Research project update of "Phenylalanine-free Protein Produced in Genetically Engineered Soybean"; Harold Trick, Department of Plant Pathology, Kansas State University (2/28/07)

One of the projects in my laboratory is produce a phenylalanine (PHE)-free protein in transgenic soybean as a source of dietary protein free of phenylalanine. This project is partially funded through MACPAD and the PKU Organization of Illinois and I would like to give a short update of our progress for the last six months.

Several individual plants from four 1st generation transgenic soybean lines have been grown in the greenhouse to establish homozygous lines, for progeny analysis, and for seed increase. Two of these lines contain the native gamma zein gene and two lines contain the PHE-free gamma zein gene. Molecular analysis was performed on these plants where DNA was extracted and the polymerase chain reaction (PCR) helped segregate the plants which contain the transgenes. Individual plants that tested positive for the selection gene and the gamma zein gene were maintained to the greenhouse for seed collection. Many of these plants are currently close to maturity and will be harvested later this spring. In addition, immature seeds were harvested from each line and PCR analysis was used to identify the homozygous plants. 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 appear to be expressing the transgenic protein and homozygous plants from each of the transgenic lines have been identified.

Mature seeds from each of these plants will be used for additional seed increase, for amino acid analysis, and to begin the refinement of our protein extraction procedures. We anticipate performing amino acid analysis on the mature seeds as well as refining our extraction techniques of the zein protein from endogenous soybean protein later this spring into the summer. A notification to Biotechnology Regulatory Services (the office of USDA which implements the regulations for genetically engineered organisms) was sent informing them we intend to increase these lines in the field this summer. (This step is necessary to comply with federal regulations concerning field testing of transgenic crops.) By increasing seeds in the field we will be able to increase to amount of seed in greater amounts than just growing in the greenhouse.

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.

Seven new transformation experiments were also initiated in the fall of 2006. The purpose for these experiments is to create additional lines which may boost the amount of PHE-free zein protein in the soybean seed. Two different versions of the modified PHE-free protein were used in these transformations with one containing an additional DNA sequence which will facilitates protein extraction from the seed. Currently these cultures are under selection.