Phenotypic Analysis of Methylmalonate Semialdehyde Dehydrogenase Knockout Mutants in *Arabidopsis thaliana*

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Abstract

The breakdown of the amino acid valine is of utmost importance in the model plant organism *Arabidopsis thaliana*. Seeds incapable of breaking down this amino acid develop a diminished ability to germinate. One enzyme involved in the molecular pathway responsible for the breakdown of valine is the mitochondrial enzyme methylmalonate semialdehyde dehydrogenase (MMSDH). In this pathway, it converts methylmalonate semialdehyde into propionyl-CoA and malonate semialdehyde to acetyl-CoA, which is the starting material for lipid biosynthesis. Seeds lacking MMSDH are incapable of breaking down valine, display a wrinkled phenotype, and must be grown on agar plates with an additional carbon source. In this study, phenotypic analyses were performed to further our understanding of this enzyme's role. It was observed that the seed coat of mutant seeds had greater mass than wild-type seeds, however the mass of the embryos from mutant seeds were less than those from wild-type seeds. It was also observed that both seed lines have similar moisture content and response to extended periods of darkness. Studies also indicted mutants display an increased sensitivity to acetate, similar to previous studies performed with propionate and isobutyrate.

Background

A greater understanding in the field of plant lipid biosynthesis and degradation could possibly provide avenues for the development of seeds with altered oil and lipid content as novel sources of biofuel or food alternatives (Maki-Arvela et al. 2009). Identifying other biochemical pathways that contribute to lipid metabolism will require evaluations of each step of these processes. One of these pathways may be the degradation of valine, in particular the enzyme that catalyzes the last step of the pathway, methylmalonate semialdehyde dehydrogenase (MMSDH). It converts methylmalonate semialdehyde into propionyl-CoA (Goodwin et al. 1989). It also converts malonate semialdehyde to acetyl-CoA, which can be used as the starting material for lipid biosynthesis (Bowsher *et al.* 2008). Phenotypic studies were conducted in an effort to learn more about MMSDH in plant growth and development, with a focus on lipid metabolism.

Materials and methods

- Fully mature *A. thaliana* Col-0 wild-type seeds and *aldh6b2* seeds based on the SALK_084428.20.85.X T-DNA insert were obtained and confirmed (Fig. 2) as mutants from the Arabidopsis Biological Resource Center at OSU.

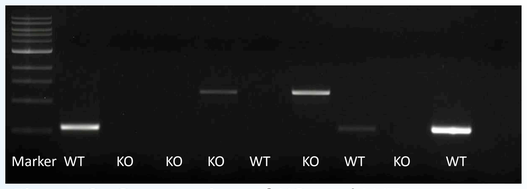

Figure 2. Genotyping of KO and WT mature plants and seeds used in this study.

- *A. thaliana* seeds were grown in 16 hour light conditions with an 8 hour dark cycle on MS media and exogenous sucrose after cold treatment for 2 days at 4°C.
- KO and WT seeds were treated with biotic (light/dark) and abiotic stresses (acetate). Seeds under normal growing conditions were used as controls.
- Dissections performed by imbibing seeds in dark at 4°C overnight and separating tissues by compressing with a glass microscope slide and dissecting under a microscope.
- Seeds (150 per replicate) were hand-counted and weighed on an AD 6000 Ultra Microbalance (Perkin Elmer, Waltham, MA).
- Water content was measured by drying seeds (150 per replicate) at 104°C overnight and calculating the percent difference between heated and unheated samples.

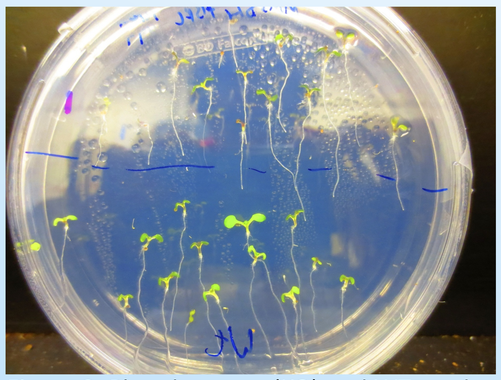

Figure 3. Plated mutant (KO) and WT seeds treated in an extended period of darkness. All seeds were plated as such before being placed into the specified growing conditions.

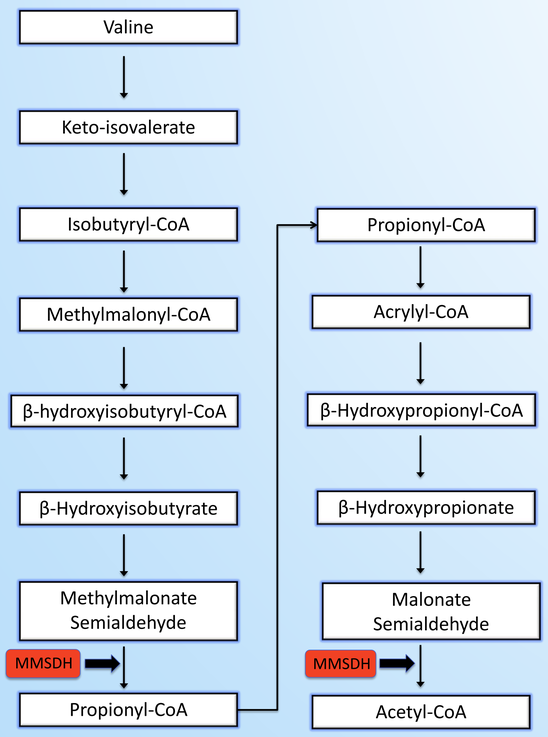

Figure 1. Pathway showing valine degradation as proposed for *A. thaliana* (Lucas, *et al*. 2007). MMSDH catalyzes the last step (conversion of methylmalonate semialdehyde to propionyl-CoA) as well as the last step of propionyl-CoA metabolism (malonate semialdhyde to acetyl-CoA).

Results

Treatment with acetate inhibited germination of mutant seedlings more strongly than wild-type seeds, which was hypothesized to rescue the mutant phenotype (data not shown).

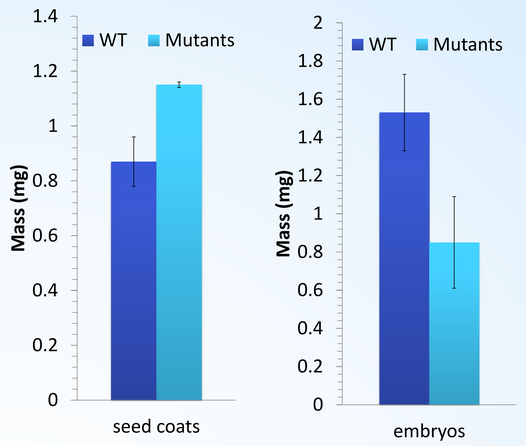

Figure 4. Comparison of 150 WT and mutant dissected and weighed seeds. Seed coats from mutant seeds weighed less than those of WT, while the embryos from mutant seeds weighed significantly less than those from WT seeds. This further supports other measurements obtained in the lab. N=3, error bars=standard deviation. (Two-sample T-test, $p<0.05$)

Table 1. Weight of dissected embryos and seed coats from WT and mutant seeds.

WT seed coats	Mutant seed coats
0.87 ± 0.09 mg	1.15 ± 0.01 mg
WT embryos	**Mutant embryos**
1.52 ± 0.21 mg	0.85 ± 0.24 mg

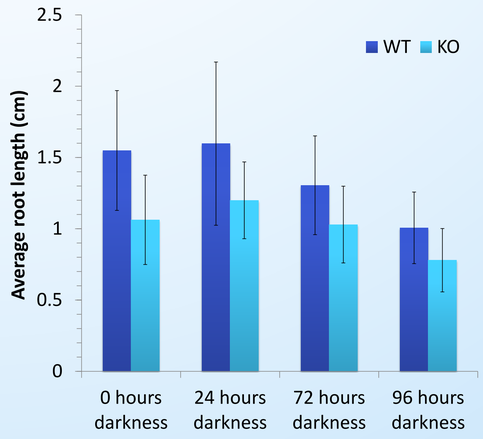

Figure 5. Comparison of WT and mutant (KO) seedlings grown in various light controlled conditions after 8 days based on average root length. When given more light, both WT and mutant seedlings grew longer roots. However, across the treatment, mutant seedlings germinated at a lower rate and had shorter roots. N=2. error bars=standard deviation. (Two-sample T-test, $p<0.05$)

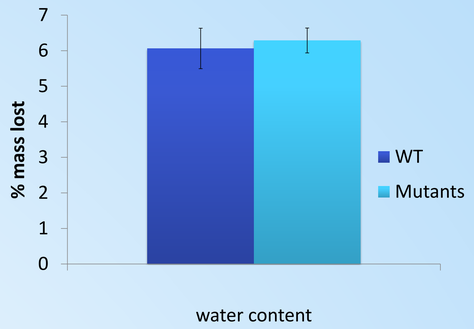

Figure 6. Comparison of 150 WT and mutant dried and weighed seeds as a measure of water content. Water content does not appear to be a factor in the growth of mutant seedlings. N=3 (WT), N=4 (mutant). error bars=standard deviation. (Two-sample T-test, $p=0.589$)

Conclusion

- Studies performed with acetate were consistent with previous experiments with propionate and isobutyrate. This further confirmed that acetate is metabolized in the peroxisomes and does not rescue the phenotypes of our mitochondrial mutant.
- Previous studies showed that mutant seeds germinate more poorly than WT seeds. Data shows that it is likely not due to water content (Fig. 6) of desiccated seeds, but that it may be due to components within the seed coat (Fig. 4). Future work will include a comparison of germination success of seeds grown with and without the seed coat.
- Mutant seeds are very sensitive to availability of sucrose. This complicated light/dark studies as it was difficult to induce valine degradation (as a result of extended darkness) in the presence of sucrose. Future studies will utilize other means for inducing the valine pathway.

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