was developed to enhance quickly and cheaply the breeding of genetically complex traits such as stem NSC partitioning (Rutkoski et al., 2011). Genomic selection can predict the breeding value of the individual based on the genomic similarity to another individual that has been phenotyped and genotyped. These methods also reduce the necessity for large-scale destructive measurements within every round of selections (Heffner et al., 2009). The main input costs are in genotyping individuals, which has become a relatively cheap and high-throughput method (Heffner et al., 2010).

Concluding thoughts

The mechanisms and agricultural applications of NSC partitioning in grass stems are diverse to say the least. This presents many challenges for this trait because there are no simple models that fit every system. Thus, there is still a long road ahead for physiologists, breeders, and plant scientists. The modification of crops must keep pace with human, ecological, and economic demands. Darwin’s pioneering work on evolution and breeding must now be approached with the methods of an engineer—accelerating the plant evolutionary processes to an unprecedented rate in order to provide food, fuel, and fibre for future generations of people on earth. Previously, constant high selection pressures have produced plants that have drastically changed in sink–source dynamics when compared with their wild relatives. That same pressure is now on the already high-yielding crops to produce the same increases as seen in the last few thousand years, in only a few decades. Improving our understanding and further optimizing whole-plant sink–source dynamics of the grass species that cover a vast portion of the earth’s surface will have profound impacts in the near future. It cannot be understated that there has been no other time in human history when the study of the fundamentals of plant evolution and physiology has become so critical.

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