Diurnal Pattern of Carbohydrates in Celery Leaves of Various Ages

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Abstract. Diurnal fluctuations in soluble carbohydrates and starch were monitored in young (expanding), mature (first fully expanded), and old (nearing senescence) celery (*Apium graveolens* L.) leaves. In all tissues, mannitol and sucrose were the carbohydrates present in the highest concentrations. In old and young leaflets and their petioles, there was little change in levels of mannitol and sucrose in 26 hours. In mature leaflets, sucrose accumulated in the light and decreased in the dark; mannitol increased slightly in late afternoon. Starch concentration, although quite low, showed definite diurnal fluctuations in mature leaflets, but only small changes in young and old leaflets. Both sucrose and mannitol were present in mature petiole phloem tissues. Mannitol concentrations were high in the adjacent storage parenchyma tissue, but sucrose was almost undetectable. These data support earlier findings that sucrose is produced, translocated, and metabolized throughout the celery plant. Mannitol is also translocated, but also serves as a major storage carbohydrate in leaf tissues, especially petiole parenchyma. Starch serves as a minor short-term storage compound in leaflets.

Sucrose and starch are primary photosynthetic products, and sucrose is the major translocated carbohydrate in many plants. Depending on the species, sucrose and/or starch also serve as temporary storage car-

bohydrates that accumulate in the light and are remobilized at night (Fondy and Geiger, 1982; Gordon et al., 1980; Sicher et al., 1984). In addition to diurnal changes, there are often leaf age-dependent differences in partitioning between sucrose and starch that are correlated with source-sink conversions (Ho et al., 1984; Ho and Shaw, 1979; Silvius et al., 1978; Turgeon and Webb, 1975).

Some plants produce, translocate, and store carbohydrates other than sucrose and starch. In celery, the two major photosynthetic products are sucrose and the sugar alcohol mannitol (Davis et al., 1988). Both are non-reducing, translocated carbohydrates that are synthesized in the cytoplasm (Rumpho et al., 1983). Little is known, however, about the roles of mannitol, sucrose, and starch in celery plants. Rates of sucrose synthesis, as determined by pulse-chase labeling, are similar in celery leaves of all ages (Davis et al., 1988). Sucrose content, however, is low in

young leaves and increases as leaves mature (Davis et al., 1988). In contrast, mannitol levels are high in all leaves, but mannitol synthesis is low in young leaves and increases with maturity. Use of mannitol and sucrose also changes with leaf development: leaves of all ages metabolize sucrose, but primarily young leaves metabolize mannitol (Fellman and Loescher, 1987). ¹⁴C-labeling studies (Davis and Loescher, 1990) show that mannitol and sucrose are both exported in the light. Sucrose is the primary translocate for several hours after sunset, but mannitol makes up the majority of the translocate stream when sucrose pools are low. Thus, there may be relatively large diurnal changes in sucrose concentrations, particularly in mature leaves, while mannitol levels may change very little. The objective of this study was to test the above hypothesis by examining diurnal changes in carbohydrate metabolism in celery leaves of various ages.

Celery (cv. Giant Pascal) was greenhouse-grown as described by Davis et al. (1988) except that 7 days before the experiment started, supplemental lighting was shut off and the plants were acclimated to the natural daylength. The day of the experiment, sunrise was at 6:03 AM and sunset at 6:33 PM. Light levels at midday ranged from 800 to 1400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Leaves referred to as young were light green, still expanding; mature leaves were dark green, just fully expanded, and old leaves were thickened and leathery and in some cases beginning to senesce. All plants had 11 to 15 leaves. Gas exchange measurements (Fox et al., 1986) and ¹⁴C-labeling data (Davis et al., 1988) indicated that leaves classified as young in this study were heterotrophic and at least partially dependent on import of carbon from mature source leaves for growth.

Starting at 5 AM, and every 2 h for 24 h, a young, a mature, and an old leaf were removed from each of three randomly selected plants (replicates). Terminal lateral leaflets were sampled from all three leaf ages, as were subtending petioles of young and old leaves. Phloem tissues were excised from the

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Fig. 1. Mannitol and sucrose concentrations in leaflets from young, developing (A), mature, fully expanded (B), and old (C) celery leaves. Sunrise was at 6:03 AM and sunset at 6:33 PM. Each value represents the mean of three replicates. Vertical bars represent $\pm$ SE.

xylem and storage parenchyma tissues of mature leaf petioles (Daie, 1986), and phloem and parenchyma tissues were analyzed. Fresh samples weighed ~500 mg each for leaflet, petiole, and petiole parenchyma, and ~200 mg for petiole phloem.

All samples were prepared for analysis according to the method of Redgwell (1980). Soluble components were dried in vacuo, dissolved in 1.0 ml pyridine containing 30 mg hydroxylamine-HCl/ml (to convert sugars to oximes) and β -phenyl-glucopyranoside (as internal standard). The solutions were heated at 70°C for 1 h. Trimethylsilyl derivatives were then prepared and analyzed by gas chromatography (Brobst and Lott, 1966) on a 3% OV-17 column (Applied Science Laboratories, State College, Pa.). Starch was determined by an amyloglucosidase/glucose oxidase method (Oakley, 1983).

In the morning, sucrose and mannitol concentrations in mature leaflets remained unchanged until after 11 AM (Fig. 1B). At 9 AM, 3 h after sunrise, there was an increase in mannitol content in mature petiole phloem and parenchyma (Fig. 2 B and C) that coincided with an increase in mannitol in the young leaflets (Fig. 1A) and a small peak in the young petiole (Fig. 2A). This pattern indicates that early in the day mannitol was synthesized in mature leaves and immediately exported. Sucrose was also synthesized in and exported from mature leaflets at this time since concentrations in leaflets ceased decreasing (as they had in the dark) (Fig. 1B) and those in the petiole phloem remained constant (Fig. 2B). Sucrose levels did not change as drastically at the beginning of the light period as in many other plant species.

Fig. 2. Mannitol and sucrose concentrations in intact young leaf petioles (A), petiole phloem preparations from mature leaf petioles (B), parenchyma from mature leaf petioles (C), and intact old leaf petioles (D). Sunrise was at 6:03 AM and sunset at 6:33 PM. Each value represents the mean of three replicates. Vertical bars represent $\pm$ SE.

For example, at the start of the light period, sucrose levels quickly increased in 6-week-old sugar beet leaves, but accumulation slowed after 2 h and concentrations remained fairly constant for the rest of the light period (Fondy and Geiger, 1985).

During the afternoon, sucrose levels more than doubled in mature leaflets and remained high for many hours (Fig. 1B). In contrast, mannitol increased only slightly. There was a small decrease in mannitol content beginning in late afternoon in mature leaflets (Fig. 1B) but there was no concomitant increase in the petiole phloem (Fig. 2B) or young leaf tissue (Fig. 1A) to indicate that this was due to increased export. The decrease can probably be attributed to reduced biosynthesis. During the night, mature leaf sucrose and mannitol levels dropped slowly, although sucrose decreased more than mannitol (Fig. 1B). The drop in mannitol content between 1 AM and 3 AM in mature leaflets coincided with a large increase in mannitol in the mature petiole phloem and parenchyma and young and old petioles (Fig. 2). This result agrees with 14 C-labeling studies (Davis and Loescher, 1990) that show mannitol to make up the majority of the transport stream after an extended dark period.

Young leaves grow rapidly and metabolize incoming sugars very quickly. Sucrose levels were very low throughout the period. Except for the 9 AM mannitol peak there was no net accumulation of any measured component on a microgram per milligram fresh-weight basis (Fig. 1A). The old leaves exhibited much lower levels of mannitol and sucrose than young or mature leaves (Fig. 1). As in mature leaves, however, there was an increase in sucrose levels in the afternoon.

Starch metabolism in mature leaflets was similar to that in many starch-storing species, i.e., levels increased in the light and decreased in the dark (Fig. 3). Even so, the importance of starch as a storage carbohydrate in celery leaves is small, because the content was very low compared with mannitol and sucrose. As in barley (Sicher et al., 1984) and sugar beet leaves (Fondy and Geiger, 1982), starch did not begin to accumulate until several hours after exposure to light, regardless of leaf age. Unlike in barley (Sicher et al., 1984), however, starch in mature celery leaves accumulated until dark and was remobilized immediately after the dark period began.

The celery petiole serves as a storage tis-

Fig. 3. Starch concentrations in leaflets from different age celery leaves. Sunrise was at 6:03 AM and sunset at 6:33 PM. Each value represents the mean of three replicates. Vertical bars represent $\pm$ SE.

sue for mannitol and sugars. Although the isolated mature petiole phloem preparation included phloem parenchyma, also sieve elements, and companion cells, it was clear that sucrose levels were much higher than in the adjacent storage parenchyma that contained only trace amounts of sucrose (Fig. 2 B and C). This substantiates our findings with ^{14}C transport (Davis and Loescher, 1990) that sucrose is hydrolyzed either with unloading or with storage in the petiole parenchyma tissue.

This study supports earlier conclusions from Y-labeling studies (Davis et al., 1988; Davis and Loescher, 1990) that sucrose is a major transport compound in celery and is readily metabolized in leaves of all ages. Mannitol is also an important transport compound but in addition serves as a long-term storage carbohydrate. Both sucrose and mannitol are synthesized in and translocated from mature and old leaflets in the light. After sunset, however, sucrose pools are depleted first, so mannitol makes up the majority of the transport stream late at night. Starch serves as a minor short-term storage carbohydrate in leaflets. Petioles of all leaves are storage organs that contain high levels of mannitol and low levels of sucrose. In isolated phloem preparations from mature leaves, however, sucrose levels were high, supporting earlier conclusions that sucrose is hydrolyzed before storage in the petiole.

Literature Cited

- Brobst, K.M. and C.E. Lott. 1966. Determination of some components in corn syrup by gas-liquid chromatography of trimethylsilyl derivatives. *Cereal Chem.* 43:35-42.
- Daie, J. 1986. Kinetics of sugar transport in isolated vascular bundles and phloem tissue of celery. *J. Amer. Soc. Hort. Sci.* 111:216-220.
- Davis, J.M., J.K. Fellman, and W.H. Loescher. 1988. Biosynthesis of sucrose and mannitol as a function of leaf age in celery (*Apium graveolens* L.). *Plant Physiol.* 86:129-133.
- Davis, J.M. and W.H. Loescher. 1990. [^{14}C] - Assimilate translocation in the light and dark in celery (*Apium graveolens*) leaves of different ages. *Physiol. Plant.* 79:656-662.
- Fellman, J.K. and W.H. Loescher. 1987. Comparative studies of sucrose and mannitol utilization in celery (*Apium graveolens* L.). *Physiol. Plant.* 69:337-341.
- Fondy, B.R. and D.R. Geiger. 1982. Diurnal pattern of translocation and carbohydrate metabolism in source leaves of *Beta vulgaris* L. *Plant Physiol.* 70:671-676.
- Fondy, B.R. and D.R. Geiger. 1985. Diurnal changes in allocation of newly fixed carbon in exporting sugar beet leaves. *Plant Physiol.* 78:753-757.
- Fox, T.C., R.A. Kennedy, and W.H. Loescher. 1986. Developmental changes in photosynthetic gas exchange in the polyol-synthesizing species, *Apium graveolens* L. (celery). *Plant Physiol.* 82:307-311.
- Gordon, A.J., G.J.A. Ryle, C.E. Powell, and D. Mitchell. 1980. Export, mobilization, and respiration of assimilates in unculm barley during light and darkness. *J. Expt. Bot.* 31:461-473.
- Ho, L.C. and A.F. Shaw. 1979. Net accumulation of minerals and water and the carbon budget in expanding leaf of tomato. *Ann. Bot.* 43:45-54.
- Ho, L.C., R.G. Hurd, L.J. Ludwig, A.F. Shaw, J.H.M. Thomley, and A.C. Withers. 1984. Changes in photosynthesis, carbon budget and mineral content during the growth of the first leaf of cucumber. *Ann. Bot.* 54:87-101.
- Oakley, E.T. 1983. Enzymatic determination of starch in fresh green, lyophilized green, and cured tobacco. *J. Agr. Food Chem.* 31:902-905.
- Redewell, R.J. 1980. Fractionation of plant extracts using ion exchange Sephadex. *Anal. Biochem.* 107:44-50.
- Rumpho, M.E., G.E. Edwards, and W.H. Loescher. 1983. A pathway for photosynthetic carbon flow to mannitol in celery leaves. *Plant Physiol.* 73:869-873.
- Sicher, R.C., D.R. Kremer, and W.G. Harris. 1984. Diurnal carbohydrate metabolism of barley primary leaves. *Plant Physiol.* 76:165-169.
- Silvius, J.E., D.F. Kremer, and D.R. Lee. 1978. Carbon assimilation and translocation in soybean leaves at different stages of development. *Plant Physiol.* 62:54-58.
- Turgeon, R. and J.A. Webb. 1975. Leaf development and phloem transport in *Cucurbita pepo*: carbon economy. *Planta* 123:53-62.