Documentation for change to proline annotations in PlantSEED

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**Current status:** At3G55610 (AtP5CS2) is annotated with two metabolic functions:

1. Gamma-glutamyl phosphate reductase (EC 1.2.1.41)

alternative name: glutamate-5-semialdehyde dehydrogenase

1. Glutamate 5-kinase (EC 2.7.2.11)

This bifunctional enzyme is linked to three reactions:

The first, rxn20617, reflects both enzymatic functions #1 and #2.

<https://modelseed.org/biochem/reactions/rxn20617>

The second reaction reflects only the ATP-dependent kinase activity performed by function #2.

<https://modelseed.org/biochem/reactions/rxn00179>

The third reflects only the NADPH-dependent reductase / dehydrogenase activity performed by function #1.

<https://modelseed.org/biochem/reactions/rxn02373>

**Proposed change:** At2G39800 (AtP5CS1) needs to be linked to the same two functions and three reactions.

**Literature support:**

Both At3G55610 and At2G39800 encode the bi-functional enzyme Δ1-pyrroline-5-carboxylate synthase. This synthase performs two steps in succession: the phosphorylation of glutamate followed by oxioreductase activity to produce glutamate-5-semialdehyde (GSA). Here is the history of how both genes were assigned this activity:

*Isolation of P5CS in plants and characterization as a bi-functional enzyme*

Hu, C. A., Ashton J. Delauney, and D. P. Verma. "A bifunctional enzyme (delta 1-pyrroline-5-carboxylate synthetase) catalyzes the first two steps in proline biosynthesis in plants." *Proceedings of the National Academy of Sciences* 89, no. 19 (1992): 9354-9358. doi: [10.1073/pnas.89.19.9354](https://doi.org/10.1073/pnas.89.19.9354)

In *E. coli*, proline biosynthesis starts with the phosphorylation of glutamate by gamma-glutamyl kinase (proB) to form gamma-glutamyl phosphate, which is then reduced to glutamic-gamma-semialdehyde (GSA) by GSA dehydrogenase (proA).

Mothbean P5CS complements *E. coli* proB, proA, and double mutants. The sequence of plant P5CS has two domains homologous to *E. coli* proB and proA, respectively. The recombinant P5CS protein from mothbean shows gamma glutamyl kinase activity.

*Isolation of P5CS in Arabidopsis*

Savouré, Arnould, Samir Jaoua, Xue-Jun Hua, Wilson Ardiles, Marc Van Montagu, and Nathalie Verbruggen. "Isolation, characterization, and chromosomal location of a gene encoding the Δ 1‐pyrroline‐5‐carboxylate synthetase in Arabidopsis thaliana." *FEBS letters* 372, no. 1 (1995): 13-19. doi: [10.1016/0014-5793(95)00935-3](https://doi.org/10.1016/0014-5793(95)00935-3)

Isolates P5CS in *A. thaliana* on chromosome 2 and performs DNA and protein sequence analysis. Concludes, based on Southern blot patterns, that there is only one copy of the enzyme in Arabidopsis. Later, this copy will be classified as P5CS1. This gene is At2G39800, the gene I am asking to change.

*P5CS is actually encoded by two differentially regulated genes in Arabidopsis*

Strizhov, Nicolai, Edit Ábrahám, László Ökrész, Stefan Blickling, Aviah Zilberstein, Jeff Schell, Csaba Koncz, and László Szabados. "Differential expression of two P5CS genes controlling proline accumulation during salt‐stress requires ABA and is regulated by ABA1, ABI1 and AXR2 in Arabidopsis." *The Plant Journal* 12, no. 3 (1997): 557-569. doi: [10.1046/j.1365-313x.1997.00557.x](https://doi.org/10.1046/j.1365-313x.1997.00557.x)

Isolating the gene in both *A. thaliana* seedlings and cell cultures reveals two distinct copies of P5CS. DNA and protein sequence analysis shows conservation of the two catalytic domains and ATP and NADPH binding sites in both copies.

P5CS1 (the copy originally isolated on chromosome 2) is expressed in most plant organs while P5CS2 (on chromosome 3) is active in dividing cell cultures. Expression of P5CS1 is induced by salt stress and regulated by ABA signalling.

*P5CS1 and P5CS2 have distinct developmental roles in proline synthesis*

Székely G, Abrahám E, Cséplo A, Rigó G, Zsigmond L, Csiszár J, Ayaydin F, Strizhov N, Jásik J, Schmelzer E, Koncz C, Szabados L. Duplicated P5CS genes of Arabidopsis play distinct roles in stress regulation and developmental control of proline biosynthesis. Plant J. 2008 Jan;53(1):11-28. doi: 10.1111/j.1365-313X.2007.03318.x. Epub 2007 Oct 27. PMID: 17971042. doi: [10.1111/j.1365-313X.2007.03318.x](https://doi.org/10.1111/j.1365-313x.2007.03318.x)

P5CS1 knockout mutations reduce stress-induced proline synthesis. P5CS2 knockout mutations are embryonic lethal and cannot be completely rescued by exogenous proline (plants can grow but are not reproductively viable). While P5CS1 is sequestered during embryonic development, P**5**CS2 is cytoplasmic.