Abstract

Aphid stylectomy and mass spectrometric techniques were used as tools for analyzing wheat phloem exudate and to determine what changes are occurring in its composition during the reproductive/grain loading phase. Further to this, the phloem composition of wheat genotypes that differ in grain Zn concentration were investigated along with nutrient changes within the phloem at different times of the day (diurnal variability). Phloem was collected from the peduncle 1cm below the head to gain insight into the phloem flux into the developing head.

Two improved methods for the accurate measurement of nano-litre volumes of phloem exudate were developed and their accuracy and precision quantified. The first method involved the use of paraffin oil to prevent evaporation due to the small sample size and the method consisted of optically measuring the droplet formed after a phloem sample had been collected and expelled into an optimised volume of paraffin oil. A change in oil volume of ±1.75 % from an optimum volume of 285 µl had a statistically significant effect on droplet measurement either under or over-estimating droplet volume due to optical effects caused by a convex or concave oil surface. The second method involved measuring exudate volumes without oil by estimating the flow-rate from photo sequences taken of droplets forming on the severed stylet during the collection period. Phloem volumes measured in air without correction were found to be on average 19.9 nl less (SD 18.87, $p < 0.001$) than those made under oil, with a strong linear relationship between volumes measured in air and those measured in oil ($R^2 = 0.942$). After correction, there was no significant difference between the volume measurement methods with the average difference between the methods being 0.5 nl ± SD 23.3 or less than 0.5 % of the average phloem sample volume.

Methods for analysing the elemental and metabolite profile were refined to enable the measuring of nl sized phloem samples. K, Mg, Zn and Fe were successfully quantified in phloem exudate using inductively coupled plasma mass spectroscopy in volumes as small as 15.5 nl. Semi-quantitative data were able to be produced for 79 metabolites using gas chromatography mass spectroscopy (GC-MS) in volumes as small as 19.5 nl. An amine group derivitization method coupled with liquid chromatography mass spectroscopy (LC-MS) based metabolomics was able to quantify 26 metabolites and semi-quantitative data were available for a further 2 metabolites. Using the LC-MS method, it was possible to quantify

the concentration of the important Fe and Zn binding metabolite nicotianamine, at a mean concentration of 255.4 µmol L^{-1} (SE 96.71, n =3).

Using the methods developed, further exploration of wheat phloem transport was possible. There were significant increases in the concentration of Mg, Zn and Fe within the phloem from the start of anthesis (1-2 days after anthesis) to the period of peak grain loading (8-12 days after anthesis). For K, there was a significant decrease across the grain loading period. Within the metabolite profile produced by GC-MS, 39 metabolites showed significant changes within the profile from the peak of grain loading to the end of grain loading (17-21 days after anthesis). Of these 39 metabolites, 21 were found to increase and 18 decreased within the phloem metabolite profile as the plant matured.

Changes within the phloem sampled at mid-day and mid-afternoon were explored and a significantly higher Fe concentration was observed in samples taken at mid-afternoon, 1-2 and 8-12 days after anthesis. There were also significantly higher concentrations of K in the phloem of samples taken at mid-afternoon only at 1-2 days after anthesis. Within the metabolite profile, 39 metabolites showed significant variability when sampled at different times of the day. Of these, the metabolites 3-hydroxybenzoic acid, glutamine, histidine and an unknown compound (UN16) had significant variability at both maturity times sampled. 22 additional metabolites had significant changes at peak grain loading and another 13 metabolites had significant variability at the end of grain loading. Of the metabolites identified, of particular interest were the metabolites glutamate, citrate and the unknown metabolite UN8. Both glutamate and citrate may play a role in Fe and Zn transport and were found to have significant changes with maturity. UN8 was found to have significant diurnal variability similar to that found for Fe concentration in the phloem and has been tentatively identified as a precursor (Cys-Gly) of the tripeptide glutathione, which is an antioxidant and phytochelatin precursor.

Finally phloem was collected from two wheat genotypes SAMNYT 16 (Zn-dense grain) and Carnamah (low Zn grain). SAMNYT 16 was found to have significantly more Fe and K in the phloem at the start of the peak grain loading period (8-12 days after anthesis) when compared to Carnamah. There were 19 metabolites that had significant genotypic differences within the metabolite profile. The metabolite 4-aminobutyric acid (GABA) was significantly higher in the metabolite profile of SAMNYT 16 than Carnamah at both maturity times sampled. At the start of peak grain loading, the metabolites proline, ornithine, glutamine, 3-

amino-piperidin-2-one, arginine and serine were significantly higher in the phloem of SAMNYT 16. The metabolites fumarate, sucrose and shikimic acid were significantly higher in the phloem of Carnamah at the same maturity time. At the end of peak micronutrient grain loading, the metabolites Glyceric acid-3-phosphate, UN14, UN17, UN21, UN23 andUN24 were significantly higher in the phloem of SAMNYT 16. The metabolites urea, trehalose and orotic acid were significantly higher in the phloem of Carnamah at the same maturity. From a mechanistic perspective, the metabolites of interest were again glutamate and UN8 as these showed significant maturity changes in SAMNYT 16 but not in Carnamah. Also of interest was the difference in the amount of GABA within the phloem of these two genotypes, with SAMNYT 16 having greater than 8 fold more GABA present within the metabolite profile of the phloem. GABA is considered to be involved in C and N cycling and possibly plays a role in signalling within the plant.

The work presented in this thesis provides tools needed for measuring and analysing nano-litre volumes of phloem. The work presented also further expands on the variability present within the phloem that needs to be accounted for to further expand our knowledge of the long-distance Zn and Fe transport pathways within plants.