Genetic Enhancement of the Antioxidant Content of Soybean Oil for Improved Food Processing, Lubricant, and Nutraceutical Properties

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Final report (covers period of 10/1/04-6/30/07)

Executive Summary:

The goal of the project was to produce soybean seeds with enhanced vitamin E antioxidant content that can be tested for improved performance in food processing, industrial lubricant, and nutritional applications. The research focused primarily on the seed-specific expression of homogentisate geranylgeranyl transferase (HGGT), which catalyzes the committed step in the synthesis of the tocotrienol form of vitamin E. By expression of a barley HGGT, transgenic soybeans were successfully generated with up to a 10-fold increase in the total content of vitamin E antioxidants (total tocopherols and tocotrienols) in their seeds. These are the highest reported levels of vitamin E antioxidants that have been engineered in soybean seeds through the use of a single transgene, and are nearly equivalent to the highest reported level of vitamin E enhancement in soybean, which was achieved by expression of four transgenes. The increased vitamin E antioxidant content was due primarily to the accumulation of the delta and gamma forms of tocotrienols, which are known to confer the highest degree of oxidative stability to vegetable oils for high temperature applications (e.g., frying, hydraulic lubricants). Soybean plants were also generated by seed-specific co-expression of HGGT and the soybean VTE4 gene that have the vitamin E composition of seeds shifted toward the more nutritionally significant alpha and beta forms of tocotrienols. Seeds produced from this research are available for functionality testing to determine the impact of genetically enhanced vitamin E antioxidant content on the oxidative stability and human and livestock nutritional properties of soybean oil.

Results:

Metabolic engineering strategies

Two approaches were used to enhance the total vitamin E antioxidant content and composition of soybean seeds (Figure 1). In the first approach, total flux into the vitamin E biosynthetic pathway was increased by seed-specific expression of homogentisate geranylgeranyl transferase (HGGT), which catalyzes the first committed step in tocotrienol biosynthesis. For these experiments, the barley HGGT gene was placed under control of the strong, seed-specific β-conglycinin promoter and linked to a hygromycin selection marker. This gene construct was then used for transformation of soybean by use of a somatic embryo-based method. In the second approach, HGGT was co-expressed in a seed-specific manner with the soybean gene for gamma-tocopherol/tocotrienol methyltransferase (VTE4) to not only increase total vitamin E levels but to also shift production toward the more nutritious alpha and beta forms of tocopherols and tocotrienols. For these experiments, the plasmid containing the HGGT

transgene was co-transformed with a plasmid containing the soybean VTE4 gene under control of the strong, seed-specific promoter for the soybean glycinin-1 gene.

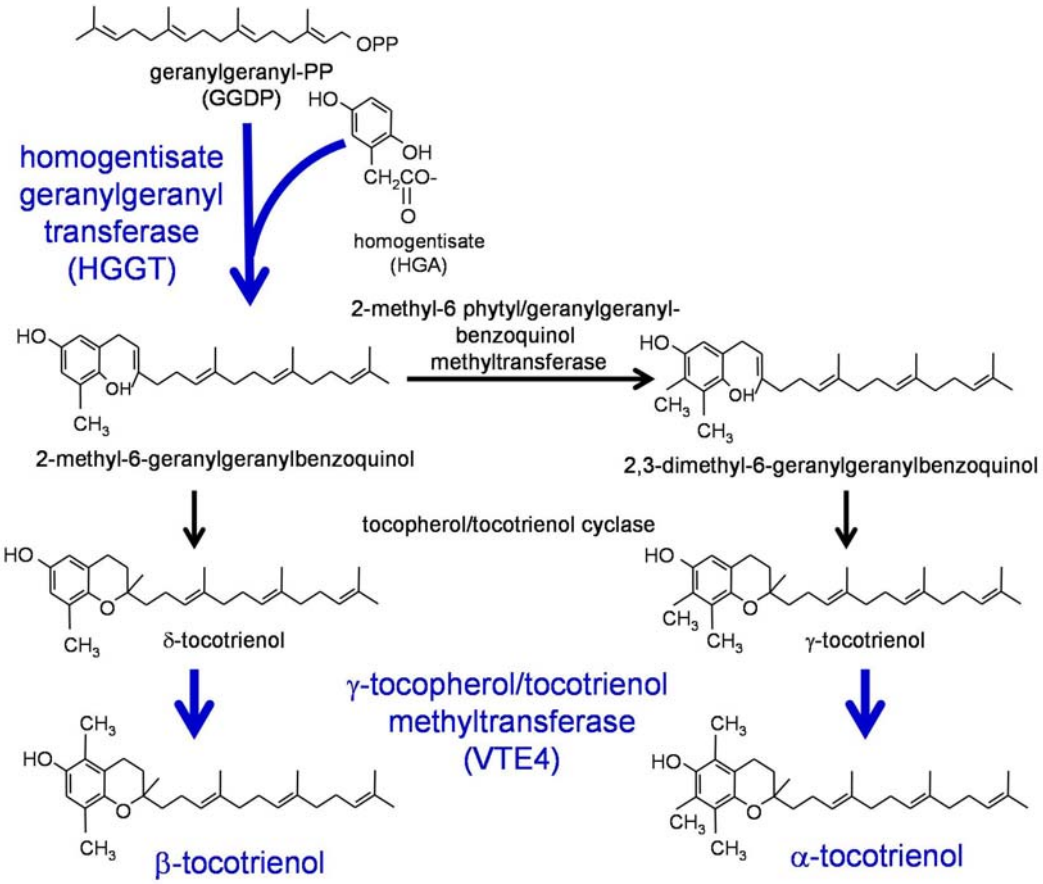

Figure 1. Metabolic engineering strategies used for enhancement of vitamin E content and composition: (1) seed-specific expression of homogentisate geranylgeranyl transferase (HGGT) and (2) seed-specific co-expression of HGGT and gamma-tocopherol/tocotrienol methyltransferase (VTE4).

Seed-specific expression of HGGT

Expression of the barley HGGT resulted in large increases in the total content of tocopherols and tocotrienols in soybean seeds (Figure 2). In the more than twenty regenerated events, the highest content of the vitamin E antioxidants observed was approximately 3,300 µg of tocopherols and tocotrienols/g seed weight. This is nearly a 10-fold increase in vitamin E antioxidants relative to seeds of non-transformed plants, which typically contain 300 to 350 µg of tocopherols and tocotrienols/g seed weight. To our knowledge, this is the highest content of vitamin E antioxidants engineered in seeds of soybean or any crop by use of a single transgene. In the top performing event (Event 574), tocotrienols accounted for ~85% of the vitamin E antioxidants in seeds, nearly all of which was present in the gamma and delta forms (Figure 3). One goal of the project was to obtain transgenic events with a range of vitamin E antioxidant levels in their seeds

for comparative use in functionality studies. As shown in Figure 2, increases in vitamin antioxidant content ranged from four- to nearly 10-fold in seeds from different events.

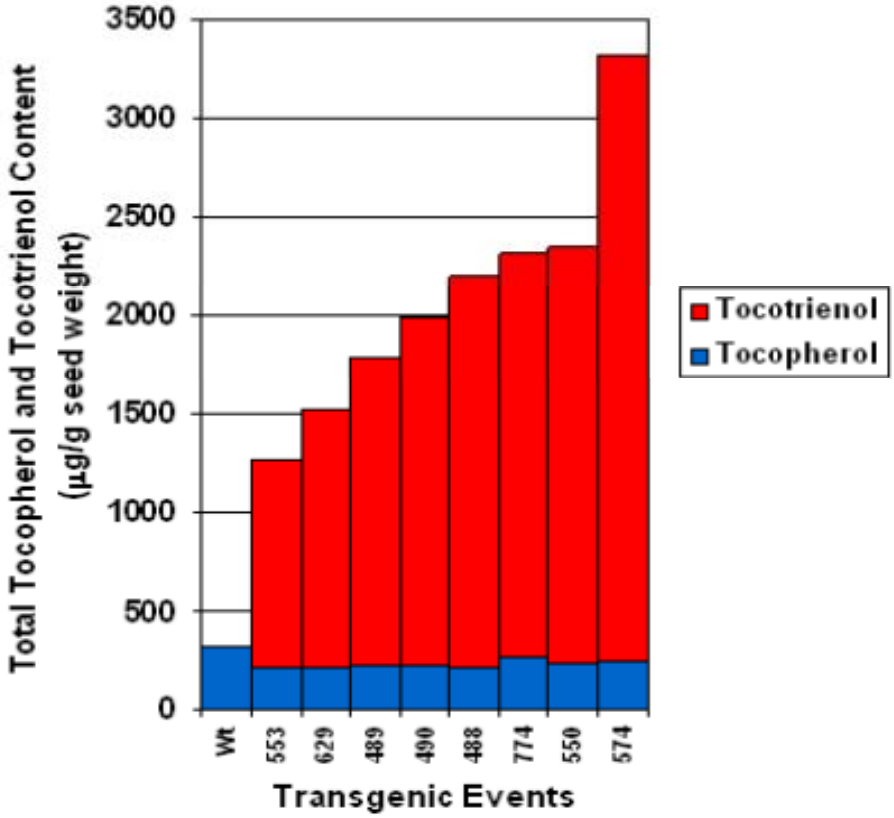

Figure 2. Examples of the vitamin E antioxidant content in seeds from transgenic events generated by seed-specific expression of HGGT.

Seed-specific co-expression of HGGT and VTE4

In the previous experiment, it was observed that at increasing levels of vitamin E production, tocotrienols accumulate predominately in the delta and gamma forms. Because these are the least methylated species of tocotrienols, this observation suggests that HGGT-mediated flux into the tocotrienol biosynthetic pathway exceeds the capacity of methyltransferases to convert delta- and gamma-tocotrienols into the more nutritious alpha and beta forms. Consistent with this, co-expression of HGGT with the soybean gamma-tocopherol/tocotrienol methyltransferase or VTE4 resulted in large increases in alpha- and beta-tocotrienols (and tocopherols) at the expense of the delta and gamma forms. In the top performing event (Event 774), the total content of tocopherols and tocotrienols in seeds was approximately 2,400 µg/g seed weight, which is a 7-fold increase in total vitamin E antioxidant content relative to seeds from non-transformed plants (Figure 3). In addition, ~85% of the vitamin E in these seeds was in the alpha and beta forms. Most notably, these seeds contained about 1,000 µg/g seed weight of alpha-tocotrienol. In comparison, this form of vitamin E was almost non-dectectable in seeds from soybeans engineered with only the HGGT transgene.

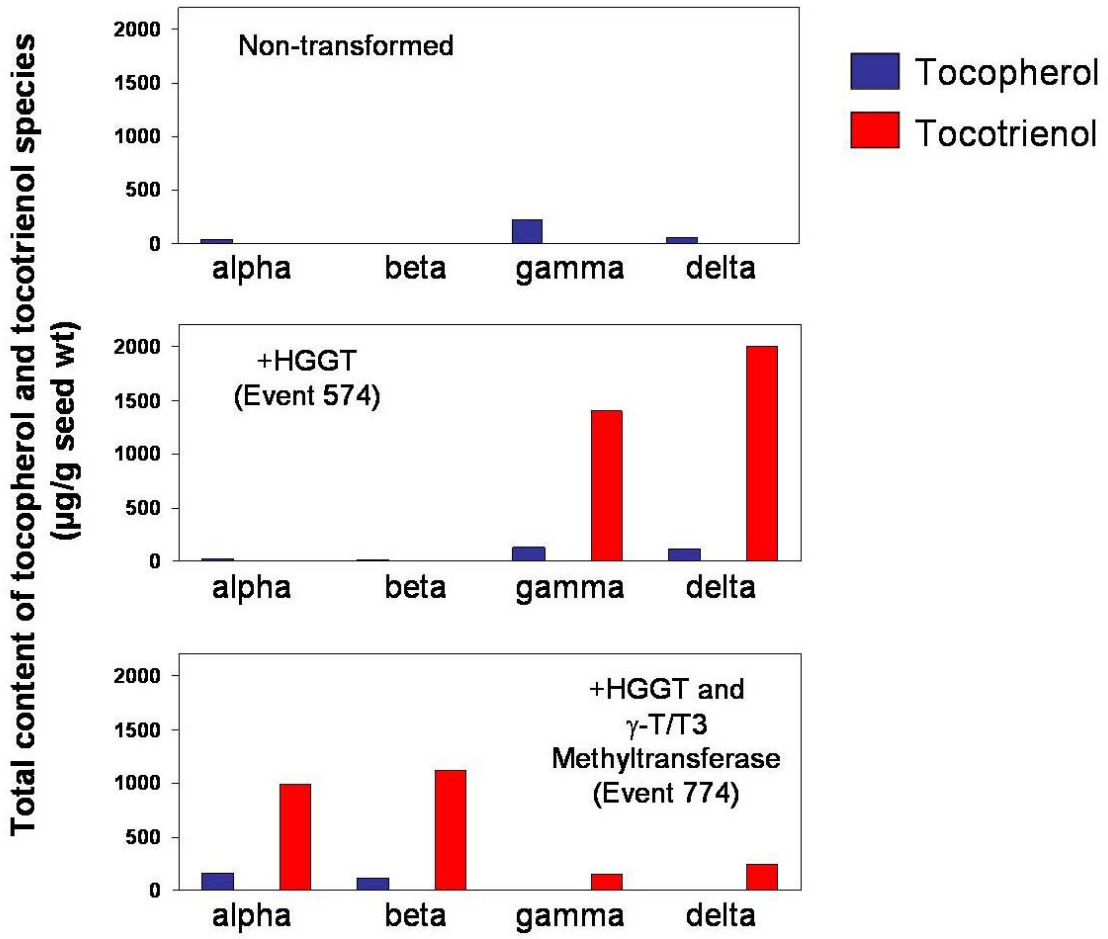

Figure 3: Comparison of the content and composition of vitamin E antioxidants in seeds from non-transformed plants, plants transformed with an HGGT transgene, and plants co-transformed with an HGGT and gamma-tocopherol/tocotrienol methyltransferase (VTE4) transgenes.

Current status of project

Seeds from selected events were used for a small field trial in Columbia, MO during the summer of 2007. The stability of the phenotypes under field conditions will be assessed pending the harvest of seeds. In addition, we are in the process of bulking up seeds from events representing a range of vitamin E levels and compositions. It is anticipated that these seeds will be used for functionality studies, including measurements of the oxidative stability of the extracted oil under high temperatures.

Presentations of IMBA-Related Research:

Cahoon EB (2005) "Toward the Biofortification of Food and Feed: Studies on the Biosynthesis of Vitamin K and the Tocotrienol Form of Vitamin E in Plants". Donald Danforth Plant Science Center, St. Louis, MO. Invited seminar, March 30, 2005.

Cahoon EB (2005) "Metabolic Engineering of Crop Plants for Improved Human Nutritional and Livestock Feed Value". Molecular and Cell Biology Department, Uniformed Services University of the Health Sciences, Bethesda, MD. Invited seminar, April 15, 2005.

Cahoon EB (2005) "Toward the Biofortification of Food and Feed: Characterization of the Biosynthesis of Vitamin K and the Tocotrienol Form of Vitamin E". Department of Crop Science, University of Illinois, Urbana-Champaign, IL. Invited seminar September 14, 2005.

Cahoon, E.B. (2005) "Toward the Biofortification of Food and Feed: Characterization of the Biosynthesis of Vitamin K and the Tocotrienol Form of Vitamin E". Department of Chemistry and Chemical Biology, Indiana University-Purdue University-Indianapolis. Indianapolis, IN. Invited seminar, November 30, 2005.

Cahoon, E.B. (2006) "Toward the Biofortification of Food and Feed: Characterization of the Biosynthesis of Vitamin K and the Tocotrienol Form of Vitamin E". Plant Sciences, University of Missouri-Columbia, Columbia, MO. Invited seminar, February 15, 2006.

Shipp, J.M., Hunter, S.C., Cahoon, E.B. (2006) "Vitamin E and Pro-Vitamin A Biofortification of Soybean". National Center for Soybean Biotechnology Second Annual Symposium. Columbia, MO. Meeting presentation, March 22, 2006.

Cahoon, E.B. (2006) "Metabolic Engineering of Antioxidant and Unusual Fatty Acid Biosynthesis in Crop Plants for Improved Nutritional, Livestock Feed, and Industrial Value". Plant Science Initiative, University of Nebraska-Lincoln, Lincoln, NE. Invited seminar, June 21, 2006.

Cahoon, E.B. (2006) "Metabolic Redesign of Vitamin E Biosynthesis in Soybean Seeds for Increased Antioxidant Content". Soy2006: Molecular and Cellular Biology of the Soybean Conference, Lincoln, NE. Meeting presentation, August 7, 2006.

Cahoon, E.B. (2007) "Characterization of Tocotrienol Biosynthesis for the Metabolic Engineering of Oilseeds with Improved Antioxidant Capacity". First International Symposium on Secondary Metabolism in Plant Seeds: Current Status and Future Applications, Potsdam, Germany. Meeting presentation, February 17, 2007.

Cahoon, E.B. (2007) "Understanding the Biosynthesis of the Tocotrienol Form of Vitamin E for the Biofortification of Crops". Phytochemical Society of North America Annual Meeting, Saint Louis, MO. Meeting presentation, July 22, 2007.

Cahoon, E.B. (2007) "Metabolic Redesign of Vitamin E Biosynthesis in Soybean Seeds for Increased Antioxidant Content". Crop Science Society of America Annual Meeting, New Orleans, LA. Meeting presentation, November 6, 2007.