

## MINIREVIEW

**The carbon reactions of photosynthesis: role of lectins and glycoregulation**A.M. NONOMURA<sup>\*,+</sup>, D. SHEVELA<sup>\*\*,\*\*\*</sup>, S.S. KOMATH<sup>#</sup>, K.Y. BIEL<sup>###,####</sup>, and G. GOVINDJEE<sup>#,†</sup>*Carbon Reactions of Photosynthesis Sector, BRANDT iHammer, Powell, Ohio, USA\***Department of Chemistry, Chemical Biological Centre, Umeå University, Umeå, Sweden\*\***ShevelaDesign AB, Umeå, Sweden\*\*\***School of Life Sciences, Jawaharlal Nehru University, New Delhi, India<sup>#</sup>**Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Russia<sup>###</sup>**Biosphere Systems International Foundation, Tucson, Arizona, USA<sup>####</sup>**Department of Plant Biology, Department of Biochemistry, and Center of Biophysics & Quantitative Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA<sup>†</sup>***Abstract**

Modulation of glycoregulation in agriculture is reviewed here with emphasis on the elucidation of previously unknown pathways involving vacuolar lectins as well as a bypass of lectins that direct free sugars toward productivity. The reversible binding sequences of the endogenous lectin cycle are compared to an induced lectin bypass, as follows. (1) In the cycle, carbohydrate ligands, with similar binding specificities that compete for binding sites on lectins, are involved in the natural cycle of sugar exchanges. (2) For the bypass, tightly bound ligands that occupy lectins prevent free sugars from binding, making them available for productivity. This bypass is induced by methyl- $\alpha$ -D-mannopyranoside, a biochemical plant growth regulator for photosynthesis. Integration of this novel technology, with structural elements crucial for ligand binding by the lectins and with nitrogen assimilation, provides the basis for successful modulation of glycoregulation in crops for enhancement of quality and quantity.

*Keywords:* agglutinin; Benson-Bassham-Calvin cycle; concanavalin A; dark reactions; mannose-binding specificity;  $\alpha$ -D-mannoside.

**Introduction**

We present our perspective on the carbon reactions of photosynthesis that includes key investigations from a series that had been initiated by Melvin Calvin and Andrew A. Benson in ‘The Path of Carbon in Photosynthesis’ (Calvin and Benson 1948; for historical reviews, see Benson 2002, Bassham 2003, Sharkey 2019, Govindjee 2020). Later investigations published in this series, starting with Nonomura and Benson (1992a) and ending with Nonomura *et al.* (2017), led to the recognition of a major role for lectins in plants (Benson and Nonomura 1992, Nonomura and Benson 1992b, 2012, 2013, 2014; Benson *et al.* 2009, Biel *et al.* 2010, Nonomura *et al.* 2011, 2012). This laid the groundwork for the development of methods for inducing the modulation of glycoregulation by lectins that affects the carbon reactions of photosynthesis (for the carbon reactions, formerly known as the dark reactions, see Buchanan 2016) involving the action of a novel biochemical class of plant growth regulators (Biel

and Fomina 2015, Nonomura *et al.* 2018a, 2020) on the path of carbohydrates, such as glucose (Glc; Fig. 1A) and methyl- $\beta$ -D-glycopyranoside ( $\beta$ MeG; Fig. 1B).

Methyl- $\alpha$ -D-mannopyranoside ( $\alpha$ MeM; Fig. 1C) is the active ingredient in the commercial plant growth regulator, *BRANDT GlucoPro*, that was recently developed for field treatment of crops (see section ‘Label information’). It induces free sugars to bypass the plant lectin cycle (for a background on this metabolic pathway, see Nonomura *et al.* 2017, 2018a, 2020; Govindjee *et al.* 2020).

We provide below an introduction to the function of lectins in the carbon reactions of photosynthesis, and then we describe the bypass in plants, with diagrams of metabolic pathways that compare it to the endogenous lectin cycle.

**The role of lectins in plants**

Lectins are proteins that possess at least one noncatalytic domain that is capable of reversibly binding free sugars

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*Abbreviations:* CBA – carbohydrate-binding agent; ConA – concanavalin A; CRD – carbohydrate recognition domain; Glc –  $\beta$ -D-glucose; Glc/Man – glucose/mannose; IAA – indoleacetic acid;  $\alpha$ MeM – methyl- $\alpha$ -D-mannopyranoside;  $\beta$ MeG – methyl- $\beta$ -D-glucopyranoside.

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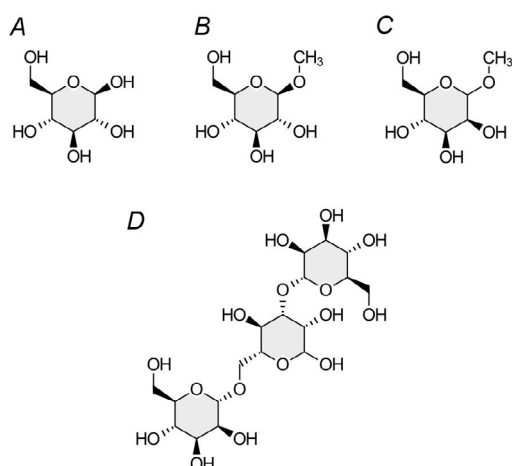


Fig. 1. Chemical structures of  $\beta$ -D-glucose (Glc) (A), methyl- $\beta$ -D-glucopyranoside ( $\beta$ MeG) (B), methyl- $\alpha$ -D-mannopyranoside ( $\alpha$ MeM) (C), and trimannopyranoside (D).

or glycans (Sharon and Lis 2007). The nomenclature and classification of lectins have evolved with the field and multiple methods of classification exist for these proteins (Komath *et al.* 2006). Based on sequence conservation amongst their carbohydrate recognition domains alone, lectins are organized into twelve evolutionarily conserved families (Van Damme *et al.* 2008).

The ubiquitous presence of different types of lectins across the plant kingdom is matched by their enormous variety of linkable carbohydrates (*see, e.g.*, Van Damme *et al.* 1998). Lectins in plants are defined by their specificities for recognition of various saccharides, primarily Glc, mannose, galactose, fucose, and their N-linked derivatives. Lectins account for as much as a tenth of the total protein dry mass in seeds, particularly in beans (Sharon and Lis 2007). Further, their localization varies according to the structures, tissues, and organelles of a given species, depending on the specific requirements and properties of the plants. For example, some nucleocytoplasmic lectins provide protective mechanisms in plants (Lannoo and Van Damme 2014) and some appear to be involved in stress signaling (Lambin *et al.* 2020). Similarly, some legume lectins provide the receptors required for *Rhizobium* bacteria to bind to root hairs and to produce nodules (Laus *et al.* 2006). Yet, other lectins may have a role in developmental regulation and immobilization of sugars (Lannoo and Van Damme 2010). However, in this minireview, we focus on the structure and function of the lectins present in vacuoles of cells in roots, shoots, and seeds of flowering plants (Nonomura *et al.* 2017).

Thylakoid membranes from several plants have been demonstrated to possess lectins and, in at least one case, they have been shown to interact with Rubisco in a galacturonic acid-dependent manner (Schröder and Petit 1992, Hincha *et al.* 1993, Aleksidze *et al.* 2002). More recently, it was shown that some lectins in stroma provide ligands to Rubisco (Kovalchuk *et al.* 2012). The significance of these observations became apparent when Nonomura and

Benson (2014) recognized that vacuolar lectins function in photosynthesis. As many of the well-characterized plant lectins are secretory proteins that tend to accumulate in the vacuoles, perhaps, one of the most spectacular roles of vacuolar lectins is in the carbon reactions of photosynthesis (Nonomura and Benson 2014, Nonomura *et al.* 2017, Govindjee *et al.* 2020) since regulation of this pathway has widespread applications in agriculture.

Historically, the first step towards the elucidation of this role of lectin was taken when  $^{13}\text{C}$ -labeled  $\beta$ MeG was identified, by *in vitro*  $^{13}\text{C}$ -NMR assays, to accumulate in sycamore cells treated with  $^{13}\text{C}$ -methanol (Gout *et al.* 2000). Significantly, Benson *et al.* (2009) established that the treatment of plants with methyl glucopyranosides (MeG) enhanced growth. Soon thereafter, Biel *et al.* (2010) demonstrated that most of the  $^{14}\text{C}$ - $\beta$ MeG given to *Beta vulgaris* remained stable within the root cells and that there was no further catabolism after exogenous  $\beta$ MeG was linked to an amine. This was a conundrum. If  $\beta$ MeG had been stored in a protein, then how could it have promoted growth?

Noting the high content of lectins in seeds, Nonomura and Benson (2013) (1) devised a rapid bioassay as a method to further study this phenomenon that was based on monitoring the germination of radish seeds; and, (2) compared responses of seedlings of various species of Brassicaceae to  $\alpha$ - and  $\beta$ -anomers of lectin substrates. From these experiments, Nonomura and Benson (2013) concluded that  $\alpha$ -D-glycopyranosides have higher potencies for enhancing seedling development than  $\beta$ -D-glycopyranosides. Furthermore,  $\alpha$ -D-mannopyranosides, too, produced rapid growth enhancement in the presence of nutrients and this was found to be not only in radish but also in other  $\text{C}_3$  plants, as well as in CAM and  $\text{C}_4$  plants, clearly suggesting that the function of lectins in this process is widespread and probably ubiquitous. In addition, the following order of effective dose response in the treatment of plants was observed:  $\alpha$ -D-trimannopyranoside (0.01 mM) >  $\alpha$ MeM (0.03 mM) >  $\beta$ MeG (309 mM) > Glc (> 309 mM) (Nonomura *et al.* 2012, Nonomura and Benson 2013, 2014). In another observation, the requirement of  $\text{Ca}^{2+}$  and  $\text{Mn}^{2+}$  for hastening the germination process in the presence of substrates was shown (Nonomura and Benson 2014).

Several plant lectins that bind to glucose/mannose (Glc/Man) already have been well-characterized and molecular details of their carbohydrate recognition, as well as ligand specificities are now available (*see, for example, Barre et al.* 2001, 2019). These include many legume and bulb lectins, such as concanavalin A (ConA), *Pisum sativum* lectin (PSA), *Lathyrus ochrus* I lectin (LOL-I), and snowdrop (*Galanthus nivalis*) lectin (GNA). Several amongst them, but not all, are known to require  $\text{Ca}^{2+}$  and  $\text{Mn}^{2+}$  in their carbohydrate recognition domains (CRDs). Since they are generally multi-subunit proteins, with the CRD of each subunit possessing extended binding sites, oligosaccharides and multivalent ligands are the preferred choices over simple monosaccharides (Schwarz *et al.* 1993, Naismith and Field 1996, Smeets *et al.* 1997,

Loris *et al.* 1998, Dam and Brewer 2007). For example, ConA is a Glc/Man binding homotetrameric lectin with greater specificity for trimannosides over simpler sugars (Naismith *et al.* 1994, Naismith and Field 1996). Each of its monomers possesses a single CRD with tightly bound  $\text{Ca}^{2+}$  and  $\text{Mn}^{2+}$  ions that participate in ligand binding.

Extrapolating from the above information, it became apparent that the  $\text{Ca}^{2+}$  and  $\text{Mn}^{2+}$  dependent Glc/Man-binding lectins were involved in the stimulation of plant growth that Nonomura and Benson (2014) had observed. These key experimental observations showing the correspondence of plant responses to lectins are summarized in Table 1.

As implied above, multi-subunit lectins bind oligosaccharides better than monosaccharides. Consistent with this, Nonomura and Benson (2014) observed the high efficacy of trimannopyranoside (10  $\mu\text{M}$ ; Fig. 1D) in stimulating growth. In contrast to this, blends of  $\alpha$ - and  $\beta$ -anomers in syrups of MeG (309 mM) were among the least potent (Benson *et al.* 2009). In general, in these investigations, oligosaccharides exhibited the highest potencies in all cases. From these trends of binding specificities, a natural cycle of chemical competition for storage and release of sugars from lectins was recognized (Nonomura and Benson 2014). When presented with two sugars of comparable affinities, a lectin would bind to the one that is present at a higher concentration. Thus, by modulating concentrations of the alternate sugars, the bound ligand may be exchanged with the competing ligand. In contrast, when a lectin is exposed to a ligand with a very strong binding affinity it would bind tightly, allowing little chance for further exchange except at extremely high concentrations of a competing ligand.

The chloroplast is well known to be a site for regulation of photosynthesis by inorganic carbon (*see*, for example, Shevela *et al.* 2012, 2020) and a site for regulation in the path of organic carbon (Armbruster and Strand 2020). Recently, it became clear that the vacuole may be a hub for the modulation of glycoregulation since the Glc/Man-binding lectins are located there (Govindjee *et al.*

2020, Nonomura *et al.* 2017). For educational purposes, we describe the endogenous plant lectin cycle as would operate in the plant before treatment with  $\alpha\text{MeM}$ , and then we show how the plant lectin bypass is induced by the treatment of crops with such a ligand of strong binding affinity.

## Endogenous lectin cycle in plants

The natural lectin cycle begins with carbon fixation (*see* Fig. 2). Atmospheric  $\text{CO}_2$ , as well as  $\text{CO}_2$  from other plant tissues already present in the vicinity, enter cells and reach the stroma, where the photosynthetic carbon reduction cycle takes place (for historical reviews, *see* Benson 2002, Bassham 2003, Fomina and Biel 2016, Sharkey 2019; and for a general background of photosynthesis, *see* Shevela *et al.* 2018). Some of the products of the photosynthetic carbon reduction cycle, such as sugars, are transported from the chloroplasts into the vacuoles of plant cells, where they bind one or more specific lectins that are involved in the carbon reactions of photosynthesis (carbon reaction lectins). We represent the sugar products of carbon reduction by Glc. The ligands, Glc and  $\beta\text{MeG}$ , have comparable affinities for binding lectins. Thus, as Glc is consumed by respiration, its concentration decreases to a point where endogenous  $\beta\text{MeG}$  is able to displace bound Glc from these lectins. Thereafter, as the concentration of Glc increases in the vacuole, due to fresh supplies from the photosynthetic carbon reduction cycle, Glc successfully outcompetes  $\beta\text{MeG}$  to, once again, bind lectins. This completes the endogenous cycle. As Glc level drops and it becomes depleted,  $\beta\text{MeG}$ , again, exchanges with Glc to occupy the CRD of the lectins, and the cycle repeats. Fig. 2 shows the endogenous lectin cycle in plant cells (as modified from Fig. 1S, *supplement*).

## Induced plant lectin bypass

Treatment of plants with highly competitive ligands of the lectins, such as  $\alpha\text{MeM}$ , induces changes in the

Table 1. Characterization of lectins corresponding to experimental results.

Lectin property	Reference
$\beta\text{MeG}$ , ligand	Benson <i>et al.</i> (2009)
$\beta\text{MeG}$ -linked protein	Biel <i>et al.</i> (2010)
$\beta\text{MeG}$ unmodified, not catabolized	Aubert <i>et al.</i> (2004), Biel <i>et al.</i> (2010)
Kinetin riboside, ligand	Nonomura <i>et al.</i> (2011)
$\alpha$ -anomer specificity greater than $\beta$ -anomer Mannose specificity greater than glucose	Nonomura <i>et al.</i> (2013)
$\alpha\text{MeM}$ , specificity Trimannopyranoside, high specificity Structural $\text{Ca}^{2+}$ and $\text{Mn}^{2+}$	Nonomura and Benson (2014)
Modulation of glycoregulation	Nonomura <i>et al.</i> (2017)
$\alpha\text{MeM}$ -induced sugars	Nonomura <i>et al.</i> (2018b)

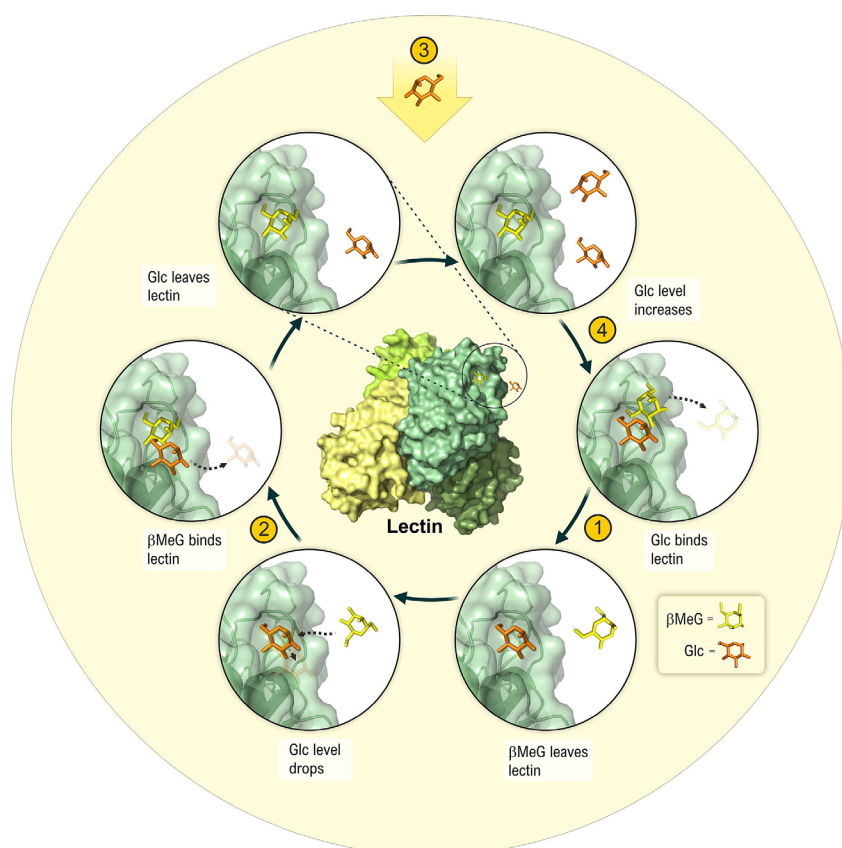


Fig. 2. Diagram of the endogenous lectin cycle in plants. It begins with  $\text{CO}_2$  entering cells, leading to the photosynthetic carbon reduction cycle. Products are transported from chloroplasts into vacuoles where some of the free sugars, represented by glucose (Glc), bind carbon reaction lectins (1). When Glc concentration drops, endogenous methyl- $\beta$ -D-glucopyranoside ( $\beta$ MeG) binds lectins. Glc leaves lectin (2). Thereafter, fresh supplies of Glc from the photosynthetic carbon reduction cycle (3) increase the concentration of Glc in the vacuole and it successfully outcompetes  $\beta$ MeG to, once again, bind lectins (4). This completes the endogenous cycle. When Glc level decreases,  $\beta$ MeG again displaces it to occupy the binding site and the cycle repeats. The circled numbers 1–4 indicate the reaction sequence of the cycle (as modified from Fig. 1S). Cells and their parts are not drawn to scale; the molecular model of lectin is based on ConA; and the structure and cofactors were generated using coordinates from the Protein Data Bank (PDB) entries 1LES and 5CNA (Naismith *et al.* 1994, Casset *et al.* 1995).

carbon reactions of photosynthesis, due to a bypass of the lectin cycle. The bypass begins within minutes after the active ingredient,  $\alpha$ MeM, has entered the cells. It binds the carbon reaction lectins tightly and displaces sugars, represented by Glc. While the binding sites of these lectins are occupied by  $\alpha$ MeM, fresh Glc generated from the photosynthetic carbon reduction cycle is locked out by the high-affinity ligand, bypassing the lectins. Fresh Glc molecules, which continue to bypass the lectins for weeks after treatment, are directed to growth and respiration and may be translocated further to plant tissues in roots and shoots, including, stems, foliage, flowers, fruits, and seeds. The induced lectin bypass in plant cells is shown in Fig. 3 (as modified from Fig. 1S; an animation of the bypass is shown in Fig. 2S, *supplement*).

### Glycoregulation

Modulation of glycoregulation has been confirmed in the field after treatments with the first commercial formulation which included the active ingredient  $\alpha$ MeM. For example, treated field-grown melons and grapes showed significant increases in soluble sugar content as compared to the controls (Nonomura *et al.* 2018a,b). Notably, during daylight hours, sugars allocated to roots or symbionts may yield the products of respiratory metabolism, water, and  $\text{CO}_2$ . Those end products, after upward transport from roots to shoots, may favor photosynthesis, particularly

with elevated  $\text{CO}_2$  in the chloroplast (for an example of transport from roots to shoots in trees, *see* Bloemen *et al.* 2013; for symbionts *see* ten Veldhuis *et al.* 2020).

### Concluding remarks

#### An abundance of lectins

Lectins are proteins with a history that can be traced to the naming of plant agglutinins by William C. Boyd in 1954. As suggested by their Latin name roots, ‘legere’ (meaning, to pick or select), lectins bind carbohydrates according to varying specificities and affinities. The ranking of lectins as some of the most important proteins in photosynthetic protists and plants is based on the view that they are translators of the glycode (Nilsson 2007) and the abundance of their presence in photosynthetic organisms may rank second only to that of Rubisco. Indeed, experiments by Benson *et al.* (2009) and genetic studies by Van Holle and Van Damme (2019) point to genomic evolution by the expansion of lectin families from algae to vascular plants. The key point in understanding the function of lectins, in the carbon reactions, is the reversibility of its carbohydrate-binding property and the ability of different sugars to simultaneously compete for and displace already bound ligands based on their available concentrations and affinities (Sharon and Lis 2007). The occupants of CRDs are, therefore, displaceable by chemical competitors.

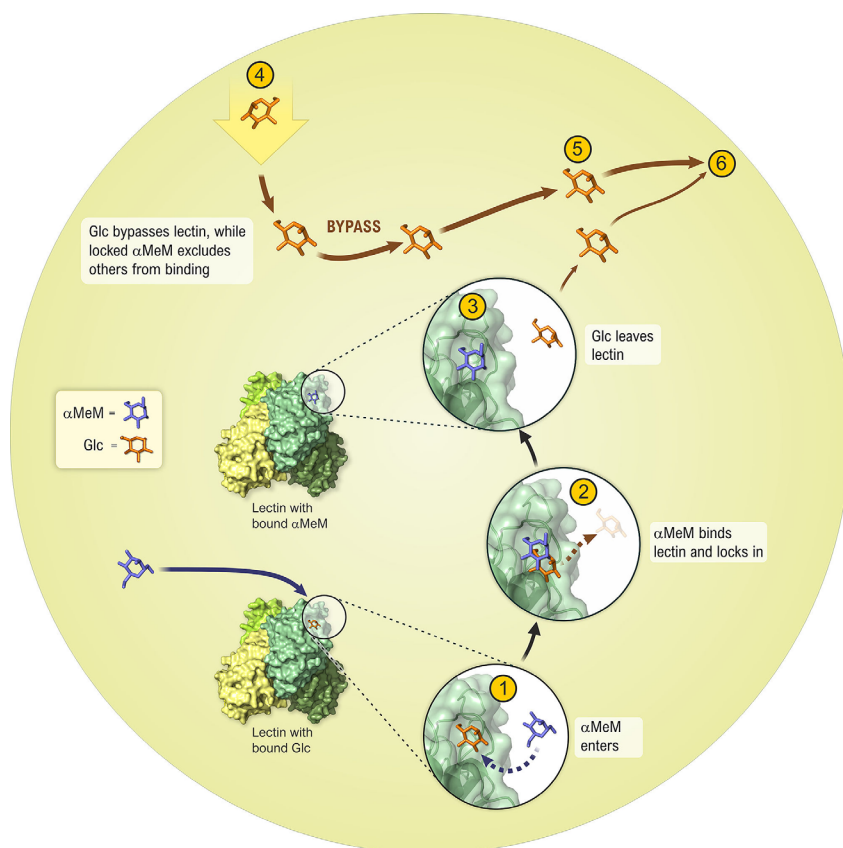


Fig. 3. Diagram of the lectin bypass in plants. Exogenous methyl- $\alpha$ -D-mannopyranoside ( $\alpha$ MeM) induces the bypass. After  $\alpha$ MeM is in the vacuole of plant cells (1), it binds carbon reaction lectins (2) and displaces Glc (3). While  $\alpha$ MeM is bound tightly and locked, fresh Glc from the photosynthetic carbon reduction cycle (4) cannot bind lectins. Instead, Glc bypasses lectins (5). This leads to growth (6). The circled numbers 1–6 indicate the reaction sequences (as modified from Fig. 1S). Sugar products are represented by Glc; cell parts are not drawn to scale; the molecular model of lectin is based on ConA; and the structure and cofactors were generated using coordinates from the PDB entries 1LES and 5CNA (Naismith *et al.* 1994, Casset *et al.* 1995).

### The vacuole as the hub of glycoregulation

Vacuoles are the largest organelles, often occupying greater than three quarters of the volume inside the plant cell, and we recognize that they are the cellular location for the storage of metabolic products, including sugars and proteins, as nutrients (Zhang *et al.* 2015). Given the abundance of vacuolar lectins, we point out that they may be essential for the role that the vacuole plays in maintaining turgor, exchange and storage of metabolites, detoxification, as well as homeostasis (Marty 1999).

When sugars are bound to lectins, they are osmotically inert, but free sugars may be active. Accumulation of free glucose in the vacuole may account for the osmotic potential required for the maintenance of turgor, pressing the tonoplast against the cell wall and driving cell expansion (Aluri and Büttner 2007). Such stiffening of plant cells (shown in an animation in Fig. 3S, *supplement*) may have contributed to the recovery from midday wilt that had been observed in crops (Nonomura and Benson 1992a, Nonomura *et al.* 2018a). Thus, the role of lectins in the modulation of glycoregulation, by controlling the concentrations of available free sugars, is important for the physical and metabolic functions that are essential for the life of the plant. As explained in this minireview, the role of lectins in the carbon reactions of photosynthesis is equally critical for the physical and metabolic well-being of the plant. For crops, vacuolar processes may contribute to the flavor of fruits and vegetables. Moreover, by enhancement

of the turgor pressure in flowers, leafy greens, and fruits, the treated end product may be crisp and deeply flavorful while also lengthening shelf life.

### The plant growth regulator, $\alpha$ MeM

Besides CRDs, many plant lectins also possess specific independent binding sites for hydrophobic ligands (Komath *et al.* 2006). Early studies by Roberts and Goldstein (1983) showed that such binding sites in lectins from *Phaseolus lunatus* (lima bean), *P. vulgaris* (kidney bean), *Glycine max* (soybean), and *Dolichos biflorus* (horse gram) could bind to plant growth regulators such as N<sup>6</sup>-benzyladenine, kinetin, zeatin, and zeatin riboside. Besides, Edelman and Wang (1978) showed that ConA from *Canavalia ensiformis* (jack bean) binds the auxin, indoleacetic acid (IAA). Further, Benson *et al.* (2009) observed growth enhancement of canola when the phytohormones, IAA and kinetin, were formulated with MeG; and Nonomura *et al.* (2011) showed the efficacy of sugar-conjugated plant growth regulators in modulating plant growth.

Thus, lectins may be regarded as the central mediators of information transfer not only due to their ability to translate the glycode via their interactions with specific monosaccharides as well as the terminal ligands of oligosaccharides, glycoproteins, and glycolipids (Nilsson 2007) but also by their interactions with plant growth regulators. Recently lectins have been subsumed under the larger group of carbohydrate-binding agents (CBAs),



which include both proteinaceous and nonproteinaceous agents as well as synthetic molecules that can specifically bind carbohydrates. However, as we note here, the definition of lectins as CBAs limits our understanding of the role of lectins within plants.

The plant growth regulator  $\alpha$ MeM, discussed in this minireview, alters the metabolic pathways of displaced compounds and fresh photosynthates from the photosynthetic carbon reduction cycle by blocking their access to their endogenous receptors on the carbon reaction lectins within the vacuoles. Enhanced flower counts, vibrant colors from sugar-based pigments such as anthocyanins, and improved yields have been observed after field treatments with the plant growth regulator formulation (Nonomura *et al.* 2018a,b). In addition to glycoregulation, we shall investigate its effect on other networks (*see*, for example, Law *et al.* 2020); furthermore, related metabolic fluxes in the carbon reactions of guard cells may lead to investigations of stomatal movement (*see*, for example, Zhang *et al.* 2018).

### Growth, productivity and more

We have focused here on carbon reactions in the vacuoles, and yet, these pathways diverge widely from points of growth that incorporate carbon backbones into the synthesis of amino acids by the different pathways of nitrogen assimilation and photorespiration (Bloom 2015). The reduction of nitrate to ammonia ( $\text{NH}_3$ ), the incorporation of  $\text{NH}_3$  into amino acids, and the carbon reduction cycle operate in the chloroplast; and it is noteworthy that carbon and nitrogen assimilation are integrated processes (Bloom 2015, Thompson *et al.* 2017). Bloom *et al.* (2002) showed that co-application of elevated  $\text{CO}_2$  with nitrogen fertilizers, particularly with the input of  $\text{NH}_3$ , promoted the synthesis of nitrogenous end-products. These observations led Benson *et al.* (2009) to begin experimenting with nitrogen supplementation of exogenous lectin ligands. In consideration of the correlation of photosynthetic capacity to nitrogen content (*see*, for example, Hymus *et al.* 2001, Jin *et al.* 2015), and the implications for drought relief in crops under elevated  $\text{CO}_2$ , by photosynthetic and nitrogen-use efficiency (Sekhar *et al.* 2020), applications of nitrogenous plant nutrition in conjunction with modulation of glycoregulation may benefit vegetative productivity in the field, particularly in the crops of leafy greens.

Historically, Hoagland and Arnon (1950) must be acknowledged for defining the essential nutrient elements required for the growth of plants. We add that plant growth regulators are known to interact with these nutrients. For example, phytohormones are involved in the regulation of nitrogen uptake systems, particularly with regard to lateral root development in response to changes in nitrogen availability (Kiba *et al.* 2011). Thus, as primary, secondary and micronutrients are co-applied with  $\alpha$ MeM to various crops, adjustments may be made to the seasonal programs to meet quality standards and marketable quantities of harvests (*see, e.g.*, Fageria *et al.* 2008, 2009; Pier and Barlow 2018).

In contrast to other plant growth regulators,  $\alpha$ MeM

modulates glycoregulation. We know that two plant nutrients,  $\text{Ca}^{2+}$  and  $\text{Mn}^{2+}$ , are also integral to carbohydrate binding by many Glc/Man specific lectins (Naismith *et al.* 1994, Loris *et al.* 1998). Nonomura and Benson (2014) found these specific cations to be a prerequisite for plant growth response to exogenous ligands. Therefore, we suggest that these elements also form the basis for recognition of the high binding specificity for locked ligands (in this case,  $\alpha$ MeM) in the carbon reaction lectins, making them essential for the transfer of this technology to agriculture.

To sum up, in this overview we have presented the discoveries and innovations that led to the elucidation of the plant lectin cycle and its bypass. We have described here how the scientific investigations have progressed from molecular (carbohydrate, protein), to cellular (chloroplast, vacuole), and to organismal (whole plant) levels. Finally, as we begin to comprehend the relationship of the structure and function of lectins to the advancement of photosynthetic efficiency, transfer to agriculture is our goal and hope of our international interdisciplinary collaborations (Biel and Fomina 2015, Nonomura *et al.* 2017, 2018a,b; Govindjee *et al.* 2020).

### Label information

The natural product  $\alpha$ MeM is registered by the United States Environmental Protection Agency (EPA) under the brand name, *BRANDT GlucoPro*, and the master label, iH026a (Nonomura *et al.* 2018b). This label is approved for foliar spray, drench, and chemical irrigation application; use on listed crops grown in fields, orchards, vineyards, greenhouses, and nurseries; use on ornamentals, flowers, lawns, commercial turf, recreational areas, and golf courses; and for use on field-grown, greenhouse-grown, nursery-grown, and hydroponically-grown ornamentals and florals. This proven plant growth regulator: increases yields, improves crop quality, enhances crop turgidity, diminishes midday wilt, promotes shoot and root growth, promotes greater root mass development, improves seed vigor, increases sweetness in fruits, increases blossoms, diminishes sun scorch, reduces the incidence of blossom end rot, enlarges foliage, encourages fuller plants, and improves stress tolerance. For general instructions on-field use of the first commercial product featuring  $\alpha$ MeM, the *GlucoPro* brochure is available at URL: <https://brandt.co/media/11012/brandt-gluco-pro-brochure.pdf>. For U.S. Patents BRANDT iHammer proprietary technologies, *see* [Brandt.Co/iHammerPatents1/](#), [Brandt.Co/iHammerPatents2/](#).

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