Toward the storage metabolome: profiling the barley vacuole

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Abstract: While recent years have witnessed dramatic advances in our capacity to identify and quantify an ever-increasing number of plant metabolites, our understanding of how metabolism is spatially regulated is still far from complete. In an attempt to partially address this question, we studied the storage metabolome of the barley (Hordeum vulgare) vacuole. For this purpose, we used highly purified vacuoles isolated by silicon oil centrifugation and compared their metabolome with that found in the mesophyll protoplast from which they were derived. Using a combination of gas chromatography-mass spectrometry and Fourier transform-mass spectrometry, we were able to detect 59 (primary) metabolites for which we know the exact chemical structure and a further 200 (secondary) metabolites for which we have strong predicted chemical formulae. Taken together, these metabolites comprise amino acids, organic acids, sugars, sugar alcohols, shikimate pathway intermediates, vitamins, phenylpropanoids, and flavonoids. Of the 259 putative metabolites, some 12 were found exclusively in the vacuole and 34 were found exclusively in the protoplast, while 213 were common in both samples. When analyzed on a quantitative basis, however, there is even more variance, with more than 60 of these compounds being present above the detection limit of our protocols. The combined data were also analyzed with respect to the tonoplast proteome in an attempt to infer specificities of the transporter proteins embedded in this membrane. Following comparison with recent observations made using nonaqueous fractionation of Arabidopsis (Arabidopsis thaliana), we discuss these data in the context of current models of metabolic compartmentation in plants.

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Supplemental Figure 1. Global comparison of all detected compounds by GC-MS and LC-MS in protoplast and vacuole. (A) Principal component analysis (PCA) using all detected peaks in GC-MS or LC-MS (260 peaks). (B) Venn Diagram to show common peaks and specific peaks.
Supplemental Figure 2. Probe ID of genes shown in the framework of co-expression network in Figure 2.
Supplemental Figure 3. Probe ID of genes shown in the framework of co-expression network in Figure 3A-C.