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Viewpoint

Polyols as biomarkers and bioindicators for 21st century plant breeding

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Abstract. Characterising changes in the plant metabolome is central to understanding adaptive responses to environmental change. New and improved quantitative and qualitative technologies have enabled the characterisation of plant metabolism at unprecedented scales and precision. New frontiers have therefore emerged for improving our understanding of the adaptability of plant metabolic networks. However, despite these advances, outcomes for 'in field' plant management remain largely based on subsets of plant metabolism due to broader scale network complexity. The synthesis and occurrence of polyols offer considerable promise as bioindicators of plant health and biomarkers for use as selective traits for plant improvement. Polyols are polyohydroxy compounds that may be either open chain (acyclic) alditols or cyclic compounds (cyclohexan-hexols), usually termed cyclitols or inositols. Here we highlight the functions of polyols in stress acclimation or amelioration and as sinks for carbon and indicate their potential for the development of integrated measures of plant function using new technologies in 21st century plant breeding.

Additional keywords: alditol, carbon partitioning, cyclitol, metabolites.

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Introduction

Global change, comprising of climate change and additional pressures arising from increased human population, pose major challenges for plants and plant breeding strategies. Principal among these challenges are increased environmental variability and homeostatic compensation for higher concentrations of atmospheric carbon dioxide. Changes in plant metabolism are undoubtedly a major component of adaptive traits to mitigate such conditions. The development of integrative tools to monitor and promote adaptive strategies of metabolic networks are therefore of great importance to agriculturalists and plant breeders.

The carbon status of leaves is considered to reflect plant growth across a range of temporal and spatial scales and is a prominent candidate for the development of tools to monitor plant processes. Recent advances in high-throughput extraction, separation and identification of plant compounds have enabled broad scale qualification and quantification of large swathes of the plant metabolome (e.g. Fiehn 2004; Trethewey 2004). Rapid expansion of 'omics' technologies and efforts to combine transcriptomic, proteomic and metabolomic data (e.g. Fiehn 2001, 2002; Sweetlove *et al*. 2008; Leakey *et al*. 2009) aim to enable 'predictive metabolic engineering' (e.g. Sweetlove *et al*. 2003). Interpretation and use of this knowledge is limited because of the complexity of plant metabolic networks with few applications developed for 'in field' plant management.

It is widely recognised that research relevant to plant breeders and landscape managers has been limited across a range of disciplines (see Passioura 2007; McClean *et al*. 2011). Similarly, large gaps remain between the molecular level science and the interpretation and application of this knowledge at the whole-plant level in the field (Araus *et al*. 2007). Development of biomarkers based on a range of quantitative morphometric, and chemical traits have led to the identification of powerful quantitative trait loci (QTL) that form the basis of plant breeding programs. Of equal importance is the development of bioindicators that reflect plant physiological status supporting plant management decisions. High-throughput technologies to monitor subsets of plant metabolism, sometimes termed 'targeted metabolic profiling', offer an alternative to complex broad scale metabolomic characterisations. Targeting subsets of plant metabolism within or close to the core metabolic reactions

of the cell, for example carbohydrates and derived metabolites, may offer significant insight into alterations in plant metabolism in response to environmental change.

The synthesis and accumulation of polyols is common to many plant tissues with discrete patterns identified among both plant taxa (Plouvier 1963; Lewis and Smith 1967; Bieleski 1982) and environments (Merchant *et al*. 2006*a*; Monson *et al*. 2006). Several functions of polyols in plant tissues have been identified, however, despite their close association with primary metabolism they are often overlooked in plant research. Here we highlight the importance of polyol synthesis in plant tissues, their physiochemical properties and how their occurrence is well suited for use as biomarkers and bioindicators of plant function. We discuss future research directions from the cellular to the plant scale and emphasise the applicability of such suggestions to broader efforts to develop metabolite based biomarkers and bioindicators of plant health.

Polyols are a major carbon sink

The immediate fate of carbon exiting the Calvin cycle has been well studied, with much of the biosynthetic pathways well characterised (for a concise review see Stitt *et al*. 2010). To reduce oxidative damage to cellular components during transport and storage, carbon exported from the Calvin Benson cycle must be 'packaged' into highly reduced forms. Major sinks for carbon exiting the cycle are starch synthesis in the chloroplast and cytosolic sucrose synthesis after export of DHAP across the thylakoid membrane. In model plants, allocation of carbon between these two competing pathways is well characterised (Sulpice *et al*. 2009; Stitt *et al*. 2010) leading to the suggestion of integrated measures of plant growth (Sulpice *et al*. 2009).

Second only to sucrose and its derivatives, polyols represent a major highly reduced sink in which plants may store and transport carbon. Polyols have been isolated from plant tissues up to 8.9% leaf DW (Richter and Popp 1992; Streeter *et al*. 2001; Monson *et al*. 2006; Merchant *et al*. 2007) up to 90% of phloem sap carbon (Moing *et al*. 1997) and often more than 50% of the carbon in the xylem sap (Richter and Popp 1992; Popp and Smirnoff 1995; Popp *et al*. 1997). The synthesis of polyols follows strong taxonomical (Bieleski and Briggs 2005; Merchant *et al*. 2007) and ecotypic (Pfundner 1993) patterns and these concentrations fluctuate in response to environmental cues (Vernon and Bohnert 1992; Wanek and Richter 1997; Streeter *et al*. 2001; Merchant *et al*. 2006*b*). Such properties suggest that the capacity to synthesise polyols under various stress conditions is ideal for use as selection criterion in plant breeding programs for enhanced drought or salinity tolerance.

Polyols are either open chain (acyclic) compounds with the general formula $HOCH_2[CH(OH)]_nCH_2OH$, or cyclic compounds (cyclohexan-hexols or -pentols), usually termed cyclitols or inositols. Although not regarded as primary metabolites, biosynthesis of polyols stems directly from glucose-6-phsophate (G-6-P). Alditols such as sorbitol (e.g. *Prunus* spp., Nadwodnik and Lohaus 2008) or mannitol (e.g. *Apium graveolens*, Sickler *et al*. 2007) are chemically reduced forms of either glucose or fructose (Fig. 1) whose synthesis are well characterised (e.g. Loescher and Everard 2000) and subject

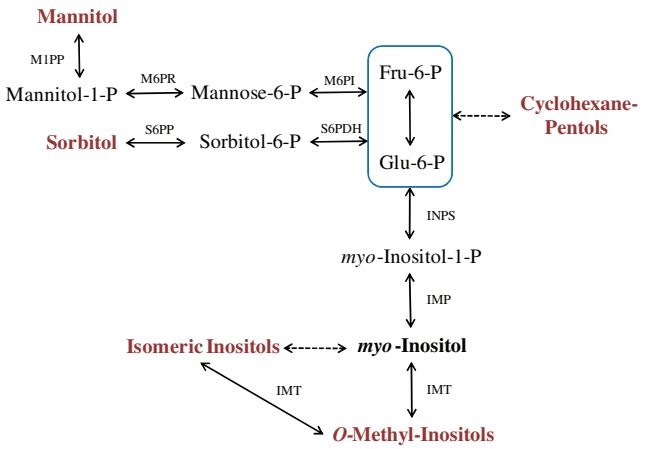

Fig. 1. Polyol biosynthesis in higher plants. Some intermediates have been omitted for simplicity. Broken line indicates hypothesised pathways. Most cyclitols are synthesised from a common cyclitol precursor *myo*-inositol. Alditols mannitol and sorbitol are synthesised from glucose-6-phosphate (Glu-6-P) and fructose-6-phosphate (Fru-6-P). Abbreviations are: INPS, inositol phosphate synthase; IMP, inositol mono phosphatise; IMT, inositol methyl transferase; S6PDH, sorbitol-6-phosphate dehydrogenase; S6PP, sorbitol-6-phosphate phosphatise; M6PI, mannose-6-phosphate isomerase; M6PR, mannose-6-phosphate reductase; M1PP, mannitol-1-phosphate phosphatase.

to many attempts to engineer stress tolerance (Tarczynski *et al*. 1993; Prabhavathi *et al*. 2002; Maheswari *et al*. 2010). Although these attempts lead to increased stress tolerance of the engineered plants, it has been questioned whether this can lead to a significant increase of whole-plant tolerance under field conditions (Bohnert and Shen 1998). In addition to alditols, a great diversity of cyclitols is commonly isolated from plant tissues due to their many substituted and dehydroxylated forms (Drew 1984). Knowledge of cyclitol biosynthesis in plants is largely based upon radio-labelling experiments by Kindl and Hoffmann-Ostenhof in the 1960s (e.g. Kindl and Hoffmann-Ostenhof 1966; Hofmann *et al*. 1969) suggesting that *myo*-inositol (a cyclitol itself) is a common precursor to all cyclitols with only one characterised exception where G-6-P is converted to *muco*-inositol via a phosphorylated intermediate in a Zannichellian seagrass (Drew 1984). Several authors have shown evidence suggesting some cyclitols (notably the cyclohexane-pentols) are derived directly from G-6-P (Kindl 1969; Popp *et al*. 1997), with further work required to determine the enzyme processes involved (Fig. 1).

Plant carbohydrate metabolism is flexible and can be altered to enable the allocation of resources towards the synthesis of alternative metabolites (Vernon *et al*. 1993; Sheveleva *et al*. 1997). Despite a close association with primary metabolism and several physiochemical properties supporting their role as a carbon sink (see below), quantifying the flux of carbon to the polyol pool has received little attention. Relative to carbohydrates, polyols are highly stable, chemically inert compounds that are not readily metabolised (Paul and Cockburn 1989; Popp *et al*. 1997; Sheveleva *et al*. 1997), thus, do not undergo significant short-term fluctuations (Paul and Cockburn 1989). Further, polyol biosynthesis is reliant on the

diversion of common intermediates of primary metabolism (Fig. 1) and is reliant only on carbon, oxygen and hydrogen as its final constituents. Relatively short metabolic pathways, closely aligned with a core set of metabolic reactions, may facilitate the rapid accumulation of polyols to high concentrations in plant cells.

Avoiding sugar-mediated downregulation of photosynthesis is thought to be significant characteristic enabling plants to tolerate changes in resource availability (Paul and Driscoll 1997; Chiou and Bush 1998; Paul and Foyer 2001) and impart 'upstream influence on primary metabolism (e.g. Paul and Driscoll 1997; Halford and Paul 2003; Smith and Stitt 2007). With the exception of *myo*-inositol, many polyols are thought not to participate in primary metabolic reactions. Allocation of carbon to the polyol pool may assist plants to avoid sugar mediated downregulation of photosynthesis by removing carbon from the carbohydrate pool and avoiding allosteric feedback effects on Calvin cycle components (Stitt *et al*. 2010). Equally, the synthesis of polyols, specifically the alditols, are a means by which plants can store 'reduction equivalents' facilitating the regeneration of $NADP^+/NAD^+$ allowing the dark reactions of photosynthesis to continue thus protecting photosystems from oxidative damage. Although both of these processes clearly have an upper limit in their ability to avoid the negative effects of plant stress, both processes can aid in tolerating shorter term variation in resource availability for example across diurnal fluctuations. These processes, with the potential for significant repercussions on primary metabolism, will require an understanding of flux through metabolite networks.

Quantifying fluxes in complex pathways represents a new frontier to understanding metabolic networks, therefore, is the subject of several recent reviews (Rontein *et al*. 2002; Schwender *et al*. 2004; Schwender 2008; Sweetlove *et al*. 2008; Allen *et al*. 2009). In cases, such as for most polyols, where little information is available regarding the kinetics of the synthetic pathways, such approaches are limited by lack of data on the parameters that govern enzyme function (e.g. Fiehn *et al*. 2000; Fiehn 2002, 2004; Trethewey 2002, 2004). Alternatively, the use of metabolic flux analysis (MFA) enables the *in vivo* measurement of co-occurring flux rates among the metabolic network thus is ideal for use in detecting 'switches' in allocation of carbon among soluble pools. Changes in the allocation of carbon from carbohydrates to polyols most likely reflect alterations in plant growth strategies in response to changes in environmental conditions. In a well characterised example, Pattanagul and Madore (1999) observed allocation of carbon to the synthesis of *O*-methyl inositols at the expense of raffinose family oligosaccharide (RFO) synthesis in response to water deficit in *Coleus*. Major shifts in allocation of carbon at such a central point in plant metabolism have significant downstream consequences and require further attention for the study of plant acclimation to changes in resource availability.

Polyol chemistry and cellular functions

In addition to the function of polyols as a carbon sink, evidence for multiple roles as stress metabolites have been gleaned from investigations across the breadth of higher plants (Paul and Cockburn 1989; Williamson *et al*. 2002). The function of polyols as 'compatible solutes' (e.g. Paul and Cockburn 1989) in plant tissues is an established mechanism of coping with low external osmotic potentials (ψ_π). As one example, the accumulation of the cyclitol D-pinitol is influenced by reduced water availability (drought, salinity, osmotic potential) among a range of crop and woody plant genera including *Glycine max* L. (Streeter 1985) *Cicer arietinum* L. (Orthen *et al*. 2000) *Vigna* spp. (Ford 1982; Wanek and Richter 1997) *Pisum sativum* L. (Streeter 1985) *Mesembryanthemum crystallinum* L. (Paul and Cockburn 1989; Vera-Estrella *et al*. 1999) coastal New Zealand species (Bieleski 1994) *Actinidia* A. Chev. (Klages *et al*. 1998, 1999) and transgenic tobacco (Vernon *et al*. 1993; Sheveleva *et al*. 1997) illustrating the potential for this group of compounds to influence the physiology among a range of herbaceous and woody plant species.

Some polyols are thought to possess protective properties towards protein structures, membranes and liposomes (Ortbauer and Popp 2008) through several modes of function. As discussed above, concentrations of many polyols do not significantly fluctuate in the short term, leading to suggestions that this improves their ability function in stabilising cellular structures (e.g. Paul and Cockburn 1989; Sheveleva *et al*. 1997). Many polyols are thought to promote the hydration of cellular structures via 'preferential exclusion' (e.g. Andersen *et al*. 2011) because of their high concentrations seemingly among all cellular components. Alternatively, methylation of many polyols incorporates a partial hydrophobicity and increases the size of the hydration shell of small zwitterionic molecules (Hare *et al*. 1998), which is thought to improve the capacity of the solute to interact with and preserve the tertiary structures of proteins.

Polyol synthesis may also assist to avoid the products of excessive photorespiration. For example, it is hypothesised that methylation of *myo*-inositol to form D-ononitol in plant tissues reduces H_2O_2 generation by photorespiration through increased demand on N^5-methelenetetrahydrofolate (Hare *et al*. 1998). Additionally, the ability of various cyclitols to scavenge hydroxyl radicals *in vitro* has been shown (Orthen *et al*. 1994) and transgenic tobacco plants that accumulate mannitol in chloroplasts and that exhibited an increased hydroxyl radical scavenging capacity have been produced (Shen *et al*. 1997).

Although polyol accumulation undoubtedly influences ψ_π and other cellular processes, the significance of subcellular compartmentalisation remains weakly characterised. Paul and Cockburn (1989) demonstrated for *M. crystallinum*, subcellular compartmentalisation of D-pinitol in the chloroplast up to 230 mol m^{-3}, compared with cytosolic concentrations of 100 mol m^{-3}. Equally, for some plants subcellular compartmentalisation of polyols may not be important or even possible due to high concentrations of solutes found in plant tissues (up to 8.9% leaf DW). Either way, significant concentrations of polyols in plant tissues would support the rapid establishment of osmotic potential under varying environmental conditions. Investigations of subcellular distributions of polyols face significant challenges owing to limited options for labelling attributable to the relatively inert properties and common constituents (C, N, O). The use of isotope labelling or cell fractionation is time consuming and hindered

by the high levels of background carbon involved in plant metabolism. A promising alternative technique may be the use of quantum dots (e.g. Eggenberger *et al*. 2010) to resolve this question. Evaluation of this approach may provide significant insight into the transport and partitioning of plant solutes from the cellular to the plant scale.

The importance of polyol partitioning at the plant scale

Many applications of metabolite analysis focus on the cellular scale with relatively little attention placed on whole-plant metabolite distributions. Partitioning of polyols between plant tissues (e.g. Noiraud *et al*. 2001*b*) and excretion from roots (e.g. Timotiwu and Sakurai 2002) perform important roles for plant function and interaction with the environment. Polyols are highly mobile among plant tissues and are involved in rapid redistributions to satisfy physiological requirements such as bud burst (Popp *et al*. 1997) or the onset of physiological stress (Richter and Popp 1992; Guo and Oosterhuis 1995). In the only known study of its type, polyol synthesis has been shown to occur in plant tissues remote to the location of polyol accumulation (Wanek and Richter 1997), highlighting the importance of whole-plant analysis.

Up to 80% of the carbon acquired in photosynthesis is, at some stage, transported in the phloem sap (Chiou and Bush 1998), of which polyols are often a major constituent. Apoplastic and symplastic modes of loading outlined for sucrose and raffinose family oligosaccharides (RFOs) are well characterised (Turgeon 1996; Turgeon 2000) and although not discounting the likely symplastic movement of polyols in to the phloem in some cases, recent studies have now isolated several genes for apoplastic loading of polyols (Noiraud *et al*. 2001*a*; Gao *et al*. 2003; Ramsperger-Gleixner *et al*. 2004; Klepek *et al*. 2005; Pommerrenig *et al*. 2007). Changes in the concentrations of sugars, and RFOs are sensitive to environmental conditions along with indirect measures of phloem sap ψ_π (Pate and Arthur 1998; Pate *et al*. 1998; Merchant *et al*. 2010), suggesting that the abundance of such compounds may be used as surrogate measures of plant physiological status. Despite such promise, movement of polyols via the phloem remains relatively unstudied with little information regarding flux. Nevertheless, movement of photoassimilates into the phloem is a recognised 'bottleneck' for carbon movement with direct consequences for the repression of photosynthesis (e.g. Adams *et al*. 2007). Uncovering the relative contributions of polyol, carbohydrate and RFO to the transport of carbon and their flexibility in response to environmental change may provide insight into the flexibility of assimilation under changing environmental conditions.

What are the priorities for future fundamental and applied research?

Polyols can ameliorate the effects of stressful conditions among a range of plant genera across a range of stress types. Although a great deal is known regarding the function of polyols in plant material, the development of tools for plant management remains unheralded. Uncovering genetic diversity in the capacity to accumulate polyols (e.g. Streeter *et al*. 2001) will be a significant advancement in the generation of selective traits for plant improvement. Underpinning such efforts will also require a more comprehensive understanding of the effects of polyol accumulation on yield in target species.

Identifying 'switches' in the allocation of carbon to polyols straddles an important concept for developing applied tools for use in plant management programs. The resilience of plant metabolism to withstand environmental change or rapid induction of polyol synthesis in response to environmental cues may represent valid reflections of fitness under particular conditions. Equally, preparedness for environmental change may be reflected in the preconditioning of plant tissues through the constitutive accumulation of polyols (e.g. Merchant *et al*. 2006*b*). Characterising such patterns under a range of environmental conditions using targeted analysis of metabolites (such as MFA) will be required to underpin the development of effective, usable tools for plant management programs.

Metabolic engineering to upregulate polyol content has been successfully achieved among a range of plant genera (e.g. Vernon *et al*. 1993; Nuccio *et al*. 1999; Sickler *et al*. 2007) leading to increased tolerance to a range of environmental conditions. Studies of this type play important roles in determining 'proof of function' for compounds involved in complex metabolic networks with the long-term objective of developing improved stress tolerance. Although metabolic engineering of polyol synthesis shows great promise, the close association of polyol synthesis with primary photosynthetic reactions and roles in long distance transport highlight the need to investigate the influence of changes in carbon allocation on plant development and yield (e.g. Teo *et al*. 2006).

Finally, a renewed focus must be made on plant-scale distributions of polyols and the transport mechanisms that underpin movement of polyols among plant tissues. The contribution of polyols to carbon transport for metabolism and the quantification of polyol exudation from root tissues and subsequent effects on root-associated micro-organisms remain relatively unstudied. Such information, although not immediately applicable to plant management programs, may provide a mechanistic basis for future breeding objectives.

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