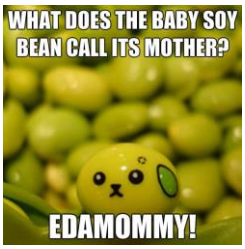
Malate Dehydrogenase In Soybean



Kathryn Dockins

4-17-17

Plant Physiology and Lab
Spring 2017

Prof. J. Bidlack

Article Summary

"Enzymes Related to Chlorophyll Biosynthesis in Mutant Soybean [*Gycine max* (L.) Merr.] Lines" authored by Aaron M. Elmer and the famous James Enderby Bidlack (2010) was an excellent reference for the Plant Physiology malate dehydrogenase experiment in soybean. This article describes the search for mechanisms of chlorosis, the loss of green pigmentation in the leaves of plants, in mutant soybean lines. Isozyme analyses of a highly mutable Asgrow w4-m soybean line suggest that three of the mitochondrial malate dehydrogenase (MDH) isozyme bands are lacking. Variants of the w4-m line posses a mutant copy of the malate dehydrogenase isozyme (mdh-1n) allele, giving rise to the idea that chlorophyll deficiency may be linked to this allele. The non-functional mutant (null) mMDH isozyme allele may relate to linkage of an upstream chlorophyll biosynthesis gene causing deficiency in chlorophyll if the mMDH null gene in the deficient lines also have a chlorophyll biosynthesis gene. The intermediate α-ketoglutarate within the Krebs cycle is a precursor of the vital metabolite glutamate for chlorophyll biosynthesis. If mMDH isozymes are lacking, the metabolite flow through the Krebs cycle and malate/aspartate shuttle will decrease and malate will not reach the chloroplast. For soybean mutant lines Asgrow w4-m, CD-1 (chlorophyll deficient), CD-2, and CD-3 were evaluated for differences in crude homogenate concentrations and enzyme activity. Dr. Elmer and Dr. Bidlack hypothesized "if the level of enzyme activities followed the same ranking as the level of chlorosis in normal and mutant lines, then decreased pigmentation may be genetically or pleitropically related to enzyme activates involved in chlorophyll biosynthesis (Elmer, AM, Bidlack JE. 2010).

The experimental methods were as follows: plant germination and maintenance; harvest and crude extract; protein determination; spectrophotometric enzyme assays using a Hewlett-Packard 8452A UV/VIS Diode Array Spectrophotometer and use of cytochrome *c* oxidase (CCO) to determine mitochondrion electron flow; microplate enzyme assays of aspartame aminotransferase (AST) using a modified colorimetric method from the Sigma Diagnostics Kit #505; and, chlorophyll quantification of chlorophyll *a*, chlorophyll *b* and carotenoid concentrations at absorbance levels of 452, 644 and 663 nm. The data for chlorophyll quantification for proteins, enzymes and pigments was analyzed using PROC GLM (Elmer, AM, Bidlack, JE. 2010).

The results of this study found significant differences were found in the analysis of variance between the four mutant soybean lines for the both the activity of MDH and CCO, and concentration levels of chlorophyll a, chlorophyll b and carotenoids. Protein concentration and AST activity had no significant difference among the four mutants. The significant differences that were found between the mutants indicate involvement of crude MDH and CCO enzymes in mechanisms from mutations in w4-m mutable lines. A decrease in glutamate production vital for chlorophyll biosynthesis could be caused by low MDH activity, leading to chlorosis in the mutant soybean lines. The data collected exhibited that a correlation between enzyme activities and chlorosis provides the likelihood that yellowing of Asgrow *w*4-*m* leaves is related to chlorophyll biosynthesis enzyme activity (Elmer, AM, Bidlack, JE. 2010).

Malate Dehydrogenase In Soybean

Introduction

Malate dehydrogenase (MDH) is an enzyme that catalyzes the oxidation of malate to oxaloacetate reversibly, producing NADH. The alteration of MDH banding patterns in soybean can be used to view those banding patterns lacking in the mitochondrial MDA (mMDH); Bands of variant soybean mMDH are absent for the mitochondrial form. This suggests that regulating chlorophyll synthesis and mMDH expression in chloroplasts in the cultivar lines w4-m, y-20-k2, and Jilin 3 is dependent upon an unknown mutable mechanism. We are referring to **J**ilin, not **G**illi**a**n. This is Gillian:

To solve this X-file, students will seek to discover which mechanism(s) relate chlorophyll deficiency and MDH mutation; two ideas have been postulated. The first suggests that a failure to produce OAA and succinate, because of decelerated activity of mMDH and glyoxysomal MDH, leads to an α-ketoglutarate deficiency and ultimately retarded chlorophyll synthesis (See Figure 1). The second idea is that chlorophyll synthesis and MDH activity are changed by a transposable element; mMDH expression inhibited by a transposon, combined with chlorophyll synthesis inhibited by the transposase could give rise to yellow soybean plants. In this experiment, students will investigate mechanisms to explain the variance of chlorophyll in MDH soybeans by gathering genetic, enzyme, and chlorophyll information from normal versus mutant soybean plants.

Procedure

Four seeds each of normal Jilin and mutant Jilin soybean seeds were planted on 2-22-17 in two separate labeled pots. On 3-22-17, after germination and four weeks of growth, five grams of tissue were removed from each mutant and normal plant for tissue homogenization in buffer stock and centrifugation to obtain supernatant containing total extract, crude mitochondria, crude microbodies, and residual supernatant; These steps will produce a resuspended pellet containing chloroplasts, and a resuspension containing washed mitochondria. Next, protein determination and MDH determination will be carried out for the prepared organelle isolations from mutant and normal Jilin 3 soybean extracts using a spectrophotometer. The final step in the experiment will be to use an SDS polyacrylamide gel electrophoresis apparatus in combination with Laemmli Running Buffer and Coomassie Blue staining solution to obtain MDH banding patterns.

Results and Discussion

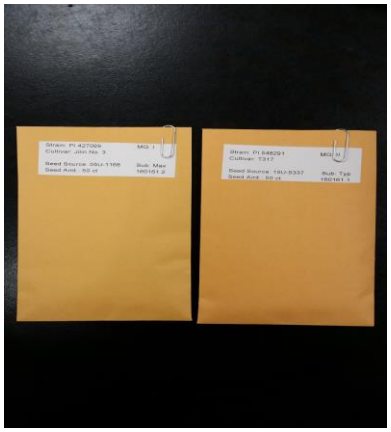


Figure 1: Left: Jilin No. 3 seeds. Right: Mutant line T317 seeds. Photo taken on March 8th.

Figure 2: Jilin 3 and mutant physical comparison. Left in each photo: mutant is taller and with a lighter coloration. Right in each photo: Jilin 3 is shorter with rich green color.

Figure 3: Fon and Kathryn posing the pellets for their photo shoot. The homogenate was put through several resuspensions. Students obtained supernatant of crude extract (A), a pellet of chloroplasts (B), a pellet of mitochondria (C), and supernatant of cytosol (D) for the evaluation of protein concentration and enzyme activity.

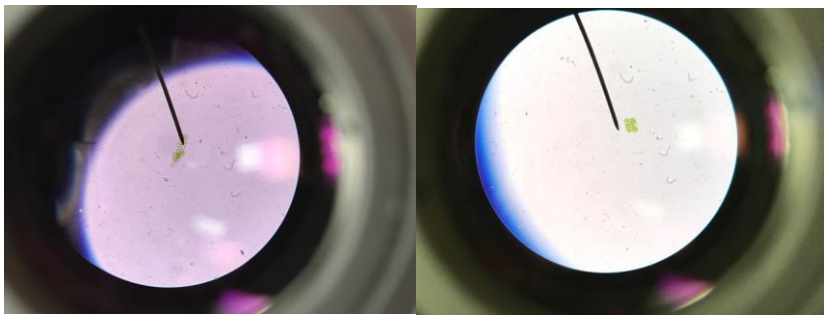
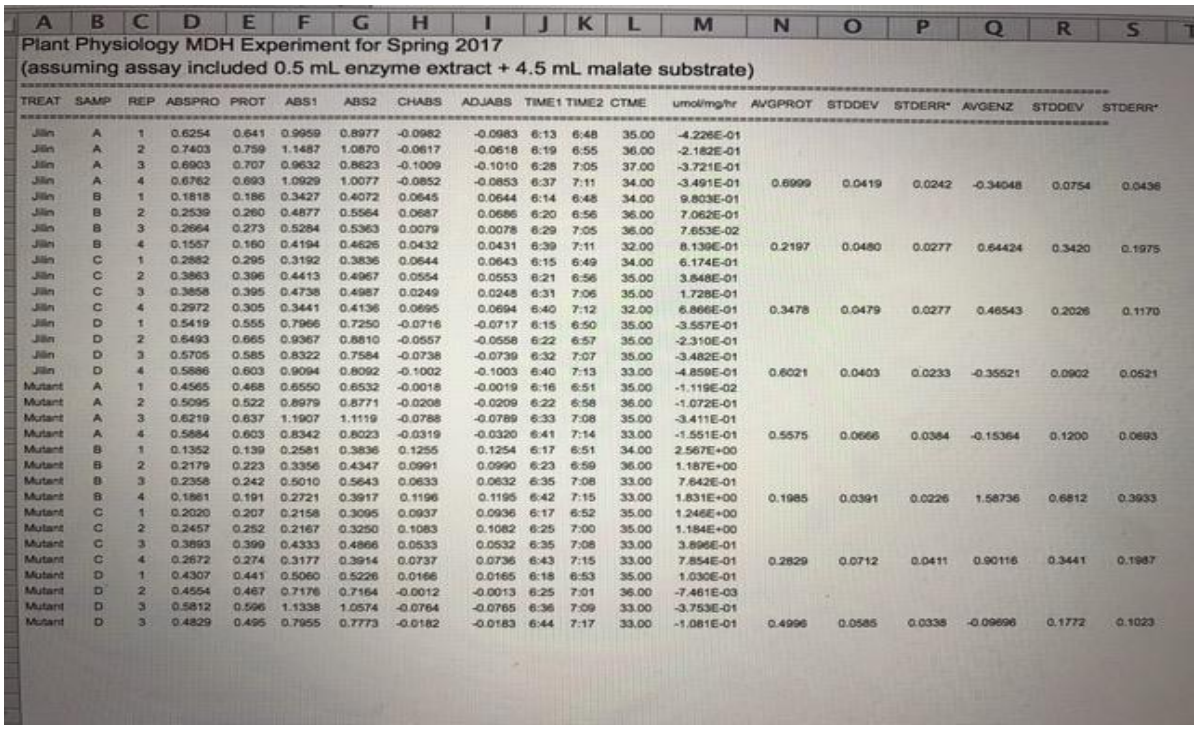


Figure: 4 Left: Mutant chloroplasts. Right: Jilin 3 chloroplasts



Plant Physiology MDH Experiment for Spring 2017
(assuming assay included 0.5 mL enzyme extract + 4.5 mL malate substrate)

TREAT	SAMP	REP	ABSPRO	PROT	ABS1	ABS2	CHABS	ADJABS	TIME1	TIME2	CTME	umol/mg/hr	AVGPROT	STDDEV	STDERR*	AVGENZ	STDDEV	STDERR*
Jilin	A	1	0.6254	0.641	0.9959	0.8977	-0.0982	-0.0983	6:13	6:48	35.00	-4.226E-01						
Jilin	A	2	0.7403	0.759	1.1487	1.0870	-0.0617	-0.0618	6:19	6:55	36.00	-2.182E-01						
Jilin	A	3	0.6903	0.707	0.9632	0.8623	-0.1009	-0.1010	6:28	7:05	37.00	-3.721E-01						
Jilin	A	4	0.6762	0.693	1.0929	1.0077	-0.0852	-0.0853	6:37	7:11	34.00	-3.491E-01	0.6999	0.0419	0.0242	-0.34048	0.0754	0.0436
Jilin	B	1	0.1818	0.186	0.3427	0.4072	0.0645	0.0644	6:14	6:48	34.00	9.803E-01						
Jilin	B	2	0.2539	0.260	0.4877	0.5564	0.0687	0.0686	6:20	6:56	36.00	7.062E-01						
Jilin	B	3	0.2664	0.273	0.5284	0.5363	0.0079	0.0078	6:29	7:05	36.00	7.653E-02						
Jilin	B	4	0.1557	0.160	0.4194	0.4626	0.0432	0.0431	6:39	7:11	32.00	8.139E-01	0.2197	0.0480	0.0277	0.64424	0.3420	0.1975
Jilin	C	1	0.2882	0.295	0.3192	0.3836	0.0644	0.0643	6:15	6:49	34.00	6.174E-01						
Jilin	C	2	0.3863	0.396	0.4413	0.4967	0.0554	0.0553	6:21	6:56	35.00	3.848E-01						
Jilin	C	3	0.3858	0.395	0.4738	0.4987	0.0249	0.0248	6:31	7:06	35.00	1.728E-01						
Jilin	C	4	0.2972	0.305	0.3441	0.4136	0.0695	0.0694	6:40	7:12	32.00	6.866E-01	0.3478	0.0479	0.0277	0.46543	0.2026	0.1170
Jilin	D	1	0.5419	0.555	0.7966	0.7250	-0.0716	-0.0717	6:15	6:50	35.00	-3.557E-01						
Jilin	D	2	0.6493	0.665	0.9367	0.8810	-0.0557	-0.0558	6:22	6:57	35.00	-2.310E-01						
Jilin	D	3	0.5705	0.585	0.8322	0.7584	-0.0738	-0.0739	6:32	7:07	35.00	-3.482E-01						
Jilin	D	4	0.5886	0.603	0.9094	0.8092	-0.1002	-0.1003	6:40	7:13	33.00	-4.859E-01	0.6021	0.0403	0.0233	-0.35521	0.0902	0.0521
Mutant	A	1	0.4565	0.468	0.6550	0.6532	-0.0018	-0.0019	6:16	6:51	35.00	-1.119E-02						
Mutant	A	2	0.5095	0.522	0.8979	0.8771	-0.0208	-0.0209	6:22	6:58	36.00	-1.072E-01						
Mutant	A	3	0.6219	0.637	1.1907	1.1119	-0.0788	-0.0789	6:33	7:08	35.00	-3.411E-01						
Mutant	A	4	0.5884	0.603	0.8342	0.8023	-0.0319	-0.0320	6:41	7:14	33.00	-1.551E-01	0.5575	0.0666	0.0384	-0.15364	0.1200	0.0693
Mutant	B	1	0.1352	0.139	0.2581	0.3836	0.1255	0.1254	6:17	6:51	34.00	2.567E+00						
Mutant	B	2	0.2179	0.223	0.3356	0.4347	0.0991	0.0990	6:23	6:59	36.00	1.187E+00						
Mutant	B	3	0.2358	0.242	0.5010	0.5643	0.0633	0.0632	6:35	7:08	33.00	7.642E-01						
Mutant	B	4	0.1861	0.191	0.2721	0.3917	0.1196	0.1195	6:42	7:15	33.00	1.831E+00	0.1985	0.0391	0.0226	1.58736	0.6812	0.3933
Mutant	C	1	0.2020	0.207	0.2158	0.3095	0.0937	0.0936	6:17	6:52	35.00	1.246E+00						
Mutant	C	2	0.2457	0.252	0.2167	0.3250	0.1083	0.1082	6:25	7:00	35.00	1.184E+00						
Mutant	C	3	0.3893	0.399	0.4333	0.4866	0.0533	0.0532	6:35	7:08	33.00	3.896E-01						
Mutant	C	4	0.2672	0.274	0.3177	0.3914	0.0737	0.0736	6:43	7:15	33.00	7.854E-01	0.2829	0.0712	0.0411	0.90116	0.3441	0.1987
Mutant	D	1	0.4307	0.441	0.5060	0.5226	0.0166	0.0165	6:18	6:53	35.00	1.030E-01						
Mutant	D	2	0.4554	0.467	0.7176	0.7164	-0.0012	-0.0013	6:25	7:01	36.00	-7.461E-03						
Mutant	D	3	0.5812	0.596	1.1338	1.0574	-0.0764	-0.0765	6:36	7:09	33.00	-3.753E-01						
Mutant	D	3	0.4829	0.495	0.7955	0.7773	-0.0182	-0.0183	6:44	7:17	33.00	-1.081E-01	0.4996	0.0585	0.0338	-0.09696	0.1772	0.1023

Figure 5: Comparison of Jilin 3 normal soybean to Mutant soybean for all four laboratory groups of the Plant Physiology and Laboratory class 2017.

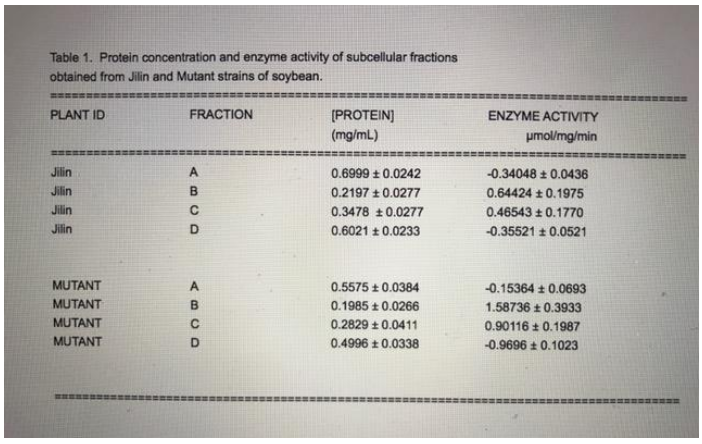


Table 1. Protein concentration and enzyme activity of subcellular fractions obtained from Jilin and Mutant strains of soybean.

PLANT ID	FRACTION	[PROTEIN] (mg/mL)	ENZYME ACTIVITY µmol/mg/min
Jilin	A	0.6999 ± 0.0242	-0.34048 ± 0.0436
Jilin	B	0.2197 ± 0.0277	0.64424 ± 0.1975
Jilin	C	0.3478 ± 0.0277	0.46543 ± 0.1770
Jilin	D	0.6021 ± 0.0233	-0.35521 ± 0.0521
MUTANT	A	0.5575 ± 0.0384	-0.15364 ± 0.0693
MUTANT	B	0.1985 ± 0.0266	1.58736 ± 0.3933
MUTANT	C	0.2829 ± 0.0411	0.90116 ± 0.1987
MUTANT	D	0.4996 ± 0.0338	-0.9696 ± 0.1023

Figure 6: A = crude; B = chloroplasts; C = mitochondria; D = cytosol
Jilin A crude had the most protein, as well as mutant A. All levels of normal Jilin has more protein compared to counterpart of mutant protein concentration.

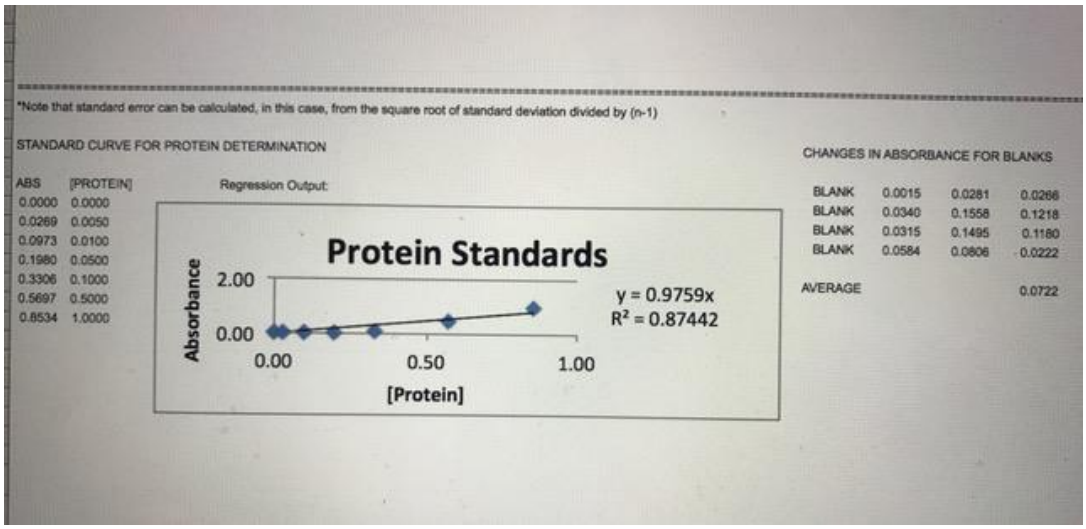


Figure 7: For perfect results the value for R^2 will equal 1. The value obtained in this experiment is close with a value of 0.87442.

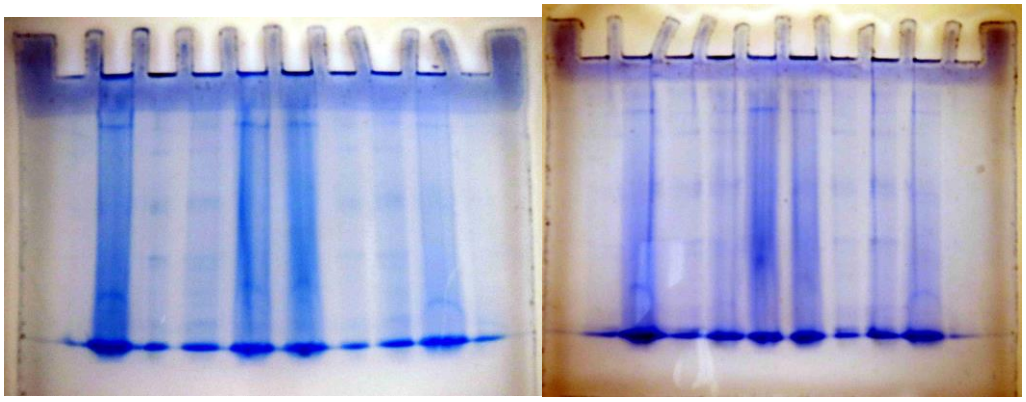


Figure 8: Left: Jillin SDS-PAGE Gell. Right: Mutant SDS-PAGE Gel
Lane 1 is standard ladder, blank. Standard most likely was subjected to temperatures too warm and proteins denatured. Jilin and mutant banding patterns compared and similar. Jilin has more blue intensity than mutant b/c had more protein than the mutant.

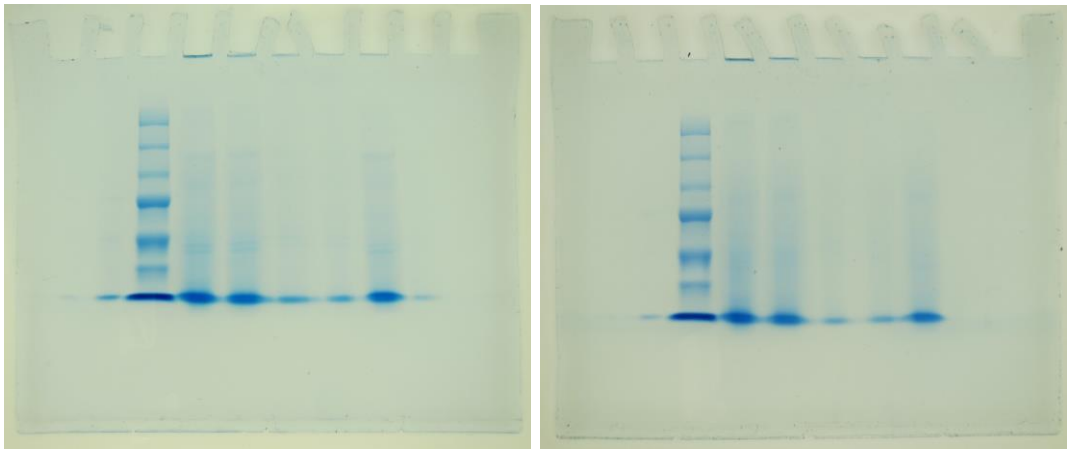


Figure 8: Gel banding patterns collected by the Plant Physiology class of spring 2016. Left: Jilin 3 bands. Right: Mutant bands.

What occurs in the mitochondrion drastically affects what occurs in chloroplast. The shipping of enzymes and proteins through the cytosol to other organelles within the plant cell will result in a high level of protein within the cytosol (D) (Figure 6). The highest levels of protein per category were found in Jilin 3 soybean. Also seen in Figure 6 is enzyme activity, which did not have good outcome from the data. The focus of the enzymatic data was for the mitochondria and chloroplasts. The values for enzymatic activity in the mitochondria were higher in the mutant, and the enzymatic values for chlorophyll in the normal plant were much higher than that of the mutant. This suggests that in the mutant plant, enzymes for chlorophyll biosynthesis within the chloroplast were hindered. Protein and enzyme absorbance values increased and decreased in the correct areas over the entire four experimenting groups (Figure 5), meaning the protein and enzyme portion of this lab was successful.

The gel samples were not clean enough for clear results (Figure 8). The hope was for a contrast of bands between the mitochondria and chlorophyll. Normal soybean gel results should have had double bands, and the mutant had smudge. Some of the bands were clear, but others could not be made out properly due to smudging.

Conclusions

This experiment focused on MDH activity in chlorophyll and mitochondria. Students were able to see how low levels of MDH, stemming from α-ketoglutarate underperformance in the mutant plant, gives rise to chlorophyll biosynthesis hindrance. Since glutamate is a precursor for chlorophyll, when α-ketoglutarate is not efficiently producing glutamic acid to be shipped out of the mitochondria and through cytosol to the chloroplast, chlorophyll production is hindered (Elmer AM, Bidlack JE. 2010).

References

Elmer AM, Bidlack JE. 2010. Enzymes related to chlorophyll biosynthesis in mutant soybean [*Glycine max* (L.) Merr.] lines. Proc. Okla. Acad. Sci. 90:11-18.