

Tougher Plants: Beating Stress by Protecting Photosynthesis in Genetically Modified Plants

by

Robin Pals-Rylaarsdam and Monica L. Tischler

Department of Biological Science

Benedictine University, Lisle, IL



Part I – Stress

Alice and Todd looked out over their 25-acre plot of tomatoes. No doubt about it, the plants looked bad. This was their first harvest after graduating from the University of Florida and blowing all their graduation money on their local natural farm venture.

“I thought the irrigation system was all we would need. We should have majored in agriculture instead of graphic design,” said Todd. “We set up a beautiful website, but we don’t have anything to sell!”

With bills coming due and, worse yet, having to ask her parents for another loan, Alice decided to call the chair of agronomy at her alma mater to see if he could help. His secretary quickly referred her to the local agriculture extension office, where a cheerful extension agent answered the phone and agreed to drive out to the tomato farm.

Question

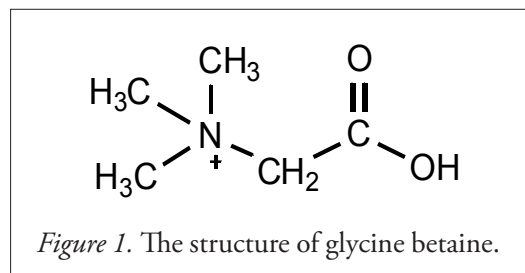
1. What are the major stresses that agricultural plants face?

Part II – Glycine Betaine

“Your plants are facing three environmental stresses out in the field,” Florida State Extension Agent Dory told the would-be farmers. “Heat in the summer, frost even in our mild Florida winters, and salt from the irrigation that’s been going on in those fields for decades. Have you considered planting a genetically modified strain of tomatoes that can help the plants survive these stresses?”

“But we want to be organic!” said Todd, horrified.

“Although the U.S. Department of Agriculture doesn’t allow genetically modified crops to be labeled as organic, you can raise a crop without using pesticides or chemical fertilizers, and still produce pesticide and chemical-free fruits and vegetables,” advised Dory. “Most consumers worry most about the chemicals. There’s been some really nice work done with using plants that make extra glycine betaine, a modified amino acid. I’ll show you some papers from the peer-reviewed literature, where scientists communicate with each other after they make discoveries. Want to take a look?”



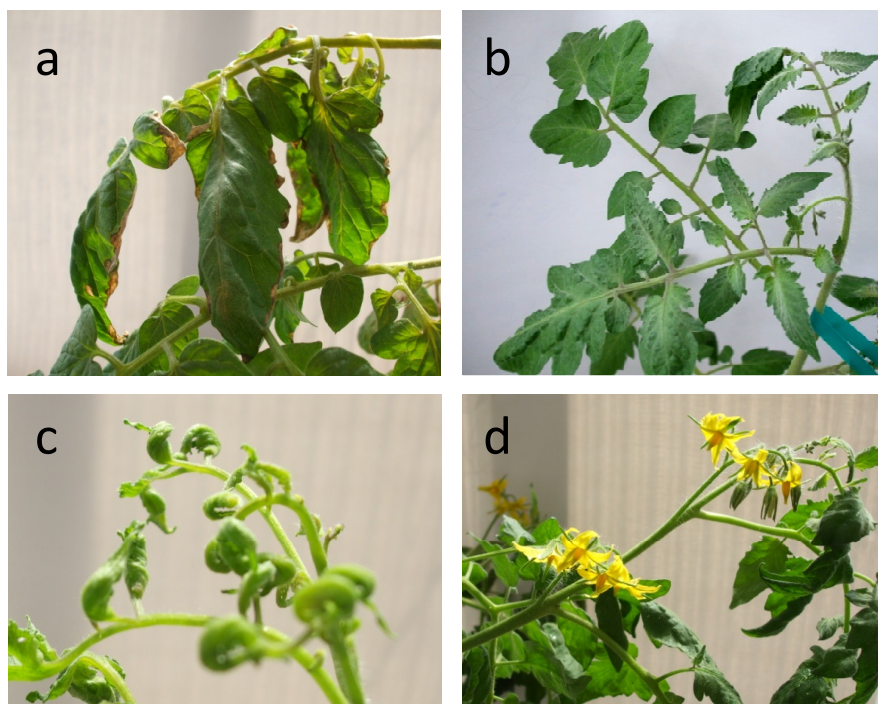
“Sure,” said Alice. “What can this glycine betaine thing do for our tomatoes?”

Questions

2. a. In Figure 2 below, what does wild type mean?
 - b. How is L1 different from wild type?
3. What does glycine betaine do for the leaves, flowers, and fruits in cold-exposed plants?

Figure 2. Susceptibility to cold stress at different plant stages. Nine week-old wildtype (a, c) and L1 transgenic plants (b, d), at stage when two or three flowers first opened fully, were incubated at 3C for 7 days (16h daylight/8h dark), then transferred to a 25C greenhouse. Leaves were observed after one day in the warm greenhouse (a, b), further flower development was observed after 2 weeks in the warm greenhouse.

Credit: Photos by Robin Pals-Rylaarsdam. This figure is intended to simulate Figure 6 in Park, E.-J., Jeknić, Z., Sakamoto, A., DeNoma, J., Yuwansiri, R., Murata, N. and Chen, T. H. H. (2004), Genetic engineering of glycinebetaine synthesis in tomato protects seeds, plants, and flowers from chilling damage. *The Plant Journal*, 40: 474–487.



Part III – Photosynthesis

“Those genetically modified plants look great,” said Todd. “How does glycine betaine give them that protection?”

Dory showed them another figure from the paper. She asked them some questions so they could understand what they were looking at.

Questions

4. What is the X-axis measuring?
5. What is the Y-axis measuring?
6. What does WT mean?
7. What are L1 and L5?

“Those are the kinds of questions you ask for any graph you see in the scientific literature,” said Dory. “But to understand this graph, we need to think a little more about photosynthesis. Do you know anything about that?”

Alice grinned. “We met and fell in love in Introductory Biology our first year of college,” she said. “I think Todd would have flunked the course if I hadn’t taught him all about photosynthesis.”

Todd pulled a face. “That’s not quite how I remember it, but yes, we used to know about photosynthesis pretty well,” he said. “The graph measures ion leakage. How can that relate to photosynthesis?”

Questions

8. Which photosynthesis process is most affected by ion leakage?
 - a. Whether the cells can capture light
 - b. Whether the Calvin cycle will function
 - c. Whether ATP synthase will function
 - d. Whether electrons will transfer from photosystem II
9. What trends can you observe in the graph? List at least two.

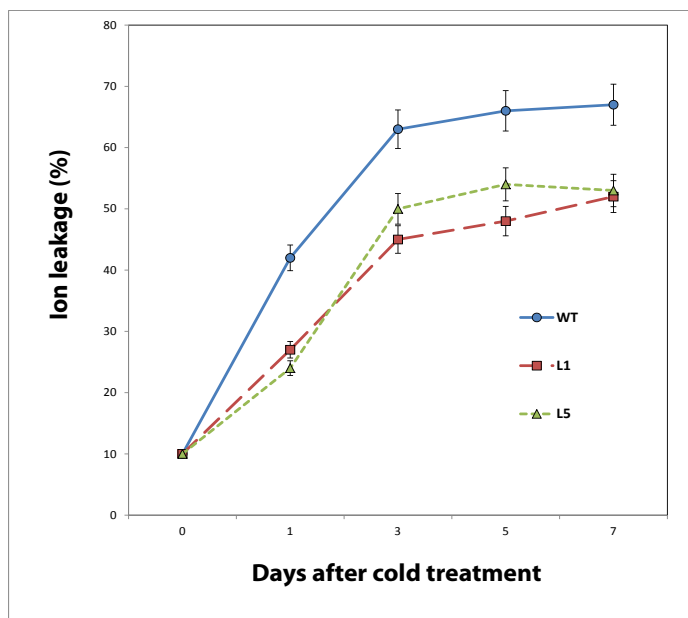


Figure 3. Effects of chilling on various growth parameters. Five-week-old greenhouse-grown wild type and independent homozygous transgenic lines (L1, L5) were chilled (3°C) for 5 days, then returned to greenhouse.

Credit: Redrawn and adapted from Figure 5(g) in Park, E.-J., Jeknić, Z., Sakamoto, A., DeNoma, J., Yuwansiri, R., Murata, N. and Chen, T. H. H. (2004), Genetic engineering of glycinebetaine synthesis in tomato protects seeds, plants, and flowers from chilling damage. *The Plant Journal*, 40: 474–487.

Part IV – Heat Tolerance

“Wow, glycine betaine is great with potential frost damage. Maybe that late frost last spring is why our plants look so terrible,” said Todd.

Dory nodded. “During the Florida growing season we have to worry about heat as well. Let me show you a study where glycine betaine helped with heat tolerance in plants. This study is with tobacco plants, but it should apply to your tomatoes too.”

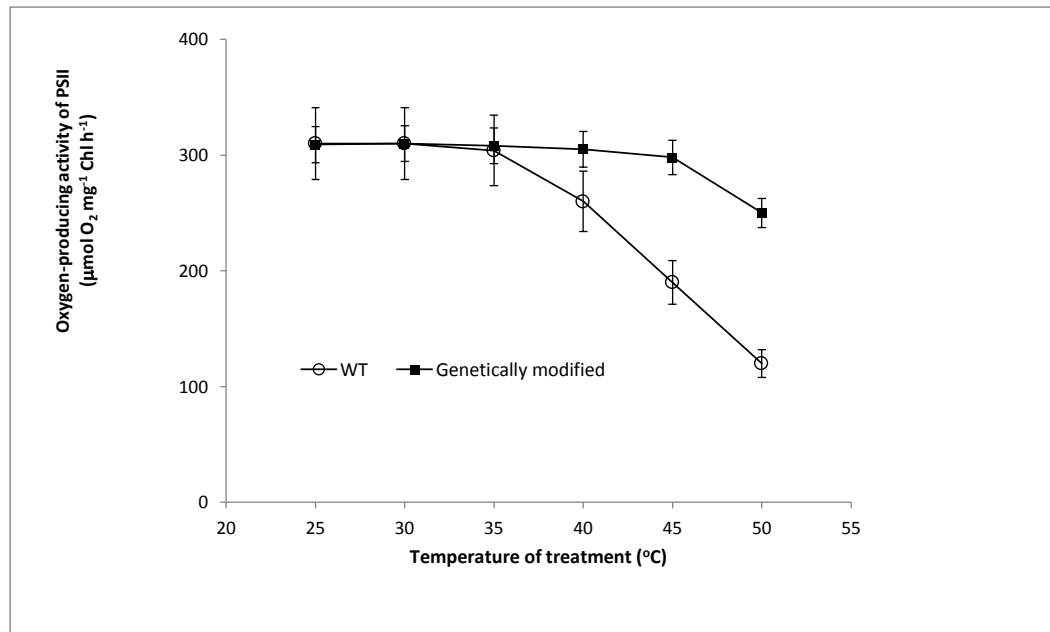


Figure 4. Changes in the oxygen-producing activity of PSII determined with thylakoid membranes isolated from leaves after exposed to different temperatures 25, 30, 35, 40, 45, or 50°C in the chambers for 4 h, in wild type and transgenic plants. The values are mean + SE of three independent experiments.

Credit: Redrawn and adapted from Xinghong Yang, Xiaogang Wen, Hongmei Gong, Qingtao Lu and Zhipan Yang, et al. (2007), Genetic engineering of the biosynthesis of glycinebetaine enhances thermotolerance of photosystem II in tobacco plants, *Planta*, Volume 225, Number 3 (2007), 719–733.

Questions

10. What is the X-axis measuring?
11. What is the Y-axis measuring?
12. What are two types of plants studied in this experiment?
13. Which process in photosynthesis produces oxygen?
14. What trends can you observe in the graph? List at least two.

Part V – Photosystem II

“Why would heat change the activity of photosystem II?” asked Alice.

“Great question,” answered Dory. “Let me show you what photosystem II looks like.” Dory pulled up the ribbon diagram of the structure of this huge protein complex. Cool, huh?” She asked them,

15. “Do you know which part of the molecule goes through the thylakoid membrane?”
 - a. Blue box
 - b. Red circles
 - c. Trick question—this is a big bunch of squiggles.

“I remember,” said Alice. “Parts of a protein in the membrane are much more stable than those inside or outside of the membrane.”

“Right,” said Dory,

16. “So how does this explain why increased temperatures decrease photosystem II activity?”
 - a. Thylakoid membranes become more permeable to ions
 - b. The chlorophyll breaks down
 - c. The peripheral proteins lose their ability to bind to the transmembrane proteins
 - d. Water cannot bind to PSII to form oxygen.

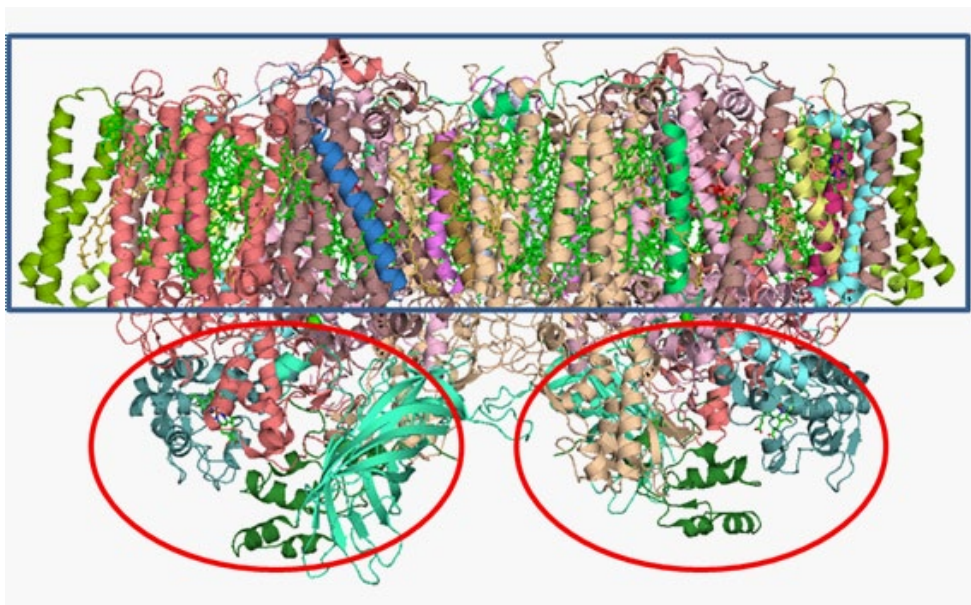


Figure 5. Structure of Photosynthesis II, PDB 2AXT.

Credit: Modified from image by Curtis Neveus, used in accordance with the Creative Commons Attribution-Share Alike 3.0 License, from Wikipedia at <http://en.wikipedia.org/wiki/File:PhotosystemII.PNG>.

Part VI – Salt

“All right, now we know that the genetically engineered tomatoes can take heat and cold. We’re set!” crowed Todd.

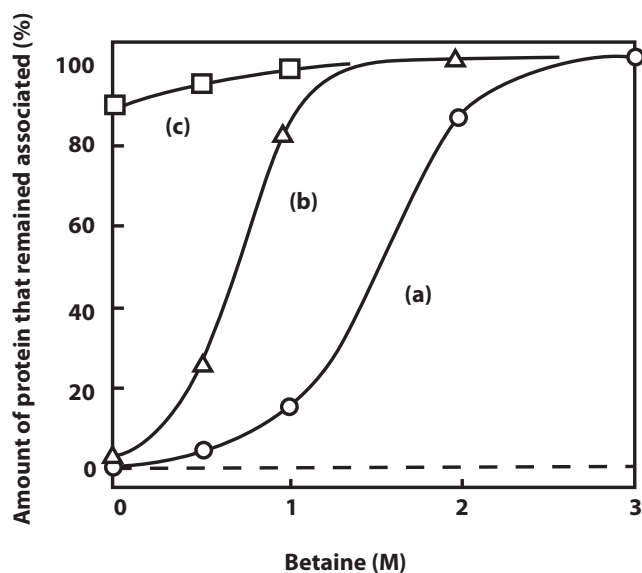
“Don’t be too hasty, Todd. There’s still one more potential stress on your tomatoes,” warned Dory. “You’ve planted on a field that had been irrigated for many years. The water evaporates in the heat and leaves behind salt. If you lived in a cold climate you’d have to worry about salt runoff from the roads in the wintertime. At least that’s not an additional problem for you guys.”

“So is there any evidence that glycine betaine can help with salty soil?” asked Alice.

“Sure, let’s take a look at this third paper,” suggested Dory. “It’s looking at isolated thylakoid membranes, but we can gain some good information from this one too.”

Figure 6. Effects of betaine on the dissociation of 18-(a), 23-(b) and 33-(c) kDa extrinsic proteins from PS2 particles by NaCl. PS2 particles were incubated in media that contained 1.2 M NaCl, 0.3 M sucrose, 0.025 M MES/NaOH (pH 6.5) plus indicated concentrations of betaine.

Credit: Redrawn from *FEBS Letters*, 296(2), N. Murata, P.S. Mohanty, H. Hayashi, G.C. Papageorgiou, Glycinebetaine stabilizes the association of extrinsic proteins with the photosynthetic oxygen-evolving complex, 187–9. Copyright (1992), with permission from Elsevier. <http://www.febsletters.org/>.



Questions

17. What is the X-axis measuring?
18. What is the Y-axis measuring?
19. What do lines a, b, and c refer to?
20. What trends can you observe in the graph? List at least two.
21. Which protein subunit of photosystem II is most stable and likely to be in the membrane?
22. Which protein subunit is least stable and likely to be a peripheral membrane protein?
23. What does glycine betaine do to protect photosystem II activity?

Todd and Alice decided to give the genetically modified tomatoes a chance. They would still need to ask her parents for a loan to tide them over, but they were hopeful that the next harvest would get them on the right track.



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