Plant Physiol. (1993) 101: 1217–1221

Elevated Levels of Both Sucrose-Phosphate Synthase and Sucrose Synthase in *Vicia* Guard Cells Indicate Cell-Specific Carbohydrate Interconversions[1]

Daniel R. C. Hite, William H. Outlaw Jr.*, and Mitchell C. Tarczynski[2]

Department of Biological Science, B-157, Florida State University, Tallahassee, Florida 32306–3050

A long series of reports correlate larger stomatal aperture size with elevated concentration of sucrose (Suc) in guard cells. To assess the role and autonomy of guard cells with respect to these changes, we have determined quantitatively the cellular distribution of the synthetic enzyme, Suc-phosphate synthase (SPS) and the degradative enzyme Suc synthase (SS) in *Vicia* leaflet. As expected for Suc-exporting cells, the photosynthetic parenchyma had a high SPS:SS ratio of approximately 45. Also as expected, in epidermal cells, which had only few and rudimentary plastids, the SPS:SS ratio was low (0.4). Of all cells and tissues measured, those that had the highest specific activity of SPS (about 4.8 μmol mg^{-1} of protein h^{-1}) were guard cells. Guard cells also had a very high relative specific activity of SS.

A stoma increases in size as a result of solute accumulation in the flanking guard-cell pair. Fluctuations in the concentration of soluble carbohydrates were proposed earlier to be the major osmotic factor in this mechanism of stomatal movements (Kohl, 1886 [cited by Yemm and Willis, 1954]). Exemplified by Sayre (1926), this so-called Classical Theory—starch (higher levels in guard cells of closed stomata) ⟷ sugar (higher levels in guard cells of open stomata)—was not uncritically accepted (e.g. Williams, 1954) and was rejected wholly because it was shown (Imamura, 1943; Yamashita, 1952; Fujino, 1959 [cited by Fujino, 1967]; Fischer and Hsiao, 1968; Humble and Raschke, 1971) that large amounts of K^+ accumulate in guard cells of open stomata. Now, the long-standing general observation that guard-cell starch contents decrease upon stomatal opening, which has been confirmed by semiquantitative (e.g. Mouravieff, 1972) and quantitative techniques (Outlaw and Manchester, 1979), constitutes evidence for a pathway leading to the accumulation of malate, a counter ion to K^+ (Raschke, 1979; Outlaw, 1982). Notwithstanding, there remain (a) the continuous history of reports (Pearson, 1973; Outlaw and Manchester, 1979; unpublished data cited by Outlaw, 1982; Reddy and Rama Das, 1986; Poffenroth et al., 1992) of a positive correlation between guard-cell sugar content and stomatal aperture size, and (b) the question of whether K salts account for all osmotic changes in guard cells (see negative perspectives, MacRobbie and Lettau, 1980a, 1980b). In addition, as discussed by Outlaw (1983), Suc may function other than as a bulk osmolyte in guard cells. Despite the importance of soluble carbohydrates in guard cells, only few investigators (refs. in Robinson and Preiss, 1985; Hite et al., 1992) have reported an enzymic basis for guard-cell carbohydrate metabolism, and none have borne directly on Suc metabolism, which we address in this paper.

MATERIALS AND METHODS

Chemicals

Analytical enzymes were from Boehringer; most other chemicals were from Sigma. Interfering contaminants in commercial UDP-Glc were removed according to the method of Outlaw et al. (1988).

Plant Material

Broad bean (*Vicia faba* L. cv Long Pod) plants were grown in pre-1990-formulated Hall's All Purpose potting mix. (Tests revealed that batches of this mix formulated more recently were unsuitable for *V. faba* culture.) Except as noted, plants were grown in a growth cabinet (nominally 600 mol m^{-2} s^{-1} PAR, 60% RH, and a 15-h photoperiod with day/night temperatures of 23/18°C). Except as noted, fully expanded bifoliates of 3- to 5-week-old plants were used in all experiments.

Enzyme Assays

SPS (EC 2.4.1.14), SS (EC 2.4.1.13), and INV (EC 3.2.1.26) were assayed (a) in a conventional way ("macroassays"), with aliquots of whole-leaf extract, and (b) by microscale methods ("microassays"), suitable for analysis of single cells. Macroassays were used primarily for methods development and validation, whereas microassays were used primarily to determine the quantitative localization of enzyme activities in *Vicia* leaflet.

[1] Supported in part by a grant from the U.S. National Science Foundation to W.H.O. and in part by a grant from the U.S. Department of Energy to W.H.O.

[2] Present address: 5 Prime → 3 Prime, Inc., 5603 Arapahoe, Boulder, CO 80303.

* Corresponding author; fax 1–904–644–0481.

Abbreviations: INV, acid invertase(s); SPS, sucrose-P synthase; SS, sucrose synthase. (Abbreviations connote protein, activity, or specific activity, depending on context.)

Macroassays

The extractions of SPS and SS were in 50 mM Mes (pH 6.9, pK = 6.1) that contained 5 mM $MgCl_2$, 1 mM EDTA, 4 mM DTT, 0.1% (w/v) BSA, and 2% (w/v) insoluble PVP. INV extraction was in 100 mM $NaPO_4$ (pH 6.9, pK = 6.7) that contained 200 mM NaCl, 1 mM EDTA, and 1 mM DTT. For all enzymes, (a) extraction was near 0°C, (b) a tissue:extraction cocktail ratio of 100 mg of fresh mass/2.5 mL of cocktail was used, (c) extracts were used immediately after centrifugation (5000*g*, 10 min), (d) substrate concentrations were set on the basis of preliminary experiments, (e) pH was optimized and tissue linearity and time linearity were confirmed, (f) stability during the assay of substrates, products, and effectors was confirmed, and (g) NADH oxidation or $NADP^+$ reduction was measured fluorometrically.

Both SPS and SS were measured in two steps, a "specific step," which was separately optimized for each activity in the synthetic direction, and an "indicator step," which was for both enzymes an enzymic measurement of UDP released. The specific step for SPS was 100 μL of 50 mM Hepes (pH 7.1, pK = 7.4), 5 mM $MgCl_2$, 10 mM Fru-6-P, ±10 mM UDP-Glc, 0.02% (w/v) BSA, ±5 mM Glc-6-P, ±2 mM Pi. The assay was conducted for 30 min at 35°C and was terminated by heating to 95°C for 2 min. The specific step for SS was 100 μL of 50 mM Tris-Cl (pH 8.3, pK = 8.1), 5 mM $MgCl_2$, 17 mM Fru, ±1.5 mM UDP-Glc, and 0.02% (w/v) BSA. The assay was conducted for 1 h at 40°C and was terminated by heating to 95°C for 2 min. The indicator steps for SPS and SS were carried out by the addition to the specific-step reagent of 1 mL of 100 mM Tris-Cl (pH 8.1), 100 μM phospho*enol*pyruvate, 5 μM NADH, 1 mM $MgCl_2$, 50 mM KCl (required for pyruvate kinase), 0.02% (w/v) BSA, 1.25 μg mL^{-1} of lactate dehydrogenase (EC 1.1.1.28), and 12.5 μg mL^{-1} of pyruvate kinase (EC 2.7.1.40; high concentration because of low affinity with UDP). Incubation was complete after 50 min at 23°C. INV was also measured in two steps. The INV-specific step was 100 μL of 100 mM acetate-Na (pH 4.8, pK = 4.8), ±10 mM Suc, and 0.02% (w/v) BSA. The assay was conducted for 1 h at 50°C. One milliliter of indicator reagent was added to the specific-step reagent, which terminated the reaction by pH shift. The INV indicator reagent (cf. Lowry and Passonneau, 1972) was 100 mM Tris-Cl (pH 8.2), 1 mM $MgCl_2$, 0.3 mM ATP, 50 μM $NADP^+$, 0.02% (w/v) BSA, 0.25 μg mL^{-1} of Glc-6-P dehydrogenase (EC 1.1.1.49), 1 μg mL^{-1} of P-Glc isomerase (EC 5.3.1.9), and 4 μg mL^{-1} of hexokinase (EC 2.7.1.1). Incubation was complete after 30 min at 23°C.

Microassays

Pieces of leaf were rapidly frozen in N_2 slurry (−210°C, to avoid the Leidenfrost effect) and then transferred to powdered dry ice. Fragments measuring from 1 to 3 mm were freeze-dried (72 h, −40°C, 10 μm Hg). Individual cells of 6 to 15 ng dry mass were dissected out and weighed. The oil-well technique was used for assays, the specific steps of which were scaled down about 2000-fold from the macroscale version described above. NAD(P) oxidation (SPS, SS) or reduction (INV) was measured by enzyme cycling (Kato et al., 1973; Lowry and Passonneau, 1972, respectively). General aspects of the quantitative histochemical methodology have been described elsewhere (Lowry and Passonneau, 1972; Outlaw, 1980; Hampp and Outlaw, 1987). In exhaustive preliminary experiments, we found protoplasts unsuitable for the analysis of SPS and SS. The present histochemical method provided complete recovery of SPS, but activities of SS were diminished compared with those of fresh extract.

RESULTS

Figure 1 shows the results of six experiments in which SPS and SS were assayed in leaflets of different ages along the axis of a *Vicia* plant. Because absolute values vary (as will be discussed below; cf. Sung et al., 1989), the specific activities were normalized to the values of the leaflets at the third node from the ground. SS was highest in young leaves and declined as the leaves matured (similar in magnitude and trend to the studies of four species by Claussen et al., 1985); these findings lend support to the idea (refs. in Dali et al., 1992) that SS is proportional to sink strength in tissues that utilize nucleoside diphosphate sugars, such as developing leaves.

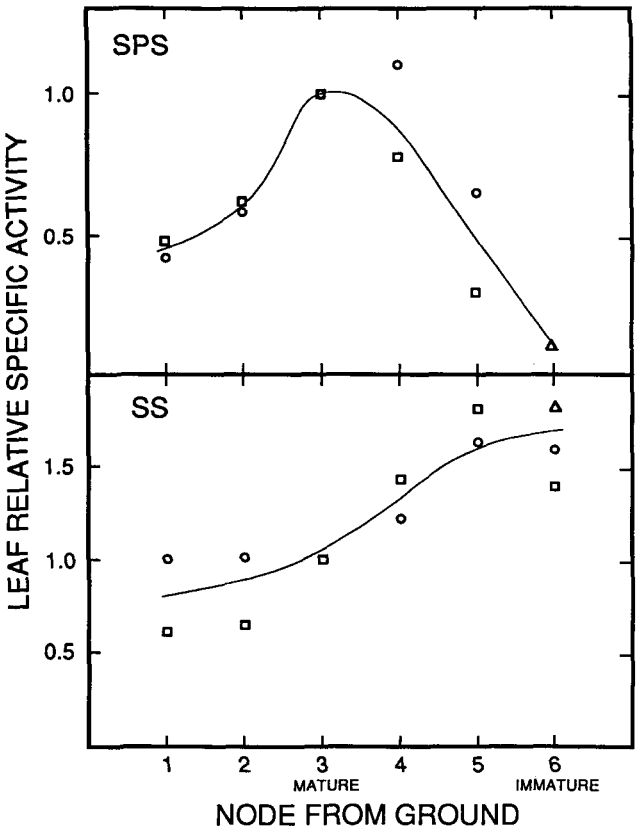

Figure 1. The relative specific activities of SPS (top) and SS (bottom) in leaves along the axis of *V. faba*. The raw values are normalized to the leaf inserted at node 3. (This leaf is similar to "Leaf Position 4" of the diagram by Outlaw et al., 1992.) The ratio, SPS:SS, in the leaf at node 3 was 2.8, whereas at node 5, the ratio was 0.27. Results shown are for three plants (indicated by different symbols), 19 to 23 d of age. Other data (not shown) from older plants confirmed this trend.

SPS was very low in immature leaves and reached the highest specific activity in fully expanded leaves. These data conform to the notion that one role of SPS is in the mobilization of newly assimilated carbon from source leaves (refs. in Worrell et al., 1991). In summary, the main value of Figure 1 is to demonstrate that SPS and SS in *Vicia* are distributed in a predictable pattern. In all other subsequent studies, mature leaves were used.

Fragments of freeze-dried leaves were assayed for SPS and SS (macroassay) or were used for dissection of nanogram tissue samples, which were assayed for these enzymes (microassay). Given the variability found in whole leaf, these data (Fig. 2) show that the microassay yielded values for various cell types that are consistent with whole-leaf values obtained conventionally. To confirm that the apparently mature leaves did not differ between the tip and base (which would reflect a difference in development, Claussen et al., 1985), we assayed leaf subsamples. Because Suc content varies considerably from cell to cell (Outlaw and Manchester, 1979), palisade samples from different fragments of the same sample were assayed. The similar variability (Fig. 2, first five bars for SPS in second column of top panel) in SPS does not prove but is consistent with the general idea that there is a correlation between SPS and Suc concentration (e.g. Fieuw and Willenbrink, 1987; Robbins and Pharr, 1987; Worrell et al., 1991). On the experimental basis, the photosynthetic

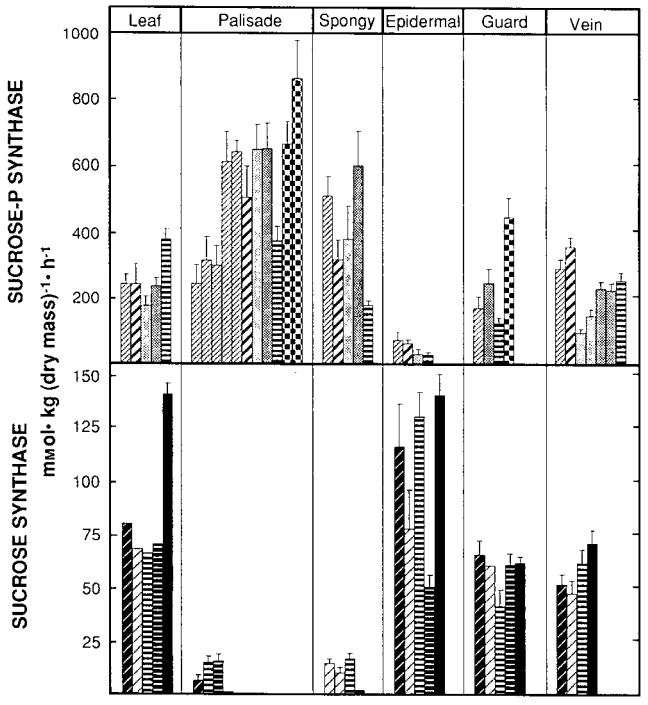

Figure 2. Summary of quantitative histochemical assays for SPS (top) and SS (bottom) activities in various cell types on the experimental basis (dry mass). The results shown above derive mainly from two paired mature leaflets each for SPS (A and B) and SS (E and F). (Data for three other leaflets are included for comparison.) SPS, leaflet A: tip, ▨; base, ▨; SPS, leaflet B: tip, ▬; base, ▬; SPS, leaflet C: ▥; SPS, leaflet D: ▦; SS, leaflet E: tip, ▩; base, ▩; SS, leaflet F: tip, ▥; base, ▥; SS, leaflet G: ■.

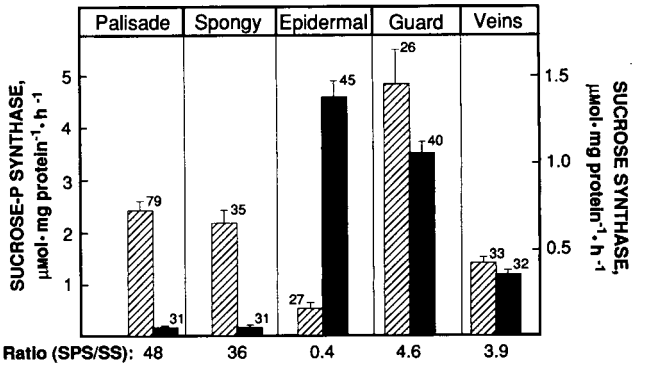

Figure 3. SPS (▨) and SS (■) in cells and tissues of *V. faba* leaflet. *n* is indicated by SE bars.

parenchyma, both types of which export sugar during photosynthesis (Outlaw and Fisher, 1975; Outlaw et al., 1975), had the highest SPS within the leaf and the lowest SS, whereas heterotrophic epidermal cells had the lowest SPS and the highest SS. These first quantitative data for single-cell localization of SPS and SS correlate well with the conclusions obtained by developmental studies (see above).

The essence of this report is found in Figure 3. SPS and SS data from the experiments reported in Figure 2 have been summarized and converted to a protein basis (conversion factors from Outlaw et al., 1985, except for vein, which was determined to be 17% protein [dry mass basis] by the Folin method). (The samples were treated equally, therefore SE are for the entire population and not the experiment averages.) Figure 3 shows the distribution of SPS and SS in *Vicia* leaflet. The photosynthetic parenchyma averaged approximately 2.3 μmol mg^{-1} of protein^{-1} h^{-1} of SPS (on a directly comparable basis, similar to maize leaf, Kalt-Torres et al., 1987). SS activity in these "bulk" tissues was low (about 0.06 μmol mg^{-1} of protein h^{-1}), a directly comparable value for *Vicia* leaf is unknown to us, and leaf SS varies widely among species from 0 to 3.4 μmol mg^{-1} of Chl h^{-1} (about 0.14 μmol mg^{-1} of protein h^{-1}) (Huber, 1981). On a protein basis, guard cells had the highest SPS (approximately 4.8 μmol mg^{-1} of protein h^{-1}), but these cells also had very high SS (about 1.1 μmol mg^{-1} of protein h^{-1}). Veins had intermediate levels of SPS and SS. Epidermal cells had a pronounced level of SS but lower SPS than any other cell or tissue assayed.

INV in guard cells was estimated to be 12 ± 2 mmol kg^{-1} of dry mass h^{-1} (n = 10). Interpretation of results with other microassays was problematic because of low specific activities, and the problem was exacerbated by the need to keep extract concentration low to maintain linearity.

DISCUSSION

Stomata open as a result of solute accumulation in the subtending guard-cell pair. The decrease in solute potential is correlated with accumulation of K salts (refs. in Outlaw, 1983). This important role of K uptake is an established fact that has been demonstrated for many species, treatment conditions, and experimental approaches. Indeed, a major current research focus in stomatal physiology is the identifi-

cation of and the elucidation of properties of ion channels (Schroeder et al., 1984, and subsequent literature) that mediate K accumulation in and dissipation from guard cells. (For a brief review, see Outlaw et al., 1992.) However, the focus on K fluctuations has diminished the impact of reports that Suc concentration increases when stomata open (see introduction).

The reasons to invoke Suc involvement in stomatal movement are compelling. First, in a series of careful studies, MacRobbie (e.g. MacRobbie and Lettau, 1980a, 1980b) concluded that K salts alone could not account for the guard-cell-solute-potential differences observed when stomata open. Second, Outlaw and Manchester (1979) reported a small but repeatable and significant increase of guard-cell Suc concentrations when stomata were opened in planta. Third, Tallman's laboratory (Poffenroth et al., 1992) has recently found that guard-cell Suc concentration increases when stomata on sonicated epidermal peels open. As a first step toward understanding this aspect of the specialized carbon metabolism of guard cells, we have quantified SPS and SS in guard cells and other leaf cells of *V. faba*. We have also established an upper limit for INV in guard cells.

The prime result of our study was finding that guard cells have very high levels of SPS and SS. On face value, these data indicate that guard cells have an enhanced capacity for synthesis and an enhanced capacity for degradation of Suc (approximately 1.4 and 0.3 pmol guard-cell pair^{-1} h^{-1} for SPS and SS, respectively), consistent with the Suc changes that occur with stomatal movement (0.27 pmol guard-cell pair^{-1}, calculated from Outlaw and Manchester, 1979). As discussed earlier, SS is an indicator of sink strength in organs and probably serves a consonant cell-specific role in epidermal cells and guard cells. As we envision it, Suc concentration increase during stomatal opening could result from Suc uptake (Reddy and Rama Das, 1986; Rohrig and Raschke, 1991) or from synthesis mediated by guard-cell SPS. Understanding temporal and environmental regulation of these enzymes in guard cells is essential to understanding carbohydrate interconversion in these cells.

Outlaw (1983) listed the myriad mechanisms through which Suc might stabilize biological structures. Here, we focus only on its potential role as an osmolyte. On a cell-average basis, the concentration of Suc is 59 mM, spongy parenchyma; 45 mM, palisade parenchyma; and 130 mM, guard cells of open stomata (data of Outlaw and Manchester, 1979), converted to cell basis (Outlaw et al., 1985) and, finally, to a molarity basis (table 2.3 of Willmer, 1983). In the present context, the important number, which to our knowledge is not known, is the leaf cytosolic Suc concentration in planta. Lacking solid data for this value in any leaf cell, we speculate that it is higher there than in the vacuole. There are two bases for this speculation. (a) The mathematical modeling done by Fisher and Outlaw (1979) with *Vicia* leaflet indicated that the palisade cytosolic Suc concentration was 3-fold that of the palisade vacuole (but see Farrar and Farrar, 1986); (b) in leaf (and some other but perhaps not all plant cells), Suc transport into the vacuole is an energy-independent process (Kaiser and Heber, 1984), indicating that the cytosol, the site of Suc synthesis, is at least as concentrated in Suc as is the vacuole (except, of course, when or if the cytosol is rapidly drained). From these observations, we infer that the guard-cell average 130 mM is the lower limit for the cytosolic concentration. In summary, our present knowledge is consistent with Suc being a significant and fluctuating compatible osmolyte. This hypothesis is testable by measurements of the nucleotide specificity of guard-cell SS, by elucidation of the transport properties of the guard-cell chloroplast, by more detailed and localized measurements of guard-cell Suc concentration, by kinetic measurements of Suc uptake into guard cells, by kinetic characterization, and characterization with respect to stomatal movements, of SS and SPS.

To our knowledge, the quantitative SPS and SS values for veins (Fig. 3; cf. Tomlinson et al., 1991) are the first from source transport tissue. In addition to the general comments made earlier, we are not presently able to ascribe a particular localized function. It is noteworthy, however, that both enzymes were measured on a tissue-averaged basis. Because SS is enriched in certain cells of the phloem (see above), the specific activity of this enzyme is obviously elevated far above that of a "source cell," prototypically the palisade parenchyma (Nolte and Koch, 1992). An obvious correlation is the need for energy ("sink") to accumulate Suc in the companion cell-sieve tube complex. As Suc fluxes in *Vicia* vein are modeled (Outlaw et al., 1975), consideration of this correlation simply depends on quantification of SS at high anatomical precision. The minimum conclusion is that the concepts of sources and sinks will be elucidated at the cell or tissue level and not at the organ level.

Received September 23, 1992; accepted January 3, 1993.

LITERATURE CITED

Claussen W, Loveys BR, Hawker JS (1985) Comparative investigations on the distribution of sucrose synthase activity and invertase activity within growing, mature and old leaves of some C_3 and C_4 plant species. Physiol Plant **65:** 275–280

Dali N, Michaud D, Yelle S (1992) Evidence for the involvement of sucrose-phosphate synthase in the pathway of sugar accumulation in sucrose accumulating tomato fruits. Plant Physiol **99:** 434–438

Farrar SC, Farrar JF (1986) Compartmentation and fluxes of sucrose in intact leaf blades of barley. New Phytol **103:** 645–657

Fieuw S, Willenbrink J (1987) Sucrose synthase and sucrose phosphate synthase in sugar beet plants (*Beta vulgaris* L. ssp. *altissima*). J Plant Physiol **131:** 153–162

Fischer RA, Hsiao TC (1968) Stomatal opening in isolated epidermal strips of *Vicia faba*. II. Response to KCl concentration and the role of potassium absorption. Plant Physiol **43:** 1953–1958

Fisher DB, Outlaw WH Jr (1979) Sucrose compartmentation in the palisade parenchyma of *Vicia faba* L. Plant Physiol **64:** 481–483

Fujino M (1967) Role of adenosinetriphosphate and adenosinetriphosphatase in stomatal movement. Sci Bull Fac Educ Nagasaki Univ **18:** 1–47

Hampp R, Outlaw WH Jr (1987) Microanalytik in der pflanzlichen Biochemie. Naturwissenschaften **74:** 431–438

Hite DRC, Bodson MJ, Outlaw WH Jr (1992) Enzymic potential for fructose 6-phosphate phosphorylation by guard cells and by palisade cells in leaves of the broad bean *Vicia faba* L. Histochem J **24:** 368–374

Huber SC (1981) Interspecific variation in activity and regulation of leaf sucrose phosphate synthetase. Z Pflanzenphysiol **102:** 443–450

Humble GD, Raschke K (1971) Stomatal opening quantitatively

related to potassium transport. Evidence from electron probe analysis. Plant Physiol **48:** 447–453

Imamura S-I (1943) Untersuchungen über den Mechanismus der Turgor-Schwankung der spaltöffnungs-Schliesszellen. Jpn J Bot **12:** 251–346

Kaiser G, Heber U (1984) Sucrose transport into vacuoles isolated from barley mesophyll protoplasts. Planta **161:** 562–568

Kalt-Torres W, Kerr PS, Huber SC (1987) Isolation and characterization of multiple forms of maize leaf sucrose-phosphate synthase. Physiol Plant **70:** 653–658

Kato T, Berger SJ, Carter JA, Lowry OH (1973) An enzymatic cycling method for nicotinamide-adenine dinucleotide with malic and alcohol dehydrogenases. Anal Biochem **53:** 86–97

Lowry OH, Passonneau JV (1972) A Flexible System of Enzymatic Analysis. Academic Press, New York

MacRobbie EAC, Lettau J (1980a) Ion content and aperture in "isolated" guard cells of *Commelina communis* L. J Membr Biol **53:** 199–205

MacRobbie EAC, Lettau J (1980b) Potassium content and aperture in "intact" stomatal and epidermal cells of *Commelina communis* L. J Membr Biol **56:** 249–256

Mouravieff I (1972) Microphotométrie des fluctuations de la teneur en amidon des stomates en rapport avec l'ouverture de l'ostiole à la lumière, en présence ou en absence de gaz carbonique. Ann Sci Nat Bot **12:** 361–368

Nolte KD, Koch KE (1992) Companion-cell-specific localization of sucrose synthase in zones of phloem loading and unloading. Plant Physiol **99:** S-237

Outlaw WH Jr (1980) A descriptive evaluation of quantitative histochemical methods based on pyridine nucleotides. Annu Rev Plant Physiol **31:** 299–311

Outlaw WH Jr (1982) Carbon metabolism in guard cells. Recent Adv Phytochem **16:** 185–222

Outlaw WH Jr (1983) Current concepts on the role of potassium in stomatal movements. Physiol Plant **59:** 302–311

Outlaw WH Jr, Fisher DB (1975) Compartmentation in *Vicia faba* leaves. I. Kinetics of ^{14}C in the tissues following pulse labeling. Plant Physiol **55:** 699–703

Outlaw WH Jr, Fisher DB, Christy AL (1975) Compartmentation in *Vicia faba* leaves. II. Kinetics of ^{14}C-sucrose redistribution among individual tissues following pulse labeling. Plant Physiol **55:** 704–711

Outlaw WH Jr, Hite DRC, Fiore GB (1988) Removal of contaminating nucleoside diphosphates from commercial preparations of uridine diphosphoglucose. Anal Biochem **171:** 104–107

Outlaw WH Jr, Hite DRC, Zhang SQ (1992) Molecular, cellular, and plant mechanisms of ABA control of stomatal aperture size. *In* CM Karssen, LC van Loon, D Vreugdenhil, eds, Progress in Plant Growth Regulation. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 474–485

Outlaw WH Jr, Manchester J (1979) Guard cell starch concentration quantitatively related to stomatal aperture. Plant Physiol **64:** 79–82

Outlaw WH Jr, Springer SA, Tarczynski MC (1985) Histochemical technique. A general method for quantitative enzyme assays of single cell "extracts" with a time resolution of seconds and a reading precision of femtomoles. Plant Physiol **77:** 659–666

Pearson CJ (1973) Daily changes in stomatal aperture and in carbohydrates and malate within epidermis and mesophyll of leaves of *Commelina cyanea* and *Vicia faba*. Aust J Biol Sci **26:** 1035–1044

Poffenroth M, Green DB, Tallman G (1992) Sugar concentrations in guard cells of *Vicia faba* illuminated with red or blue light. Plant Physiol **98:** 1460–1471

Raschke K (1979) Movements of stomata. *In* W Haupt, ME Feinleib, eds, Encyclopedia of Plant Physiology (New Series), Vol 7. Springer-Verlag, Berlin, pp 383–441

Reddy AR, Rama Das VS (1986) Stomatal movement and sucrose uptake by guard cell protoplasts of *Commelina benghalensis* L. Plant Cell Physiol **27:** 1565–1570

Robbins NS, Pharr DM (1987) Regulation of photosynthetic carbon metabolism in cucumber by light intensity and photosynthetic period. Plant Physiol **85:** 592–597

Robinson N, Preiss J (1985) Biochemical phenomena associated with stomatal function. Physiol Plant **64:** 141–146

Rohrig K, Raschke K (1991) [^{14}C]-Sucrose uptake by guard cell protoplasts of *Pisum sativum*, argenteum mutant (abstract No. 317). Plant Physiol **96:** S-47

Sayre JD (1926) Physiology of stomata of *Rumex patientia*. Ohio J Sci **26:** 233–266

Schroeder JI, Hedrich R, Fernandez JM (1984) Potassium-selective single channels in guard cell protoplasts of *Vicia faba*. Nature **312:** 361–362

Sung SJS, Xu DP, Black CC (1989) Identification of actively filling sucrose sinks. Plant Physiol **89:** 1117–1121

Tomlinson PT, Duke ER, Nolte KD, Koch KE (1991) Sucrose synthase and invertase in isolated vascular bundles. Plant Physiol **97:** 1249–1252

Williams WT (1954) A new theory of the mechanism of stomatal movement. J Exp Bot **5:** 343–352

Willmer CM (1983) Stomata. Longman, London, UK

Worrell AC, Bruneau JM, Summerfelt K, Boersig M, Voelker TA (1991) Expression of a maize sucrose phosphate synthase in tomato alters leaf carbohydrate partitioning. Plant Cell **3:** 1121–1130

Yamashita T (1952) Influences of potassium supply upon various properties and movement of the guard cell. Sieboldia **1:** 51–70

Yemm EW, Willis AJ (1954) Stomatal movements and changes of carbohydrate in leaves of *Chrysanthemum maximum*. New Phytol **53:** 373–397