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Profiling of spatial metabolite distributions in wheat leaves under normal and nitrate limiting conditions

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ABSTRACT

The control and interaction between nitrogen and carbon assimilatory pathways is essential in both photosynthetic and non-photosynthetic tissue in order to support metabolic processes without compromising growth. Physiological differences between the basal and mature region of wheat (*Triticum aestivum*) primary leaves confirmed that there was a change from heterotrophic to autotrophic metabolism. Fourier Transform Infrared (FT-IR) spectroscopy confirmed the suitability and phenotypic reproducibility of the leaf growth conditions. Principal Component–Discriminant Function Analysis (PC–DFA) revealed distinct clustering between base, and tip sections of the developing wheat leaf, and from plants grown in the presence or absence of nitrate. Gas Chromatography-Time of Flight/Mass Spectrometry (GC-TOF/MS) combined with multivariate and univariate analyses, and Bayesian network (BN) analysis, distinguished different tissues and confirmed the physiological switch from high rates of respiration to photosynthesis along the leaf. The operation of nitrogen metabolism impacted on the levels and distribution of amino acids, organic acids and carbohydrates within the wheat leaf. In plants grown in the presence of nitrate there was reduced levels of a number of sugar metabolites in the leaf base and an increase in maltose levels, possibly reflecting an increase in starch turnover. The value of using this combined metabolomics analysis for further functional investigations in the future are discussed.

1. Introduction

Nitrogen is a major plant nutrient, being an essential component of amino acids, peptides and proteins, chlorophyll, nucleic acids and many cofactors and plant defence compounds. For most higher plants, particularly when growing in well-aerated soils, nitrate is the primary source of inorganic nitrogen. Nitrate is reduced to nitrite, then ammonium, prior to assimilation into amino acids, in a series of reactions that are highly compartmentalised within cells and tissues (Tobin and Yamaya, 2001). Nitrogen assimilation interacts with carbon assimilation and degradation in a complex network that adjusts the balance between N and C according to the physiological status of the tissue and the environmental conditions (Nunes-Nesi et al., 2010), in both photosynthetic and non-photosynthetic tissue (Smirnoff and Stewart, 1985).

Nitrate assimilation and amino acid biosynthesis require a supply of reductant (NAD(P)H and/or reduced ferredoxin) and ATP as well as a range of organic acids to act as carbon skeletons. In photosynthetic cells, reductant and ATP can be derived from photosynthesis, while mitochondrial respiration also provides supplementary ATP and reductant even in light (Kromer, 1995; Nunes-Nesi et al., 2010). Carbon skeletons can be produced from newly synthesised carbohydrates that are converted into organic acids via respiration (glycolysis, TCA cycle and oxidative pentose

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[1] Dr. Surya Chandra who had performed many of the classical plant physiological measurements and assays within this research programme as part of his MPhil studies, was tragically killed in a road traffic accident shortly after his return to Bangkok Thailand following completion of his MPhil. Surya showed the ability to perform high quality research and was a warm character within our research groups making his loss even more tragic and we feel worthy of such a dedication.

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phosphate pathway), or from stored organic compounds (Nunes-Nesi et al., 2010; Sweetlove et al., 2010). In non-photosynthetic cells, reductant and ATP are supplied by respiration, with carbohydrates being imported, as sucrose from photosynthetic tissue or released from reserves. The cost of transporting sucrose to the roots, as well as respiration to generate ATP and reductant, makes roots and other non-photosynthetic tissue more energetically 'costly' as sites for nitrate assimilation. The energetic advantages of photosynthetic tissue becomes increasingly compromised as light intensities fall to the point where photosynthesis becomes light-limited. Under these conditions nitrate assimilation competes with the Calvin cycle for reductant and ATP, leading to a reduced rate of carbon assimilation (Canvin and Atkins, 1974). If carbohydrate concentrations fall it can result in a depletion of amino acid pools, either due to a limited supply of carbon skeletons for amino acid synthesis or due to the catabolism of amino acids to maintain respiration (Matt et al., 1998; Usadel et al., 2008). Even under high light, the presence of nitrate can shift the flow of photosynthetic carbon towards amino acid (Champigny and Foyer, 1992) and organic acid synthesis (Scheible et al., 1997), while carbohydrate synthesis is decreased, and a greater proportion of assimilated carbon is incorporated into organic and amino acids (Stitt et al., 2002). These examples illustrate the need for nitrogen and carbon assimilation pathways to be coordinated in order that there is an adequate supply of carbon to support amino acid biosynthesis without compromising growth. They also indicate significant differences in the metabolic networks that exist within non-photosynthetic compared to photosynthetic tissue.

To date there have been some extremely informative integrative 'omics' approaches for assessing nitrogen status in the model dicotyledonous plant Arabidopsis. For example Hirai et al. (2004) combined transcriptomics and metabolomics to gain a better understanding of nutritional stress responses in Arabidopsis. Whilst Albinsky et al. (2010) over expressed rice full-length cDNA clones in Arabidopsis and then performed transcriptome and metabolome analyses to learn more about the processes related to nitrogen metabolism. A more sophisticated experimental design and the measurement of relevant enzyme activities, in addition to classical targeted metabolite quantification, allowed Tschoep et al. (2009) to interpret Arabidopsis nitrogen deficiency phenotypes. Such studies in Arabidopsis have, by necessity, not accounted for the fact that certain tissues of the leaf are undergoing different metabolic processes with respect to autotrophic and heterotrophic metabolism. Also all these studies have used multiple leaf pools from multiple plants meaning that it is not possible to compare respiring versus photosynthetic tissues and no consideration can be given to leaves from different positions and of different ages.

In order to identify metabolic networks and their fluctuations in response to changing N supply and C assimilation, we have used the natural developmental gradient that exists within the wheat primary leaf. This system has advantages over comparisons between leaf and root assimilation because it provides tissue that is anatomically comparable (i.e. composed of mesophyll, vascular and epidermal cells) and is readily characterised. As cell division is restricted to a basal meristem, this generates a measurable gradient of cell age and development along the leaf blade with a transition from non-photosynthetic cells at the base to fully photosynthetic cells at the leaf tip (Tobin et al., 1985). Hence, within a single tissue we are able to identify distinct changes in metabolic networks as the pathways for nitrogen assimilation operate within cells that are transitioning from wholly respiratory to fully photosynthetic.

In this paper initial studies were carried out to characterise the physiological differences between basal and mature regions of wheat primary leaves of nitrate-grown plants. Following characterisation by metabolite fingerprinting with Fourier Transform Infrared (FT-IR) spectroscopy, non-photosynthetic, semi-autotrophic and fully photosynthetic leaf sections were taken from plants grown in the presence or absence of nitrate and subjected to metabolite profiling using Gas Chromatography-Time of Flight/Mass Spectrometry (GC-TOF/MS). The metabolite data were analysed using multivariate chemometric approaches, point-by-point (univariate) data interpretation, as well as by Bayesian network (BN) based correlation analyses.

We discuss the extent to which these metabolomics approaches are able to distinguish the different tissues and treatments and we identify major changes in metabolite networks during the transition from heterotrophic to fully photosynthetic metabolism in response to increased N supply. The value of this approach when undertaking functional investigations of plants grown in different scenarios is also considered.

2. Results

2.1. Changes in metabolism during leaf development

In wheat plants grown in the presence of a continuous N supply by growing on compost, mesophyll cell number was highest in the basal 5 mm of the leaf blade (approximately 11×10^7 cells g^{-1} fwt), and then rapidly decreased to a constant lower number of ca. 2×10^7 cells g^{-1} fwt beyond 20 mm from the leaf base (Fig. 1), confirming that cell division is restricted to the basal 5 mm and the mesophyll cell elongation zone occurred within the basal 20 mm of the leaf (0–20 h old). Cell age increases rapidly over the basal region, where the cells are actively dividing, and then increases at a constant rate with distance from the leaf base (Fig. 1).

The total chlorophyll concentration markedly increased from the leaf base to the tip (Fig. 2a and b). The data are plotted in alternative forms to show how distance along the leaf from the base (Fig. 2a) equates to cell age in hours (Fig. 2b). In subsequent graphs the data are presented against cell age. There is a 'switch over' from heterotrophic metabolism, where respiration predominates up until the end of the elongation zone of the leaf (Fig. 2d), to autotrophic metabolism, where photosynthesis predominates towards the leaf tip (Fig. 2c). Photosynthetic activity reaches its maximum at the leaf tip, coinciding with the maximum size and development of the chloroplasts (Figs. 2c, S1). Based on this metabolic distinction, metabolite fingerprinting and profiling of the basal, mid and terminal 20 mm sections of the developing wheat leaf allows a comparison to be made between heterotrophic, 'semi-autotrophic' and fully autotrophic metabolism. Basal tissue contains cells up to 24 h old, which includes all the meristematic cells as well as those undergoing elongation. Although they contain some chlorophyll

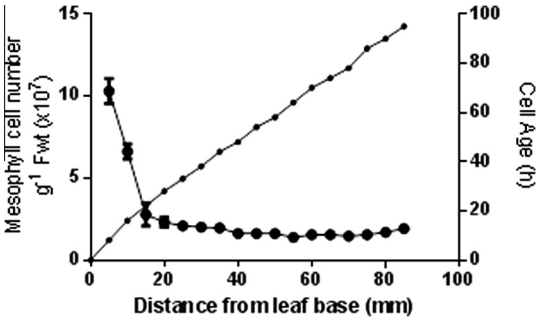

Fig. 1. Mesophyll cell number and age along the developing wheat leaf. Mesophyll cell numbers (large closed circle) and cell age (small closed circle) along the length of 7 day old primary leaves. Data points represent the mean of 5 independent growth studies, sampling 5 seedlings per replicate. Error bars show ±SE of the mean.

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