Chloroplast Proteome of Maize Leaf

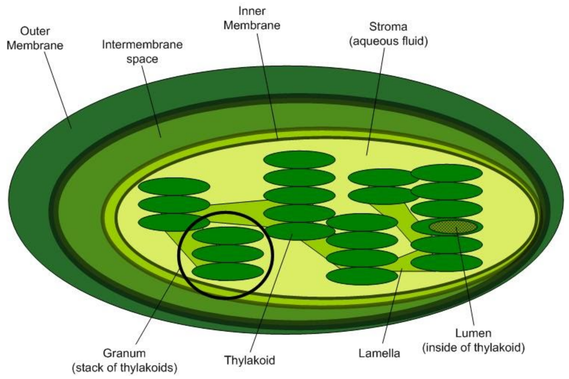
Samantha Kan, Vedika Shenoy, Zhouxin Shen and Steven P. Briggs

The Division of Biological Sciences, University of California San Diego, 9500 Gilman Drive, La Jolla, California

Abstract

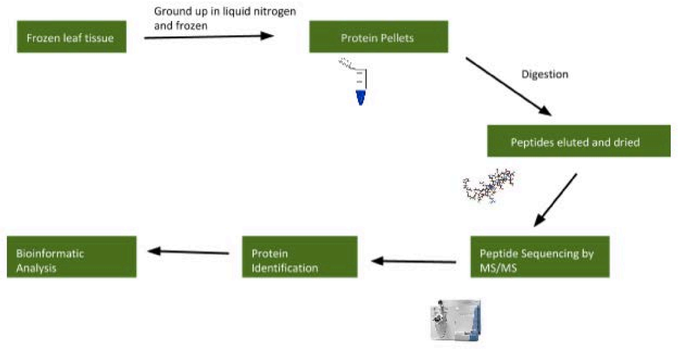
The objective of this investigation was to observe and analyze various proteins present during the stages of chloroplast maturation. Through mass spectrometry, databases, and multiple data analysis programs, we were able to identify 2207 potential proteins in the chloroplast and construct a pathway of photosynthesis. We also pinpointed 569 of the 2207 proteins as chloroplast proteins after input in Agrigo. In addition, we observed the abundance of individual proteins in various chloroplasts throughout leaf development, which was then represented in a graph and a hierarchical cluster diagram. We also discovered that chloroplasts from the middle and base of the leaf are more closely related than those from the tip. This finding was supported through investigation of certain C4 enzymes present in the data. Furthermore, we detected 48 proteins not previously identified in the chloroplast.

Chloroplast Anatomy and Function



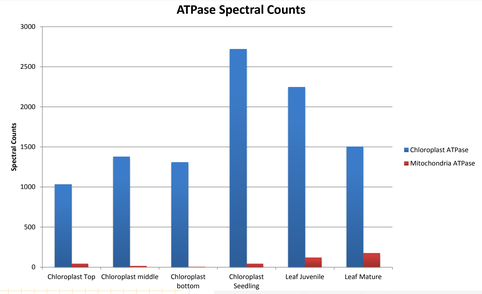
Chloroplasts are the organelles in which photosynthesis takes place. The chloroplast is composed of two membranes: the outer membrane and the inner membrane. The inner membrane contains several grana, which are composed of stacked thylakoids. Thylakoids are flattened sacs that contain a type of pigment called chlorophyll. The inner membrane also has stroma, which is a dense fluid that contains enzymes essential to the process of photosynthesis. Chloroplasts are primarily found in mesophyll cells, which make up the interior of the leaf, and there are generally 30-40 chloroplasts per cell. Image from https://upload.wikimedia.org/wikipedia/commons/1/11/Chloroplast-new.jpg

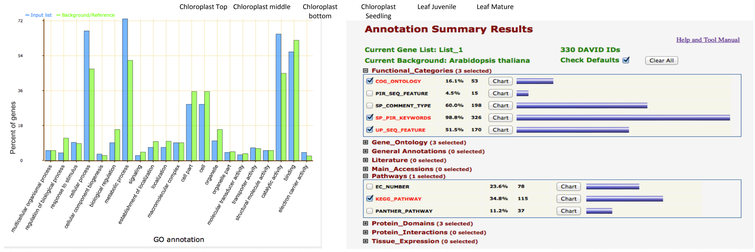
Methodology



Frozen leaf tissues were washed with methanol and acetone after being ground in liquid nitrogen for 15 minutes. Protein pellets were then extracted and dried. Chloroplast sample protein pellets were also extracted and dried. The pellets were then submerged in a buffer, reduced, and alkylated. The protein concentration was checked through a Bradford assay and digested with trypsin twice. The digested peptides were purified and BCA was used to check peptide concentration. Mass spectrometry was then used to quantify the spectra and LC-MS/MS analysis was performed. After analysis, the proteins were identified through database search.

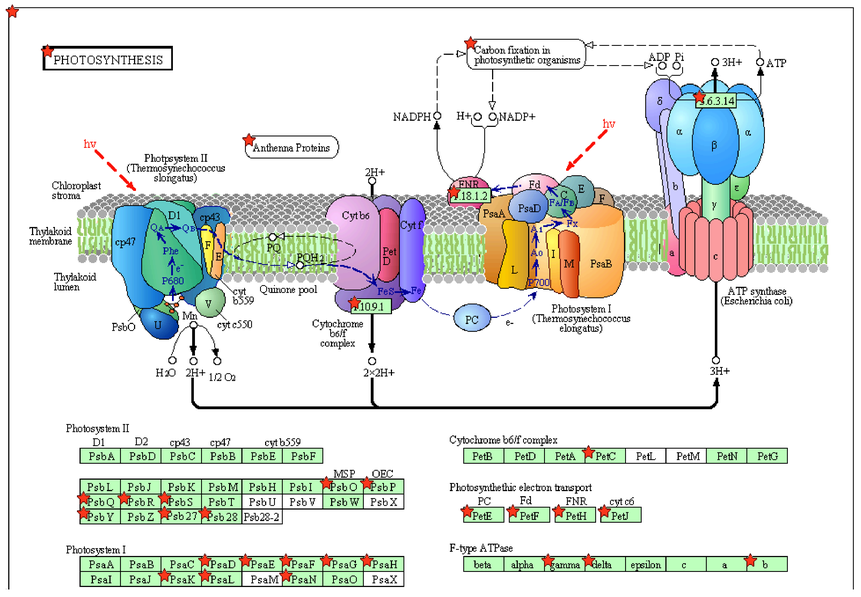
Chloroplast Proteome Data and Tools





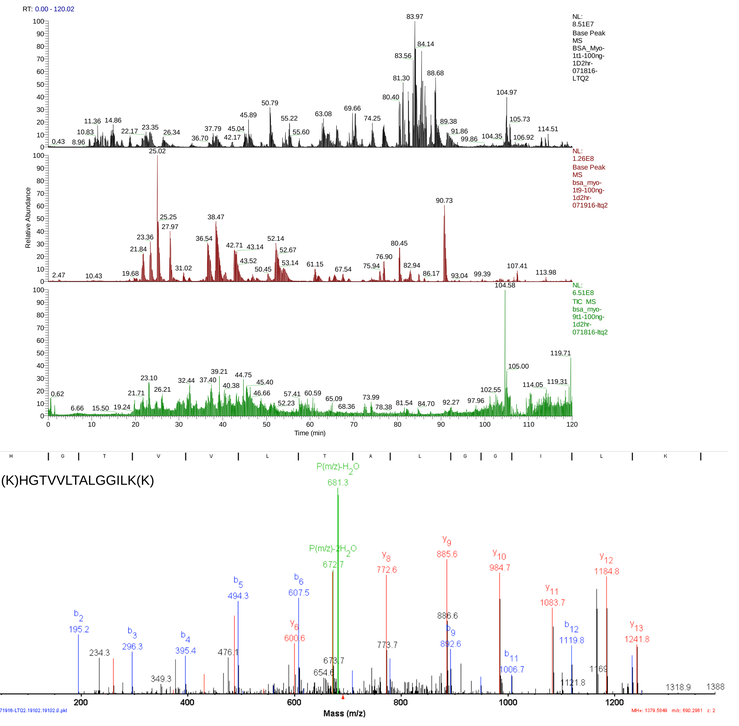
The ATPase diagram demonstrates how the chloroplast data is pure, since there is significantly more chloroplast ATPase than contaminant mitochondria ATPase. In order to process the data, we used several tools and databases. One source that we used was Agrigo, which is an online database maintained in China that provides agricultural gene ontology. By using Agrigo, we were able to identify which proteins from the data were specifically from chloroplasts. For each data point, we also had a description from MapMan, an online database that specifies protein function, which supplemented our findings in Agrigo. To generate diagrams and trees, we used the database David and the program Gene Cluster 3.0; the latter was supported by Java TreeView. Furthermore, we used Microsoft Excel to sort and view the data.

Photosynthesis Pathway



The protein pathway of photosynthesis was produced based on leaf data we collected. To create the image, the top 500 protein entries in Excel were used. The red stars indicate the presence of specific proteins in our data within the proteins known to be in photosynthesis. The considerable number of red stars confirms that many of the proteins we extracted are part of photosynthesis. Through the diagram, it can be assumed that a significant number of the proteins we collected are from chloroplasts, since photosynthesis occurs in the chloroplast. The image was generated by David.

Hands-On Experiment to Generate Mass Spectrometry Data



Three solutions with various ratios of two proteins (1 BSA: 1 myo; 9 BSA: 1 myo; 1 BSA: 9 myo) were produced. The proteins were digested by trypsin and LC-MS/MS analysis was performed. After quantifying the spectra, an online database search was done to identify the proteins.

Discussion

We were able to detect 2207 potential chloroplast proteins from previously gathered data by applying a log transformation. The data was gathered through protein extraction, trypsin digestion, and LC-MS/MS analysis. Many of the proteins we identified were part of the photosynthetic process, such as the light-dependent reactions and the Calvin cycle. Furthermore, of the 2207 proteins, 569 chloroplast proteins with hypothetical photosynthetic functions were identified. The other proteins may have come from contamination in the process of isolating the chloroplast proteins, since proteins from the mitochondria and chromosome were also found in the data. We also discovered 48 proteins that were not previously known to be in the chloroplast. However, more research must be conducted to confirm whether they are contaminants or new chloroplast proteins.

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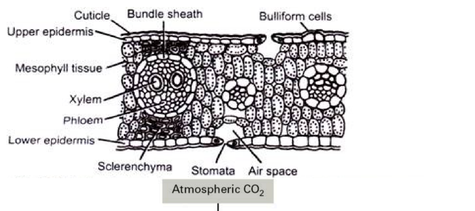
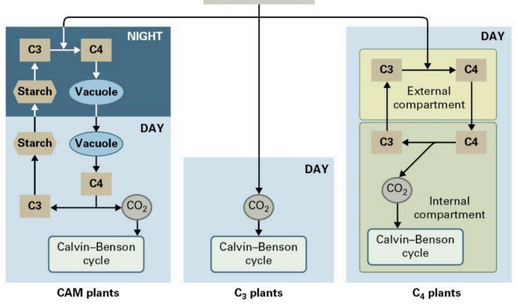
The Stages of Chloroplast Maturation

The leaf samples we took were from three sections: base, middle, and tip. The leaf has a tip-to-base differentiation, which means that bundle-sheath and mesophyll cells begin developing at the tip due to light inducement. As a result, chloroplasts from the tip are more complex and mature than those from the middle and the base. Furthermore, more proteins are found in chloroplasts from the tip than chloroplasts from the middle and base. We attempted to identify various protein concentration gradients, pinpointing the proteins that were more prevalent in the tip than the base. By doing so, we were able to compare which proteins were significant in the leaf's mature stage in relation to the leaf's juvenile stage.

Maize Leaf Anatomy and Plant Pathways





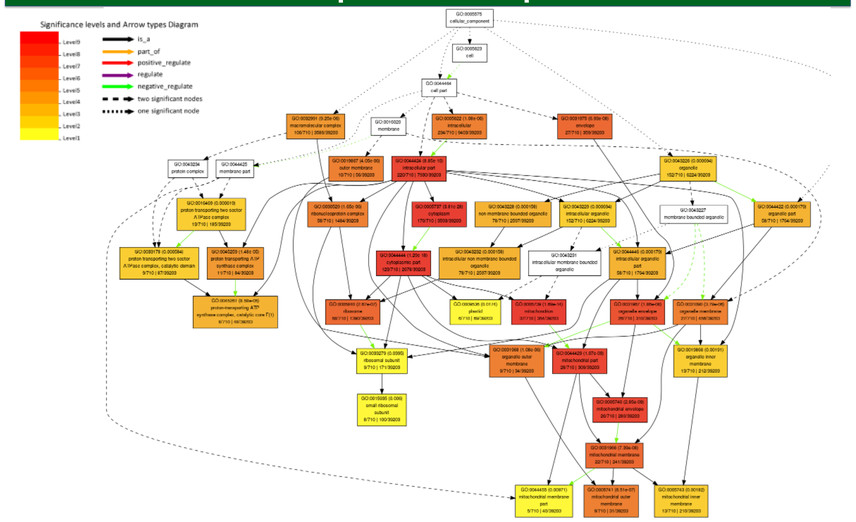
The structure of a maize leaf can be classified by Kranz anatomy, which occurs when the vein is surrounded by a layer of bundle-sheath cells and a layer of mesophyll cells. In addition, maize cells use the C_4 pathway of incorporating carbon into the Calvin cycle of photosynthesis, which is made possible due to Kranz anatomy. The C_4 pathway is an efficient way of fixing carbon, because it prevents photorespiration by assimilating carbon dioxide into four-carbon compounds in mesophyll cells. The compounds are then transferred to the bundle-sheath cells, where the carbon dioxide is used in the Calvin cycle. Images from http://www.biologydiscussion.com/wp-content/uploads/2014/09/clip_image0101.jpg and http://www.wiley.com/legacy/wileychi/buchanan/

List of C_4 Pathway Proteins

	A	B
1	Accession	MapMan Term
2	GRMZM2G306345_P05	'gluconeogenese/ glyoxylate cycle.pyruvate dikinase'
3	GRMZM2G097457_P01	'gluconeogenese/ glyoxylate cycle.pyruvate dikinase'
4	GRMZM2G129513_P01	'TCA / org. transformation.other organic acid transformaitons.cyt MDH'
5	GRMZM2G306345_P01	'gluconeogenese/ glyoxylate cycle.pyruvate dikinase'
6	GRMZM2G131286_P01	'not assigned.unknown'
7	GRMZM2G162200_P02	'PS.calvin cycle.rubisco interacting'
8	GRMZM2G085019_P01	'TCA / org. transformation.other organic acid transformaitons.malic'
9	GRMZM2G113033_P02	'PS.calvin cycle.rubisco small subunit'
10	GRMZM2G418752_P01	'PS.calvin cycle.rubisco interacting'
11	GRMZM5G803490_P01	'amino acid metabolism.synthesis.branched chain group.leucine specific'
12	GRMZM2G104613_P01	'amino acid metabolism.synthesis.branched chain group.leucine specific'
13	GRMZM2G122479_P01	'TCA / org. transformation.other organic acid transformaitons.malic'
14	GRMZM2G083841_P01	'glycolysis.cytosolic branch.phospho-enol-pyruvate carboxylase (PEPC)'
15	GRMZM2G004880_P01	'not assigned.unknown'
16	GRMZM2G154595_P01	'gluconeogenesis.Malate DH'
17	GRMZM2G466833_P01	'gluconeogenesis.Malate DH'
18	GRMZM2G068455_P03	'gluconeogenesis.Malate DH'
19	GRMZM2G118770_P01	'TCA / org. transformation.other organic acid transformaitons.malic'
20	GRMZM2G404237_P01	'TCA / org. transformation.other organic acid transformaitons.malic'
21	GRMZM5G886257_P01	'TCA / org. transformation.other organic acid transformaitons.malic'
22	GRMZM2G159724_P03	'TCA / org. transformation.other organic acid transformaitons.malic'
23	GRMZM2G018566_P01	'TCA / org. transformation.other organic acid transformaitons.IDH'
24	GRMZM2G120857_P02	'TCA / org. transformation.other organic acid transformaitons.IDH'
25	GRMZM2G161245_P01	'gluconeogenesis.Malate DH'
26	GRMZM2G415359_P02	'TCA / org. transformation.other organic acid transformaitons.cyt MDH'
27	GRMZM2G085747_P01	'TCA / org. transformation.other organic acid transformaitons.malic'
28	GRMZM2G406672_P01	'TCA / org. transformation.other organic acid transformaitons.malic'
29	GRMZM2G001696_P02	'gluconeogenese/ glyoxylate cycle.PEPCK'

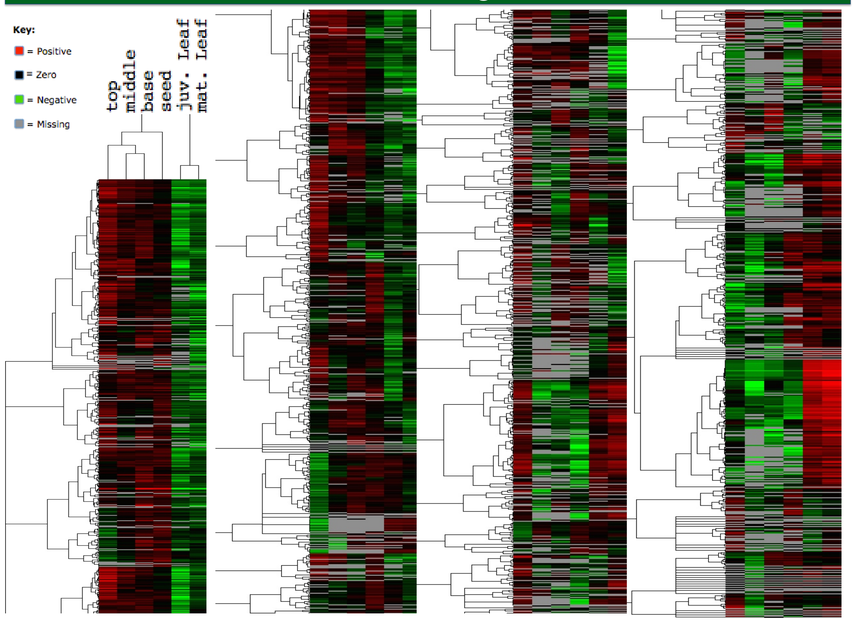
C_4 pathway enzymes, such as NADP⁺-malic enzyme, were used to identify corresponding proteins. In particular, NADP⁺-malic enzyme was mainly used since it differentiates C_3 from C_4 photosynthesis. However, PEP carboxylase and pyruvate-orthophosphate dikinase (PPDK) were also used as identifiers.

Diagram of Various Proteins Present in the Stages of Chloroplast Development



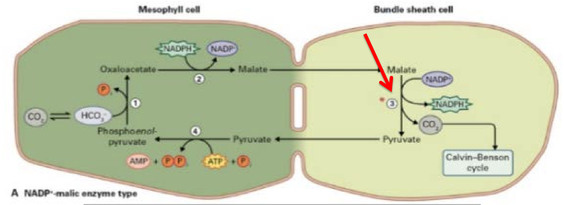
The graph above depicts the top-to-bottom chloroplast ratios in leaves. Using a 2-fold cutoff, chloroplast proteins that were abundant at the top were identified through a sort. The boxes in red show the significance of the proteins in our data, with either higher or lower enrichment. As shown by the diagram, chloroplast proteins concentrated at the top of the leaf are either highly enriched or de-enriched for mitochondrial parts and cytoplasm. This image was generated by Agrigo.

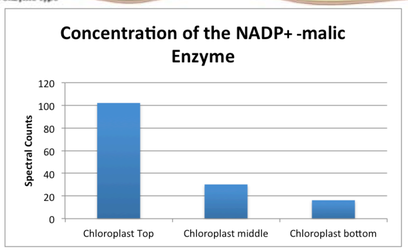
Hierarchical Cluster of Protein Concentrations During Each Stage



The cluster diagram above shows the chloroplast protein concentrations in respect to the chloroplast's location in the leaf. As indicated by the tree, chloroplast top, middle, and base samples are more closely related to each other than to seed and leaf samples. This conclusion was expected because chloroplast samples would be more similar to each other than to samples from seeds or leaves. Furthermore, the cluster diagram also suggests that the samples from the middle and base are more closely related than those from the top. This finding provides insight into the possible curve of differentiation during chloroplast maturation. The diagram also indicates the presence of proteins that are more enriched in whole leaves than in chloroplasts. This image was produced by Gene Cluster 3.0 and Java TreeView.

C_4 Pathway Enzymes





The NADP⁺-malic enzyme is located on step three of the cell diagram. The bar graph also shows concentration of the enzymes in the tip, middle, and base. Since the NADP⁺-malic enzyme is integral to C_4 photosynthesis, it is expected that it would be more prominent in the tip, where C_4 photosynthesis mainly takes place. In addition, we observed three other proteins with highly similar clustering patterns to that of the NADP⁺-malic enzyme and concluded that they may also play a role in photosynthesis. Image from http://www.wiley.com/legacy/wileychi/buchanan/

Discussion and Conclusions

From inputting the data in Agrigo, we were able to confirm that many chloroplast proteins were involved in metabolic and cellular processes. In addition, we created a hierarchical cluster diagram to demonstrate how many proteins had a higher concentration in the tip than in the middle and base. This discovery supports the tip-to-base differentiation concept and shows how chloroplasts from the tip are more complex than those from the middle and base. Furthermore, we detected 29 proteins that were involved in the C_4 pathway from our data. This discovery is significant because it further confirms that our data is from the chloroplast.
From our research, we were able to identify 569 chloroplast proteins from a list of 2207 potential ones. We also performed trypsin digestion and LC-MS/MS analysis that was similar to how the chloroplast data was produced. In addition, we identified proteins involved in the C_4 pathway and their concentrations from the tip to the base of the leaf. Finally, we were able to pinpoint 48 proteins that were not previously determined to be located in the chloroplast. If they are not contaminants, it is likely that these proteins are involved in some aspect of photosynthesis. Further research may include constructing a co-expression network and confirming whether or not the 48 proteins are indeed new chloroplast proteins.

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