

United States Department of Agriculture
Project Initiation

Project Data for Accession Number 1032540

Project Title

Engineering soybeans and pennycress for increased vitamin B2 contents using a mutant riboflavin kinase/FMN hydrolase

Project Details

Sponsoring Institution National Institute of Food and Agriculture	Project Status ACTIVE
Funding Source NIFA Non Formula	Grant Year 2024
Grant No. 2024-67013-42713	Cumulative Award Amt. \$650,000.00
Proposal No. 2023-08194	Multistate No. (N/A)
Project Start Date Jul 01, 2024	Project End Date Jun 30, 2028
Program Code [A1103] Foundational Knowledge of Plant Products	

Project Director

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Recipient Organization

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Performing Department

(N/A)

Non Technical Summary

Our long-term goal is to develop strategies for engineering of plants enriched in nutritionally available vitamin B2. The main dietary sources of vitamin B2 in Western diets are milk and dairy products, followed by fortified cereals, meat, and fatty fish. Plants are poorer sources of this vitamin, typically containing around 5-30% the amount compared to dairy products and meat. The Recommended Dietary Allowance (RDA) for adult men and women is 1.3 mg and 1.1 mg daily of vitamin B2, respectively, so to satisfy the RDA by eating plant-based foods, an average woman would need to eat daily about 2 lbs of broccoli, 5 lbs of tomatoes, or 15 lbs of potatoes. Vitamin B2-enriched plants are expected to offer a critical solution to the poor dietary intake of this essential nutrient in humans, reducing dependency on meat and dairy consumption, and providing an overall healthier diet, thus benefiting the society. Like humans, many farmed animals need vitamin B2 in their diets. As a result, nearly 80% of the total commercial production of

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riboflavin is now used to supplement animal feed. An alternative to this excessive supplementation can thus be feeding engineered plants enriched in vitamin B2. We here propose to generate such plants by increasing the biosynthesis of the B2 vitamers riboflavin, FMN, and FAD. To accomplish our engineering goal, we will take advantage of a mutation in one of the enzymes on the vitamin B2 biosynthesis pathway in plants. This mutation causes a huge increase in vitamin B2 accumulation in seeds of the model plant species Arabidopsis. We will determine if the same mutation can be used to increase vitamin B2 accumulation in seeds of the crop species soybean and pennycress. Soybean is one of the most important crops grown in the US on a large scale, and is an excellent model species for other legume crops. Pennycress (*Thlaspi arvense*) is being developed for use as a rotation crop for corn and soybeans, to be grown in the Corn Belt and other suitable areas in the late fall, winter, and early spring when the other crops cannot be grown. The creation of crops with enhanced vitamin B2 contents is expected to provide means for improving nutritional uptake of this vitamin in humans, and to offer an alternative to supplementing animal feed with riboflavin.

Animal Health Component

Animal Health Component: 0%

Research Effort Categories

Basic	50%
Applied	25%
Developmental	25%

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
206	1820	1000	27%
204	1820	1010	12%
201	1820	1040	12%
206	1440	1000	25%
204	1440	1010	12%
201	1440	1040	12%

Knowledge Area

- [206] Basic Plant Biology
- [204] Plant Product Quality and Utility (Preharvest)
- [201] Plant Genome, Genetics, and Genetic Mechanisms

Subject Of Investigation

- [1820] Soybean
- [1440] Cole crops (includes cabbage, kale, broccoli, brussels sprouts, cauliflower, kohlrabi)

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Field Of Science

[1000] Biochemistry and biophysics

[1010] Nutrition and metabolism

[1040] Molecular biology

Keywords

Biosynthesis, FAD, FMN, Riboflavin, Vitamin B2, pennycress, soybean, Flavins

Goals / Objectives

Our long-term goal is to develop strategies for engineering of plants enriched in nutritionally available riboflavin (vitamin B2). The main dietary sources of vitamin B2 in Western diets are milk and dairy products, followed by fortified cereals, meat, and fatty fish. Plants are poorer sources of this vitamin, typically containing around 5-30% the amount compared to dairy products and meat. The Recommended Dietary Allowance (RDA) for adult men and women is 1.3 mg and 1.1 mg daily of vitamin B2, respectively, so to satisfy the RDA by eating plant-based foods, an average woman would need to eat daily about 2 lbs of broccoli, 5 lbs of tomatoes, or 15 lbs of potatoes. Vitamin B2-enriched plants are expected to offer a critical solution to the poor dietary intake of this essential micronutrient in humans, reducing dependency on meat and dairy consumption, and providing an overall healthier diet, thus benefiting the society. Like humans, many farmed animals need vitamin B2 in their diets. As a result, nearly 80% (~2400 tons per year) of the total commercial production of riboflavin is now used to supplement animal feed. An alternative to this excessive supplementation can thus be feeding engineered plants enriched in vitamin B2. We here propose to generate such plants by increasing the biosynthesis of the B2 vitamers riboflavin, FMN, and FAD. The creation of such plants is expected to provide means for improving nutritional uptake of this vitamin in humans, and to offer an alternative to supplementing animal feed with riboflavin. Toward that goal, we here propose the following objectives: Objective 1. Test strategies for increasing vitamin B2 contents in soybean and pennycress seeds by overexpressing the Melinoe mutant form of the bifunctional enzyme riboflavin kinase/FMN hydrolase from Arabidopsis. We here propose to produce and analyze transgenic soybean and pennycress plants that overexpress under control of appropriate seed promoters the Melinoe mutant form of the AtFMN/FHy enzyme from Arabidopsis, with or without knocking down in seeds the native FMN/FHy enzymes from soybean and pennycress. The PI Roje lab will also verify the enzymatic activities for the putative FMN/FHy enzymes from soybean and pennycress, which have not yet been cloned and biochemically characterized. Objective 2. Determine whether the Melinoe mutation causes the increase in riboflavin production because of a change in the AtFMN/FHy enzymatic activity, or because of a change in regulation of the riboflavin biosynthesis pathway, or both. To accomplish this aim, we here propose to (1) attempt rescuing the Melinoe mutant with the riboflavin kinase domain of FMN/FHy, (2) test whether overexpression of the Melinoe FMN hydrolase domain confers the same phenotype to the wild type plants as the full-length Melinoe mutant protein, (3) look for changes in the transcript abundance and enzyme activity of the enzymes on the riboflavin biosynthesis pathway in siliques and mature seeds in the Melinoe mutant vs. wild type plants, and (4) look for proteins that interact with the wild type and Melinoe forms of AtFMN/FHy in siliques and seeds. We propose to conduct the Objective 1 in the crop species soybean and pennycress. Soybean is one of the most important crops grown in the US on a large scale, and is an excellent model species for other legume crops. It is transformable and its whole genome sequence is available. Pennycress (*Thlaspi arvense*) is being developed for use as a rotation crop for corn and soybeans, to be grown in the Corn Belt and other suitable areas in the late fall, winter, and early spring when the other crops cannot be grown. It is easily transformable, and represents an excellent model species for other Brassica crops such as Canola and Camelina. It is likely that the results of this study will be transferrable to other major crops, as genomic evidence suggests that flavin metabolism is highly conserved among angiosperms, including major staple crops such as corn, rice, and potatoes.

Project Methods

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Objective 1 is to test strategies for increasing vitamin B2 contents in soybean and pennycress seeds by overexpressing the Melinoe mutant form of the bifunctional enzyme riboflavin kinase/FMN hydrolase from Arabidopsis. To accomplish this objective, we will first verify the enzymatic activities for the putative FMN/FHy enzymes from soybean and pennycress. Next, we will generate the transgenic plants, and phenotype them with respect to the vitamin B2 contents and the growth and development parameters. Objective 2 is to determine whether the Melinoe mutation causes the increase in riboflavin production because of a change in the AtFMN/FHy enzymatic activity, or because of a change in regulation of the riboflavin biosynthesis pathway, or both. To accomplish this objective, we will:

(a) Test if the Melinoe mutation causes the riboflavin kinase domain to be less functional in vivo by determining if the Arabidopsis Melinoe mutant can be rescued with the riboflavin kinase domain of AtFMN/FHy. (b) Test if the AtFMN/FHy protein may have a moonlighting non-enzymatic role as a regulator of flavin biosynthesis by:

- Determining if overexpression of the FMN hydrolase domain carrying the Melinoe mutation confers similar phenotype to the wild type plants as the Melinoe mutation in the wild type gene.
- Determining if changes exist in the transcriptional regulation and enzyme activity of enzymes on the riboflavin biosynthesis pathway in siliques and mature seeds in the Melinoe mutant vs. wild type plants.
- Discovering proteins that interact with the wild type AtFMN/FHy and the Melinoe mutant in siliques and seeds.