Eliminating Cross Contamination of Maize With 'BIOTECH Corn' Pollens To Add Food Safety and Boost the Economy

Project GREEEN No.: GR04-039

Team Leader: Mariam Sticklen, MSU Dept. of Crop and Soil Sciences

Statement of Challenge

Safety is an issue for all modern technologies, regardless whether it is an auto technology, information/internet technology or biotechnology. For example, appropriate road signs keep automobiles safely on the pavement and the most sophisticated software prevent access to private information transmitted online. Safety of transgenic crops is still lagging while consumer concerns are skyrocketing. The main public/consumer concern is whether non-transgenic crops could stay away from being contaminated with transgenes. This PI decided to transfer the *Arabidopsis* Flowering Locus C (*flc*) gene in plant to test her hypothesis of delaying flowering to bypass the normal corn pollination time. The *flc* gene encodes a novel protein that acts as a repressor of flowering. This gene has been cloned and characterized and confirmed causing delay in flowering after it was transferred to an early-flowering *Arabidopsis*. Using this gene, this PI planned to assure consumer confidence for human safety by delaying the corn flowering time so the pollens of transgenic plants would not cross contaminate the other cross breedable crops in the fields.

Objectives

The goal of the study was to test the hypothesis of bioconfining the pollens of BIOTECH corn by 3-4 weeks of delay in flowering time. To test this hypothesis, the PI tested her concept through the objectives listed below:

1. Transformed the model plant (tobacco) and co-transformed corn with the *flc* and the *bar* selectable marker (BASTA resistance) gene driven by a strong promoters using the Biolistic® device.
2. Selected for herbicide resistant plantlets, grew plants and confirmed the integration and expression of the *flc* transgene in plants.
3. Confirmed the level of production of FLC enzyme, and delay in flowering time of the transgenic plants as compared to non-transgenic plants.

Results and Accomplishments

This project has been completed and two grants for total of $300,000 were awarded to the PI ($50,000/6 month, and $250,000/24 months) from DOE through Edenspace Limited LLC. Four refereed publications (see below) and a licensing with a private sector also resulted from this Project GREEEN one-year funds.

Resulting Refereed Publications

1. Salehi H., Ransom C., Oraby H., and Sticklen MB. Delay in flowering and increase in biomass of plants expressing the Arabidopsis floral repressor gene FLC (FLOWERING LOCUS C). J. Plant Physiology. 2005. In press.
2. Salehi H., Seddighi, Z., C. Ransom, H. Oraby, R. Ahmad and M. Sticklen (2005). Convergence of goals: Expression of flowering locus C in an *Acidothermus cellulolyticus* endo-1,4-*β*-D-glucanase (E1) transgenic tobacco (*Nicotiana tobacum* L.) and its effect on delay in flowering, increase in biomass and phytoremediation. Applied Biochemistry and Biotechnology Submitted.
3. Sticklen M. B. and H. Oraby (2005). Shoot apical meristem: A sustainable explant for genetic engineering of cereal crops. In Vitro Cellular & Developmental-PLANT. In press.
4. Kirk T., Carlson J., Ellstrand N., Kapuscinski, Lumpkin T., Magnus D., Nester E., Peloquin J, Snow A., Sticklen M., and Turner P. (2004). Biological Confinement of Genetically Engineered Organisms. NRC Report. The Natl. Acad. Press. Washington, DC. 255p.

Invited speeches at other institutions and conferences

Oct. 2004	The United States National Academies Workshop on Global Challenges for Guiding and Managing Biological Technologies: "Opportunities to Improve Food Security and Human Nutrition: Coping with Challenges of Human Health in Developing Nations." The U.S. Natl. Academies Bldg. Washington, DC. USA.
Sept. 2004	The 2nd Ukrainian Workshop on Biofuels and Biobased Industrial Material: Production of Biofuel Ethanol and PHB Biodegradable Plastic from Transgenic Biomass Crops. The Ukrainian Natl. Academies Bldg. Kiev, Ukraine.
Aug. 2004	MSU Genetics Program Student Orientation. "Cell and Molecular Genetics Improvement of Cereal Crops."
July 2004	United States Department of Agriculture/Agricultural Research Center, Albany, CA, USA. "Evolution in development of safe GM crops: New molecular tools for biological confinements to protect human health and environmental integrity."
Feb. 2004	Consortium for Plant Biotechnology Research Annual Conf., Washington, DC. "Production of valuable proteins from transgenic maize."
March 3, 2005	Annual meeting of the Consortium for Plant Biotechnology Research (CPBR), Washington DC. "Increasing the crop biomass through delay in flowering via genetic engineering"
March 10, 2005	Oxford University, Oxford, U.K. "Producing the Microbial Hydrolysis Enzymes within the Biomass Crops via Genetic Engineering" Department of Plant Sciences,
May 2, 2005:	Genetics Program Forum, MSU, East Lansing, MI. "Convergence of goals: Genetically engineering of biomass crops for biofuels and for phytoremediation."

May 4, 2005. 27th Symposium on Biotechnology for Fuels and Chemicals... Denver, CO. "The Development of Transgenic Biomass Crops for Renewable Energy and Cleaner Environment."

Technical Results

The PI laboratory transformed both tobacco as a plant model system with the *flc* and co-transformed the corn with a combination of flc and bar genes controlled by 35S promoter and Nos terminator. Results on production of FLC-transgenic tobacco showed up to 36 days delay in flowering and a significant increased in crop biomass. When plantlets were small, we used the Polymerase Chain Reaction and confirmed the presence of the *flc* gene in the plant genome. Southern blots were performed to confirm the integration and the copy number of *flc* transgene in these plants. To confirm the level of translation product of the *flc* gene in plants, we performed western blot analysis. We grew the T0 and T1 plants in our greenhouses, collected sets of data on delay in flowering, increase in biomass and in fact uptake of the lead by transgenic plants. Results of these data collections are reported in the above refereed publications.

What has changed because of this proposal

This project has proven to have potentials to change the public concerns on cross contamination of GM crops with non-GM crops in the fields.

Impacts

Over 36% of corn crop is BIOTECH (GMO) corn. The PI has proven that one could block the transfer of transgenic pollens to non-transgenic crops simply by 3-4 weeks of delay in flowering of transgenic corn.

Summary Statement

The PI and her team were able to prove that one could indeed transfer a single delay in flowering gene in BIOTECH crops, and delay the flowering of corn by up to 36 days. This delay is the simplest method of bioconfinement in order to block the cross contamination or transfer of transgenic pollen grains to other corn plants in the field.

Funding Partnerships

1. DOE STTR Phase I: $50,000 (6 month) to PI. June 2004 to Dec 2005.

2. DOE STTR Phase II: $250,000 (24 month) to PI effective July. 1, 2005 to June 31, 2007.

a

b

c

Figure 2. *FLC* transgenic tobacco plants delayed flowering 2 or more weeks. a) Right plant is *FLC* transgenic and left plant is untransformed control. b) Plants from line 4 (right) compared to control plants (left). c) *FLC* transgenic versus control flowers from the same age. (A = anther, S = stigma). Note the pollen grains on control anthers and stigma.