

**Guidelines for MAFMA Final Report**  
Final Reports due 3 months after completion of project

Project Title Development of Phenylalanine-free Zein Protein in Transgenic Soybean as a Value-added Trait for PKU Patients

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Co-PI (s) \_\_\_\_\_

Academic Institution: Kansas State University

Award Date July 15, 2006

**Please complete all questions below and attached form**

1. Objective Summary (1-2 sentence summary)

A) Continue to analyze the protein expression of our transgenic soybean lines and advance these transgenic lines to successive generations

B) Refine protein extraction methods and begin large scale extraction of the zein protein from soybean seed.

2. Objective Accomplishments

(If objectives were not met, what extenuating circumstances contributed to that factor?)

Convey all of your progress on this project including that obtained with the industry and other matching funds.

Originally our proposal called for a two year funding cycle where in Year 1 we would continue to analyze the protein expression of our transgenic soybean lines and advance these transgenic lines to successive generations and in Year 2 we would refine protein extraction methods and begin large scale extraction of the zein protein from soybean seed. As funding was only for Year 1 we did not make significant progress on Objective B, the large scale extraction of the zein protein.

Four T1 transgenic soybean lines have been grown in the greenhouse to establish homozygous for progeny analysis and for seed increase. Two of these lines contain the native gamma zein gene and two contain the PHE-free gamma zein gene. Several individual seeds from each line were planted, DNA was extracted, and PCR analysis was performed for a segregation analysis. Individual plants that tested positive for the selection gene and the gamma zein gene were grown to maturity. Immature seeds were harvested from each line and PCR analysis identified the homozygous lines. Immature seeds were also taken from these plants and protein was extracted for western blot analysis. Both the native gamma zein and PHE-free lines are expressing the transgenic protein. Homozygous plants from each of the transgenic lines have been identified and are at various stages of maturity. T3 generation of the native zein lines and

T2 generation of the modified zein lines have been obtained. We were unable to plant in the field this summer for seed increase as our seed harvest due to the lateness in identifying homozygous lines with our spring greenhouse crop. However we currently have one greenhouse dedicated to seed increase for these lines.

Rabbit polyclonal antibodies have been made to the gamma zein protein to aid in the detection of the protein as we refine the extraction methods. This antibody reacts to both the native gamma zein as well as the modified PHE-free gamma zein. Seeds from T3 homozygous native zein lines and seeds from T2 homozygous modified zein lines were ground into flour and used for protein extractions. Protein extractions using the procedure described by our lab (Li, Z., et al. 2005. *Molecular Breeding* 16:11-20) were performed on our transgenic lines. Transgenic lines (both the native and the MDZ gene expressing lines) continued to have a significant increase in alcohol soluble protein with a 5- to 20-fold increase compared to non transgenic control seeds. Typical yield from a one gram sample was 300 mg of alcohol soluble protein. This fraction was sent out for amino acid analysis however the results were not available in time for this report.

3. Unexpected findings, if any

No unexpected findings have been encountered.

4. Practical impacts of research efforts. Include: implementation of accomplishments by industry partners (if any), identification of economic impacts, and any further pursuit by PI of research in area of this project whether MAFMA or not.

- a. Short Term Impacts
- b. Long Term Impacts

PKU is a genetic disease that if left unchecked, phenylalanine accumulates at high levels in the blood and will lead to mental retardation. Individuals diagnosed with the disorder must maintain a strict life-long diet low in PHE. The current diet of PKU patients consists of a malodorous and distasteful medical formulation of dietary amino acids supplemented with foods low in protein and phenylalanine. The use of soybean-derived PHE-free protein would replace the malodorous and distasteful medical formulas and help patient to have strict low phenylalanine diet. It is estimated that over 28,000 people are affected with PKU in the US and this **PKU diet supplement market is estimated to be 84 to 168 million dollars annually and offers a new opportunity for soybean producers.** Aside from the medical and nutritional benefits, Kansas agriculture would benefit from our proposal by creating a new market for soybean commodity. Additionally, this type of research will have a tremendous impact in the public acceptance of GM crops because there is a direct consumer benefit.

5. If you are also making reports to other funding agencies in the course of this research work, please include a copy of that report.

6. a. If any publications resulted from the research, a copy must be included. Please note we were notified by the USDA/CSREES National Program Leader for the Midwest Advance Food Manufacturing Alliance (MAFMA) that all publications resulting from research that was funded

by MAFMA must include the following wording **“The project was supported by the USDA Cooperative State Research, Education and Extension Service, special research grant number 200X-34328-xxxxx.**

No publications were submitted at the time of this report.

**b. If any patents (pending or granted) resulted from the research, please include the patent information.**

No patent were filed.