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MINIREVIEW

The carbon reactions of photosynthesis: role of lectins and glycoregulation

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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-α-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; α-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. 1*A*) and methyl-β-D-glycopyranoside (βMeG; Fig. 1*B*).

Methyl-α-D-mannopyranoside (αMeM; Fig. 1*C*) 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 – β-D-glucose; Glc/Man – glucose/mannose; IAA – indoleacetic acid; αMeM – methyl-α-D-mannopyranoside; βMeG – methyl-β-D-glucopyranoside.
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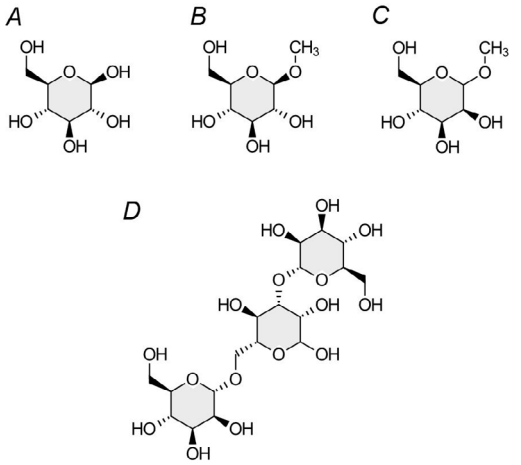

Fig. 1. Chemical structures of β-D-glucose (Glc) (*A*), methyl-β-D-glucopyranoside (βMeG) (*B*), methyl-α-D-mannopyranoside (αMeM) (*C*), and trimannopyranoside (*D*).

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}C-labeled βMeG was identified, by *in vitro* ^{13}C-NMR assays, to accumulate in sycamore cells treated with ^{13}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}C-βMeG given to *Beta vulgaris* remained stable within the root cells and that there was no further catabolism after exogenous βMeG was linked to an amine. This was a conundrum. If βMeG 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 α- and β-anomers of lectin substrates. From these experiments, Nonomura and Benson (2013) concluded that α-D-glycopyranosides have higher potencies for enhancing seedling development than β-D-glycopyranosides. Furthermore, α-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 C_3 plants, as well as in CAM and 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: α-D-trimannopyranoside (0.01 mM) > αMeM (0.03 mM) > βMeG (309 mM) > Glc (> 309 mM) (Nonomura *et al.* 2012, Nonomura and Benson 2013, 2014). In another observation, the requirement of Ca^{2+} and 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 Ca^{2+} and 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 Ca^{2+} and Mn^{2+} ions that participate in ligand binding.

Extrapolating from the above information, it became apparent that the Ca^{2+} and 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 μM; Fig. 1*D*) in stimulating growth. In contrast to this, blends of α- and β-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 αMeM, 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 CO_2, as well as 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 βMeG, have comparable affinities for binding lectins. Thus, as Glc is consumed by respiration, its concentration decreases to a point where endogenous βMeG 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 βMeG to, once again, bind lectins. This completes the endogenous cycle. As Glc level drops and it becomes depleted, βMeG, 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 αMeM, induces changes in the

Table 1. Characterization of lectins corresponding to experimental results.

Lectin property	Reference
βMeG, ligand	Benson *et al.* (2009)
βMeG-linked protein	Biel *et al.* (2010)
βMeG unmodified, not catabolized	Aubert *et al.* (2004), Biel *at al*. (2010)
Kinetin riboside, ligand	Nonomura *et al.* (2011)
α-anomer specificity greater than β-anomer Mannose specificity greater than glucose	Nonomura *et al.* (2013)
αMeM, specificity Trimannopyranoside, high specificity Structural Ca^{2+} and Mn^{2+}	Nonomura and Benson (2014)
Modulation of glycoregulation	Nonomura *et al.* (2017)
αMeM-induced sugars	Nonomura *et al.* (2018b)

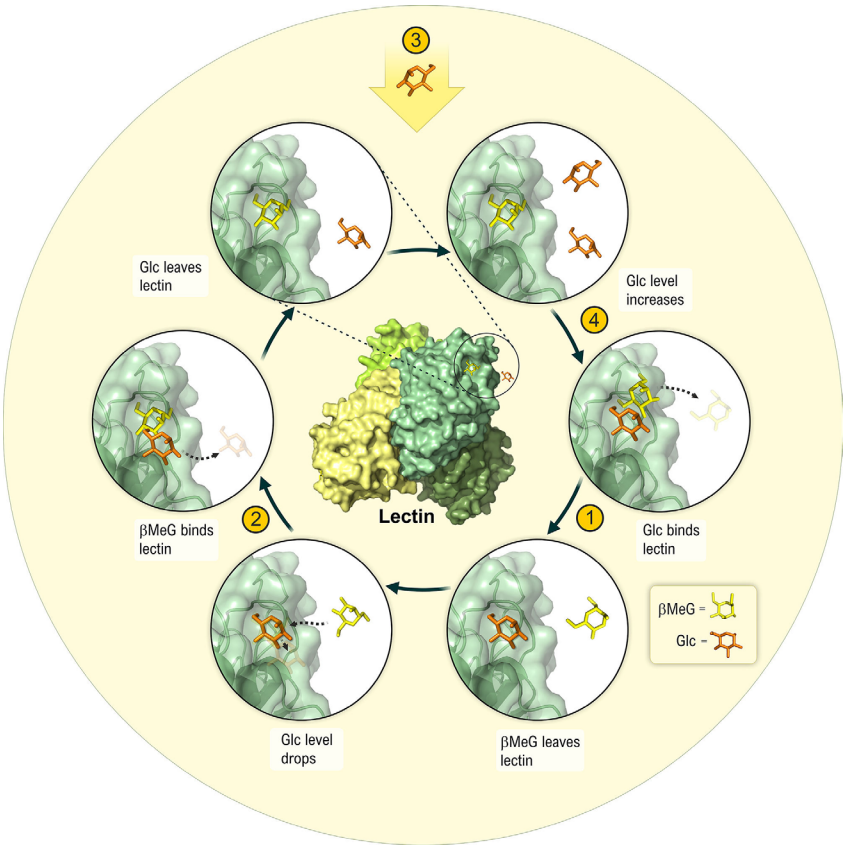

Fig. 2. Diagram of the endogenous lectin cycle in plants. It begins with CO_2 entering cells, leading to the photosynthetic carbon reduction cycle. Products are transported from chloroplasts into vacuoles where some of the free sugars, represented by glucose (Glc), bind carbon reaction lectins (1). When Glc concentration drops, endogenous methyl-β-D-glucopyranoside (βMeG) 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 βMeG to, once again, bind lectins (4). This completes the endogenous cycle. When Glc level decreases, βMeG 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, αMeM, 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 αMeM, 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 αMeM. 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 CO_2. Those end products, after upward transport from roots to shoots, may favor photosynthesis, particularly with elevated 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 glycocode (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.

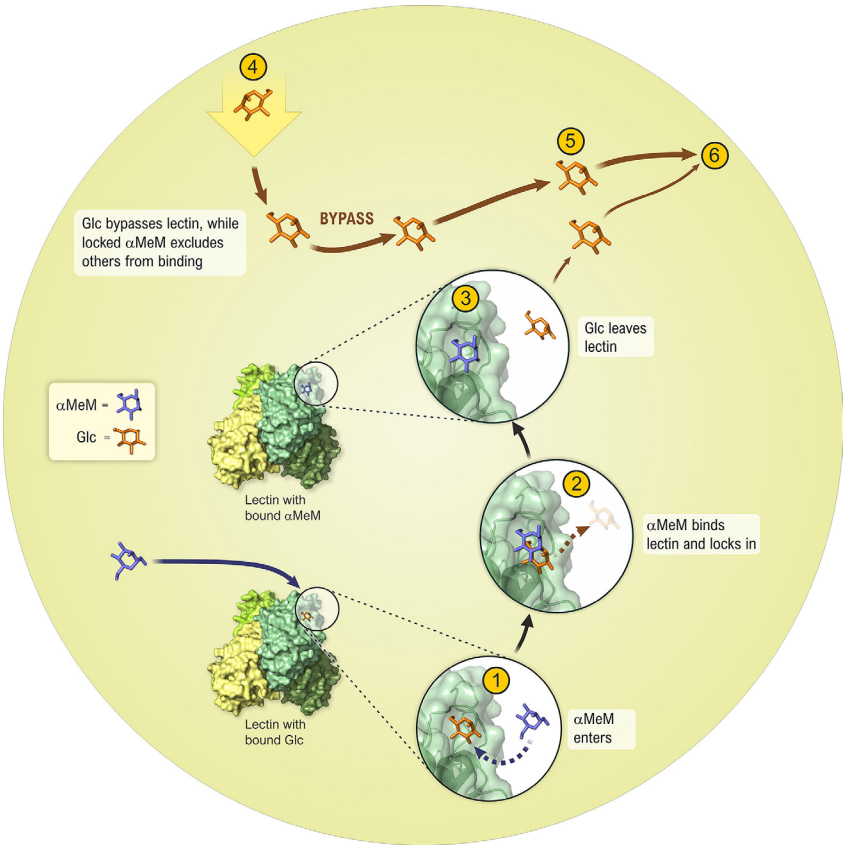

Fig. 3. Diagram of the lectin bypass in plants. Exogenous methyl-α-D-manno-pyranoside (αMeM) induces the bypass. After αMeM is in the vacuole of plant cells (1), it binds carbon reaction lectins (2) and displaces Glc (3). While αMeM is bound tightly and locked, fresh Glc from the photosynthetic carbon 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, αMeM

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^6-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 glycocode *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 αMeM, 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 (NH_3), the incorporation of 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 CO_2 with nitrogen fertilizers, particularly with the input of 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 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 αMeM 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, αMeM modulates glycoregulation. We know that two plant nutrients, Ca^{2+} and 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, αMeM) 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 αMeM 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 αMeM, the *GlucoPro* brochure is available at URL: https://brandt.co/media/11012/brandt-glucopro-brochure.pdf. For U.S. Patents BRANDT iHammer proprietary technologies, *see* Brandt.Co/iHammerPatents1/, Brandt.Co/iHammerPatents2/.

References

Aleksidze G. Ya., Litvinov A.I., Vyskrebentseva E.I.: The model of Calvin cycle enzyme organization on thylakoid membranes with the involvement of the Photosystem I lectin. – Russ. J. Plant Physl+ **49**: 137-141, 2002.

Aluri S., Büttner M.: Identification and functional expression of the *Arabidopsis thaliana* vacuolar glucose transporter 1 and its role in seed germination and flowering. – P. Natl. Acad. Sci. USA **104**: 2537-2542, 2007.

Armbruster U., Strand D.D.: Regulation of chloroplast primary metabolism. – Photosynth. Res. **145**: 1-3, 2020.

Aubert S., Choler P., Pratt J. *et al*.: Methyl-β-D-glucopyranoside in higher plants: Accumulation and intracellular localization in *Geum montanum* L. leaves and in model systems studied by ^{13}C nuclear magnetic resonance. – J. Exp. Bot. **55**: 2179-2189, 2004.

Barre A., Bourne Y., Van Damme E.J.M. *et al*.: Mannose-binding plant lectins: different structural scaffolds for a common sugar-recognition process. – Biochimie **83**: 645-651, 2001.

Barre A., Bourne Y., Van Damme E.J.M., Rougé P.: Overview of the structure–function relationships of mannose-specific lectins from plants, algae and fungi. – Int. J. Mol. Sci. **20**: 254, 2019.

Bassham J.A.: Mapping the carbon reduction cycle: a personal retrospective. – Photosynth. Res. **76**: 35-52, 2003.

Benson A.A.: Following the path of carbon in photosynthesis: a personal story. – Photosynth. Res. **73**: 29-49, 2002.

Benson A.A., Nonomura A.M.: The path of carbon in photosynthesis: methanol inhibition of glycolic acid accumulation. – In: Murata N. (ed.): Research in Photosynthesis, Proceedings of the IX International Congress on Photosynthesis. P-522. Kluwer, Dordrecht 1992.

Benson A.A., Nonomura A.M., Gerard V.A.: The path of carbon in photosynthesis. XXV. Plant and algal growth responses to glycopyranosides. – J. Plant Nutr. **32**: 1185-1200, 2009.

Biel K.Y., Fomina I.R.: Benson-Bassham-Calvin cycle contribution to the organic life on our planet. – Photosynthetica **53**: 161-167, 2015.

Biel K.Y., Nonomura A.M., Benson A.A., Nishio J.N.: The path of carbon in photosynthesis. XXVI. Uptake and transport of methylglucopyranoside throughout plants. – J. Plant Nutr. **33**: 902-913, 2010.

Bloemen J., McGuire M.A., Aubrey D.P. *et al*.: Transport of root-respired CO_2 via the transpiration stream affects aboveground carbon assimilation and CO_2 efflux in trees. – New Phytol. **197**: 555-565, 2013.

Bloom A.J.: Photorespiration and nitrate assimilation: a major intersection between plant carbon and nitrogen. – Photosynth. Res. **123**: 117-128, 2015.

Bloom A.J., Smart D.R., Nguyen D.T., Searles P.S.: Nitrogen assimilation and growth of wheat under elevated carbon dioxide. – P. Natl. Acad. Sci. USA **99**: 1730-1735, 2002.

Buchanan B.B.: The carbon (formerly dark) reactions of photosynthesis. – Photosynth. Res. **128**: 215-217, 2016.

Calvin M., Benson A.A.: The path of carbon in photosynthesis. – Science **107**: 476-480, 1948.

Casset F., Hamelryck T., Loris R. *et al*.: NMR, molecular modeling, and crystallographic studies of lentil lectin-sucrose interaction. – J. Biol. Chem. **270**: 25619-25628, 1995.

Dam T.K., Brewer C.F.: Fundamentals of lectin-carbohydrate interactions. – In: Kamerling J.P., Boons G.J., Lee Y.C. *et al*. (ed.): Comprehensive Glycoscience. Pp. 397-452. Elsevier, Oxford 2007.

Edelman G.M., Wang J.L.: Binding and functional properties of concanavalin A and its derivatives. III. Interactions with indoleacetic acid and other hydrophobic ligands. – J. Biol. Chem. **253**: 3016-3022, 1978.

Fageria N.K., Baligar V.C., Li Y.C.: The role of nutrient efficient plants in improving crop yields in the twenty first century. – J. Plant Nutr. **31**: 1121-1157, 2008.

Fageria N.K., Barbosa Filho M.P., Moreira A., Guimarães C.M.: Foliar fertilization of crop plants. – J. Plant Nutr. **32**: 1044-1064, 2009.

Fomina I.R., Biel K.Y.: Photosynthetic carbon metabolism: strategy of adaptation over evolutionary history. – In: Allakhverdiev S.I. (ed.): Photosynthesis: New Approaches to the Molecular, Cellular, and Organismal Levels. Pp. 233-326. Scrivener Publishing, Austin 2016.

Gout E., Aubert S., Bligny R. *et al*.: Metabolism of methanol in plant cells. Carbon-13 nuclear magnetic resonance studies. – Plant Physiol. **123**: 287-296, 2000.

Govindjee G., Nonomura A., Lichtenthaler H.K.: Remembering Melvin Calvin (1911–1997), a highly versatile scientist of the 20th century. – Photosynth. Res. **143**: 1-11, 2020.

Hincha D.K., Bakaltcheva I., Schmitt J.M.: Galactose-specific lectins protect isolated thylakoids against freeze-thaw damage. – Plant Physiol. **103**: 59-65, 1993.

Hoagland D.R., Arnon D.I.: The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular 347. The College of Agriculture, University of California, Berkeley 1950.

Hymus G.J., Baker N.R., Long S.P.: Growth in elevated CO_2 can both increase and decrease photochemistry and photoinhibition of photosynthesis in a predictable manner. *Dactylis glomerata* grown in two levels of nitrogen nutrition. – Plant Physiol. **127**: 1204-1211, 2001.

Jin X., Yang G., Tan C., Zhao C.: Effects of nitrogen stress on the photosynthetic CO_2 assimilation, chlorophyll fluorescence, and sugar-nitrogen ratio in corn. – Sci. Rep.-UK **5**: 9311, 2015.

Kiba T., Kudo T., Kojima M., Sakakibara H.: Hormonal control of nitrogen acquisition: roles of auxin, abscisic acid, and cytokinin. – J. Exp. Bot. **62**: 1399-1409, 2011.

Komath S.S., Kavitha M., Swamy M.J.: Beyond carbohydrate binding: new directions in plant lectin research. – Org. Biomol. Chem. **4**: 973-988, 2006.

Kovalchuk N.V., Melnykova N.M., Musatenko L.: Role of phytolectin in the life cycle of plants. – Biopolym. Cell **28**: 171-180, 2012.

Lambin J., Asci S.D., Dubiel M. *et al*.: OsEUL lectin gene expression in rice: stress regulation, subcellular localization and tissue specificity. – Front. Plant Sci. **11**: 185, 2020.

Lannoo N., Van Damme E.J.M.: Nucleocytoplasmic plant lectins. – BBA-Gen. Subjects **1800**: 190-201, 2010.

Lannoo N., Van Damme E.J.M.: Lectin domains at the frontiers of plant defense. – Front. Plant Sci. **5**: 397, 2014.

Laus M.C., Logman T.J., Lamers G.E. *et al*.: A novel polar surface polysaccharide from *Rhizobium leguminosarum* binds host plant lectin. – Mol. Microbiol. **59**: 1704-1713, 2006.

Law S.R., Kellgren T., Björk R. *et al*.: Centralization within sub-experiments enhances the biological relevance of gene co-expression networks: a plant mitochondrial case study. – Front. Plant Sci. **11**: 524, 2020.

Loris R., Hamelryck T., Bouckaert J., Wyns L.: Legume lectin structure. – BBA-Protein Struct. Mol. Enzymol. **1383**: 9-36, 1998.

Marty F.: Plant Vacuoles. – Plant Cell **11**: 587-599, 1999.

Naismith J.H., Emmerich C., Habash J. *et al*.: Refined structure of concanavalin A complexed with methyl α-D-mannopyranoside at 2.0 Å resolution and comparison with the saccharide-free structure. – Acta Crystallogr. D **50**: 847-858, 1994.

Naismith J.H., Field R.A.: Structural basis of trimannoside recognition by concanavalin A. – J. Biol. Chem. **271**: 972-976, 1996.

Nilsson C.L.: Lectins: Analytical Technologies. Pp. 442. Elsevier, Amsterdam 2007.

Nonomura A.M., Benson A.A.: The path of carbon in photosynthesis. XXIV. Improved crop yields with methanol. – P. Natl. Acad. Sci. USA **89**: 9794-9798, 1992a.

Nonomura A.M., Benson A.A.: The path of carbon in photosyn-

thesis. Methanol and light. – In: Murata N. (ed.): Research in Photosynthesis, Proceedings of the IX International Congress on Photosynthesis. P-589. Kluwer, Dordrecht 1992b.

Nonomura A.M., Benson A.A.: The path of carbon in photosynthesis. XXIX. Glass microbeads. – J. Plant Nutr. **35**: 1896-1909, 2012.

Nonomura A.M., Benson A.A.: The path of carbon in photosynthesis. XXX. α-Mannosides. – In: Dubinsky Z. (ed.): Photosynthesis. Pp. 3-22. InTech, Rijeka 2013.

Nonomura A.M., Benson A.A.: The path of carbon in photosynthesis. XXXI. The role of lectins. – J. Plant Nutr. **37**: 785-794, 2014.

Nonomura A.M., Benson A.A., Biel K.Y.: The path of carbon in photosynthesis. XXVII. Sugar-conjugated plant growth regulators enhance general productivity. – J. Plant Nutr. **34**: 653-664, 2011.

Nonomura A.M., Biel K.Y., Fomina I.R. *et al*.: Benson's protocol. – In: Fomina I.R., Biel K.Y., Soukhovolsk V.G. (ed.): Complex Biological Systems: Adaptation and Tolerance to Extreme Environments. 2nd Edition, extended. Pp. 463-508. John Wiley & Sons, Inc., Hoboken 2018a. doi: 10.1002/9781119510390.ch11.

Nonomura A.M., Cullen B.A., Benson A.A.: The path of carbon in photosynthesis. XXVIII. Responses of plants to polyalkylglycopyranose and polyacylglycopyranose. – In: Najafpour M.M. (ed.): Advances in Photosynthesis: Fundamental Aspects. Pp. 259-271. InTech, Rijeka 2012.

Nonomura A.M., Holtz B., Biel K.Y. *et al.*: The paths of Andrew A. Benson: a radio-autobiography. – Photosynth. Res. **134**: 93-105, 2017.

Nonomura A.M., Pedersen A., Brummel D.P. *et al*.: Brandt iH026a plant growth regulator. – Photosynthetica **56**: 411-417, 2018b.

Nonomura A.M., Shevela D., Komath S.S. *et al*.: A plant growth regulator for photosynthesis. Govindjee's Educational Poster Series, 2020. doi: 10.13140/RG.2.2.30634.44480.

Pier J., Barlow D. (ed.): Western Fertilizer Handbook. 3rd Edition. Western Plant Health Association. Pp. 400. Waveland Press, Long Grove 2018. ISBN: 1-4786-3846-x.

Roberts D.D., Goldstein I.J.: Adenine binding sites of the lectin from lima beans (*Phaseolus lunatus*). – J. Biol. Chem. **258**: 13820-13824, 1983.

Schröder W.P., Petit P.X.: Flow cytometry of spinach chloroplasts. – Plant Physiol. **100**: 1092-1102, 1992.

Schwarz F.P., Puri K.D., Bhat R.G., Surolia A.: Thermodynamics of monosaccharide binding to concanavalin A, pea (*Pisum sativum*) lectin, and lentil (*Lens culinaris*) lectin. – J. Biol. Chem. **268**: 7668-7677, 1993.

Sekhar K.M., Kota V.R., Reddy T.P. *et al*.: Amelioration of plant responses to drought under elevated CO_2 by rejuvenating photosynthesis and nitrogen use efficiency: implications for future climate-resilient crops. – Photosynth. Res., 2020. doi: 10.1007/s11120-020-00772-5.

Sharkey T.D.: Discovery of the canonical Calvin-Benson cycle. – Photosynth. Res. **140**: 235-252, 2019.

Sharon N., Lis H.: Lectins. 2nd Edition. Pp. 454. Springer, Dordrecht 2007.

Shevela D., Björn L.O., Govindjee G.: Photosynthesis: Solar Energy for Life. Pp. 204. World Scientific Publishing, Singapore 2018. doi: 10.1142/10522.

Shevela D., Do H.-N., Fantuzzi A. *et al*.: Bicarbonate-mediated CO_2 formation on both sides of Photosystem II. – Biochemistry **59**: 2442-2449, 2020.

Shevela D., Eaton-Rye J.J., Shen J.R., Govindjee: Photosystem II and unique role of bicarbonate: a historical perspective. – BBA-Bioenergetics **1817**: 1134-1151, 2012.

Smeets K., Van Damme E.J.M., Verhaert P. *et al*.: Isolation, characterization and molecular cloning of the mannose-binding lectins from leaves and roots of garlic (*Allium sativum* L.). – Plant Mol. Biol. **33**: 223-234, 1997.

ten Veldhuis M.C., Ananyev G., Dismukes G.C.: Symbiosis extended: exchange of photosynthetic O_2 and fungal-respired CO_2 mutually power metabolism of lichen symbionts. – Photosynth. Res. **143**: 287-299, 2020.

Thompson M., Gamage D., Hirotsu N. *et al*.: Effects of elevated carbon dioxide on photosynthesis and carbon partitioning: a perspective on root sugar sensing and hormonal crosstalk. – Front. Physiol. **8**: 578, 2017.

Van Damme E.J.M., Lannoo N., Peumans W.: Plant lectins. – Adv. Bot. Res. **48**: 107-209, 2008.

Van Damme E.J.M., Peumans W.J., Pusztai A., Bardocz S.: Handbook of Plant Lectins: Properties and Biomedical Applications. Pp. 466. John Wiley & Sons, New York 1998. ISBN: 978-0-471-96445-2.

Van Holle S., Van Damme E.J.M.: Messages from the past: new insights in plant lectin evolution. – Front. Plant Sci. **10**: 36, 2019.

Zhang C., Hicks G.R., Raikhel N.V.: Molecular composition of plant vacuoles: important but less understood regulations and roles of tonoplast lipids. – Plants-Basel **4**: 320-333, 2015.

Zhang J., Wang N., Miao Y. *et al*.: Identification of SLAC1 anion channel residues required for CO_2/bicarbonate sensing and regulation of stomatal movements. – P. Natl. Acad. Sci. USA **115**: 11129-11137, 2018.