Carbon Partitioning and Source-Sink Interactions in Plants, *Monica A. Madore* and *William J. Lucas*, eds, Copyright 1995, published by The American Society of Plant Physiologists

Sugar Alcohol Metabolism in Source Leaves

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Roles in Photosynthesis, Transport, and Storage

Sugar alcohols account for as much as 30% of global primary photosynthetic production (Bieleski, 1982). Sugar alcohols were first shown to be primary photosynthetic products in higher plants approximatelty 35 years ago, after earlier work had established similar roles in algae. Rapid glucitol labeling was demonstrated in apple and plum leaves, as was rapid $^{14}CO_2$ incorporation into mannitol in *Fraxinus* and *Syringa* and into allitol in *Itea*. These, and later studies, showed that $^{14}CO_2$ assimilation generally resulted in two major soluble products, e.g. in apple, approximately 70% of the fixed carbon was distributed between sucrose and glucitol, and in celery, 80 to 90% between mannitol and sucrose. When galactosyl-sucrose oligosaccharides were significant products, sucrose and the sugar alcohol collectively accounted for less of the newly fixed carbon (Bieleski and Redgwell, 1977; Flora and Madore, 1993; Loescher *et al.*, 1992). In all sugar-alcohol-producing higher plants studied to date, sucrose is present and is frequently the major photoassimilate. This dual, or multiple, pattern of primary products is not, however, universal; in diatoms and brown algae, mannitol may be the sole soluble product.

Like sucrose, sugar alcohols are also phloem transported, and frequently serve as storage compounds in fungi, algae, and higher plants. They may also be major photosynthetic carbon pools in leaves and other vegetative tissues, as well as in fruits and other storage organs. Sugar alcohols are additionally involved in heterotrophic nutrition, osmoregulation, and transport, occurring in mammals, bacteria, yeasts, fungi, parasitic plants, and in nonphotosynthetic

stages of green plants, e.g. developing and germinating seeds and seedlings. However, space does not permit covering these topics here. For further discussion and for a list of extensive reviews of sugar alcohol physiology, distribution, and chemistry, see Loescher and Everard (1995).

Sugar Alcohol Biosynthesis

Compared to 15 years ago, sugar alcohol enzymology in higher plants is now better understood. Numerous synthetic and degradative enzymes have been identified—some have been purified, with antibodies available, and two critical genes in plant mannitol and glucitol biosynthesis have been sequenced, as has a step in mannitol degradation. Progress began when it was shown that glucitol synthesis in the family Rosaceae was accompanied by the appearance of hexose and hexitol phosphates (Bieleski and Redgwell, 1977). An NADPH-dependent aldose 6-phosphate reductase (A6PR) was discovered in *Eriobotrya* (Hirai, 1979), in apple cotyledons (Yamaki, 1980), and mature leaves of apple, peach, pear, and apricot (Negm and Loescher, 1981). A similar enzyme was found in *Trollius* in the Ranunculaceae (Chen and Negm, unpubl. data). An NADPH-dependent mannose 6-phosphate reductase (M6PR) was reported in celery in the Apiaceae (Loescher *et al.*, 1982; Rumpho *et al.*, 1983) and a ribitol-synthesizing NADPH-dependent ribose 5-phosphate reductase in *Adonis* in the Ranunculaceae (Negm and Marlow, 1985). Other reductases include a galactitol-synthesizing, NADPH-dependent aldose reductase in *Euonymus* leaves in the Celastraceae (Negm, 1986), and an NADH-dependent ketose reductase in maize endosperm (Doehlert, 1987). For further discussion of these and related enzymes, see Loescher and Everard (1995). Also, although A6PR has been cloned, sequenced, and otherwise well characterized (Kanayama *et al.*, 1992, only M6PR will be discussed in any detail here.

Mannitol is the most widely distributed of the sugar alcohols (Bieleski, 1982), and M6PR is the critical step in higher plant mannitol biosynthesis (Loescher *et al.*, 1992). M6PR has been isolated, characterized, and purified (Loescher *et al.*, 1992), polyclonal antibodies prepared, and the gene cloned and sequenced (J. Everard, C. Cantini, R. Grumet, and W. Loescher, unpubl. data). M6PR is predominantly cytosolic and not at all vacuolar or associated with any membrane system or organelle, although it may be present in nuclei. M6PR (and thus mannitol biosynthesis) is also primarily restricted to green, palisade, and spongy parenchyma tissues and bundle sheath cells in celery, but not vascular parenchyma (Everard *et al.*, 1993). Similarly, labeling studies in olive (*Olea*) suggested spatial separation of stachyose and mannitol biosynthesis, with rapid mannitol synthesis occurring within the photosynthetic mesophyll tissues and slower raffinose synthesis occurring closer to, probably within, minor veins (Flora and Madore, 1993).

Conclusions regarding sugar alcohol synthesis are supported by several kinds of evidence, e.g. the above-mentioned localization studies, as well as developmental, source-sink transition studies in celery (Davis and Loescher, 1990) and peach (Merlo and Passera, 1991) and studies of the parasites *Orobanche* in the Orobanchaceae (Harloff and Wegmann, 1993) and *Thesium* in the Santalaceae (Simier *et al.*, 1994). But, very little is known about regulation at the enzyme level, even less is known about regulation of transport and utilization, and nothing is known about regulation at the gene level.

Roles of Sugar Alcohols in Stress Tolerance in Higher Plants

Although sugar alcohols are clearly involved in photosynthesis, transport, and storage, in most species, these roles are played by sucrose and starch, and sucrose is apparently translocated in all higher plants (Zimmerman and Ziegler, 1975). What then is the advantage of sugar alcohols? Work on algae (Cowan *et al.*, 1992), lichens (Honegger, 1991), and fungi (Brown, 1978) suggests roles as compatible solutes in tolerance of environmental stresses, especially those related to salinity and drought. Several biophysical explanations have been proposed for compatible solutes and their interactions with biological structures (Galinski, 1993). The prevailing view suggests uneven distribution of compatible solutes in a protein solution where compatible solutes are excluded from protein hydration spheres; in some way, bulk water structure is ordered so that biological structures become preferentially hydrated and conformation is maintained as the bulk water activity is otherwise lowered.

Plants adapted to high salts have various mechanisms by which deleterious effects are avoided or minimized. Exclusion is one mechanism, but in most tolerant species, inorganic ions entering the cells are usually sequestered in the vacuole. In such salt-tolerant plants, ions may accumulate such that the water potential of the vacuole balances that of external milieu, thus maintaining turgor. In the cytoplasm, however, ions are usually maintained at low concentrations, yet this compartment must remain in osmotic equilibrium with the vacuole and the external medium. Compatible solutes play a role since they may accumulate in the cytoplasm without disrupting biological processes.

In theory, compatible solutes preferentially accumulate in the cytoplasm, but sugar alcohols are apparently predominantly stored in the vacuole (Keller and Matile, 1989). This implies that salt stress causes a reallocation of sugar alcohol to the cytoplasm. Although little is known about reallocation of sugar alcohols in response to stress, the extravacuolar/vacuolar ratio of proline has been reported to increase with water stress in tobacco protoplasts (Pahlich *et al.*, 1983). In celery, petiole parenchyma mannitol is stored predominantly in the vacuole (81%), with lesser amounts in the cytosol (19%), but cytosolic concentrations may reach 300 mM (Keller and Matile, 1989).

Evidence that sugar alcohols play roles in plant stress tolerance, especially to salt stress, is compelling in marine algae (Kirst, 1990) and in the euryhaline

green algal flagellate *Dunaliella*, where glycerol is involved in osmoregulation across the entire NaCl solubility range (see Ginzburg, 1987, and Cowan *et al.*, 1992, for reviews). However, few studies link the acyclic hexitols to salt tolerance in higher plants. In a distributional study, mannitol (as well as cyclitols) dominated instead of sugars in several salt-secreting mangrove species; whereas, their nonmangrove counterparts typically lacked hexitols and cyclitols, but there was little correlation between accumulation and increasing salt load (Popp, 1984). In salt-tolerant members of the Plantaginaceae, however, glucitol accumulated strikingly with salinity stress and declined when the stress was relieved (Briens and Larher, 1983).

Several recent studies show that salt or nutrient stress increases sugar alcohol accumulation in higher plants, i.e. mannitol in celery (Stoop and Pharr, 1994*a*, *b*) and olive (Romani *et al.*, 1992) and glucitol in *Plantago* (Briens and Larher, 1983), but the cellular mechanisms involved were not investigated. In another celery study, however, increasing salt (NaCl) stress not only increased mannitol pools (and decreased other carbohydrates), but also increased relative levels of M6PR (Everard *et al.*, 1994). That study also showed that in mature leaves, the rate of mannitol biosynthesis was maintained at control levels even at 300 mM NaCl, despite a 70% reduction in photosynthesis. Labeling of sucrose and starch declined commensurate with photosynthesis. In another study, celery exposed to high macronutrient levels also showed increases in mannitol and M6PR in mature leaves and, in addition, decreases in mannitol 1-oxidoreductase in sink tissues (Stoop and Pharr, 1994*b*).

RESULTS AND DISCUSSION

Here, we report some results of a developmental study which shows that salt effects depend distinctly on leaf age. Salt treatments had no effect on total assimilation rates in young leaves, although older leaves senescenced prematurely (Fig. 1), but ^{14}C-labeling patterns in young leaves were altered dramatically with the proportion in mannitol increasing from 10% in controls to 23 and 37% in 150- and 300-mM treated plants, respectively (Table I). As in mature leaves (Everard *et al*, 1994), increased mannitol labeling occurred at the expense of sucrose, but the difference between the two leaf ages was that the rate of mannitol biosynthesis in young leaves increased 1.8- and 3-fold over controls in 150- and 300-mM treated plants, respectively. Increased mannitol synthesis in young leaves was also associated with much higher M6PR activities (Everard *et al.*, 1994) and M6PR protein (data not shown). Our conclusion is that salt treatments induced earlier expression of mannitol biosynthesis in leaf tissues.

Table I. *^{14}C-Labeling of Young, Still Expanding Celery Leaves*

See Everard *et al.* (1994) for experimental protocols. Values followed by the same letter are not significantly different at the 5% level.

	^{14}C-Labeling		Rate	
Treatment	Sucrose	Mannitol	Sucrose	Mannitol
	%		*(μCi/g fresh wt) x 10^3*	
Control	82.2 a	10.2 a	44.3 a	5.6 a
150 mM NaCl	68.4 b	23.3 b	29.9 a	10.2 ab
300 mM NaCl	59.1 c	36.9 c	26.7 a	16.7 b

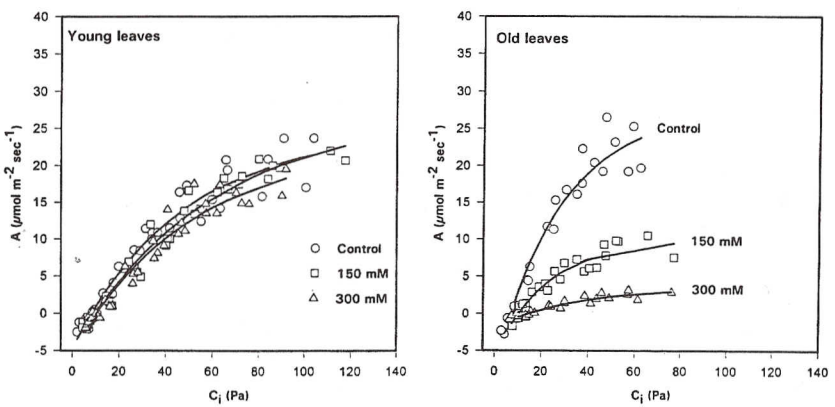

Figure 1. Salt effects on A-Ci curves for young (expanding) and old celery leaves. See Everard *et al.* (1994) for experimental protocols and further descriptions of leaf ages.

These results suggest a role for mannitol in stress tolerance, but do not resolve whether it is in osmoregulation or as a compatible solute. Genetic transformations have begun to address these same questions. For example, a mannitol 1-phosphate dehydrogenase, from the *E. coli mtl* operon, was recently introduced into tobacco. Although transformants produced only very low (micromolar) levels of mannitol in most tissues, the plants apparently showed enhanced salt tolerance (Tarczynski *et al.*, 1993). However, an important next step will be genetic transformation of a salt-tolerant, sugar-alcohol-synthesizing species to see if lack of the hexitol diminishes salt tolerance.

As compatible solutes, sugar alcohols may play a general role in stress tolerance. Work on insects strongly suggests a role in cold hardiness (Storey and Storey, 1991). The evidence in plants is less convincing (see review by Loescher and Everard, 1995). However, glucitol content and A6PR activity increased in late autumn and decreased in spring in evergreen leaves of loquat (*Eriobotrya*) and in seedlings exposed to low temperatures (Hirai, 1983). Low-temperature acclimation in *Opuntia* increased soluble sugars two- to nine-fold, including a nearly three-fold increase in mannitol in the hardiest species (Goldstein and Nobel, 1994).

Various salt, drought, and cold stress effects suggest that sugar alcohol metabolism might be highly regulated by stress at the gene level. Such relationships are clear in other organisms (Jennings and Burke, 1990), but in higher plants, the evidence more often involves increased capacity for cyclitol synthesis (Vernon and Bohnert, 1992). In barley embryos, however, desiccation tolerance was accompanied by expression of an ABA- and GA-promoted gene with high structural homology to mammalian genes encoding NADPH-dependent aldose reductases involved in sorbitol synthesis (Bartels *et al.*, 1991), but the barley gene product's substrate specificity and product were undefined, and presence of sorbitol in barley has not been established. Interestingly, the celery M6PR sequence also has similarities to several aldose

Table II. *DNA Similarities Between Celery M6PR and Genes Catalogued in the BLAST Nonredundant Database*

81%	-	rat aldose reductase
71%	-	*C. elegans* cDNA sequence
66%	-	apple aldose 6-phosphate reductase
60%	-	rat dihydrodiol dehydrogenase
60%	-	rat lens aldose reductase
60%	-	rat hydroxysteroid dehydrogenase
58%	-	mouse aldose reductase

reductases (Table II), but further relationships to stress tolerance mechanisms remain to be determined.

Conclusions and Considerations for Future Research

Understanding of sugar alcohol metabolism has progressed significantly in the last 15 years for both glucitol and mannitol. Nonetheless, little is yet known about regulation of biosynthesis or degradation, and even less is known about the basic metabolism of other sugar alcohols in higher plants. Sugar alcohol metabolism is very tightly controlled developmentally, but we have yet to describe any of the mechanisms involved. The systems involved are complex for two major reasons: (*i*) The need to regulate carbon partitioning in plants has been discussed in detail at this and other conferences, and all the arguments for the need to regulate carbon flow in starch and sucrose synthesizers are equally relevant to sugar alcohol synthesizers, with the added complication of another synthetic pathway (with additional regulatory steps) competing for the same substrates and intermediates. And (*ii*), with the increasing probability that sugar alcohols are involved in stress tolerance in higher plants, this almost certainly requires still further modulation of metabolism, storage, and transport. However, despite the complexities and difficulties, as we answer these problems we should develop insight not only into regulation of carbon partitioning, but also into mechanisms of stress tolerance, with the result the potential to increase crop productivity.

ACKNOWLEDGMENTS

This work was supported in part by a USDA-NRI grant #940-1439 to W.H.L., J.D.E., and R.G.

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