

## CRADA Final Report

v2010 Aug 24

**Date:** February 4, 2013

**PI :** Tamas Torok, Ph.D.

**CRADA No.** UFCRA006535

**LBNL Report Number** \_\_\_\_\_

- Parties:** Pioneer Hi-Bred International, Inc. A DuPont Company  
P.O. Box 1000  
7100 NW 62<sup>nd</sup> Avenue  
Johnston, IA 50131-1000  
Attn: Ms. Amy Cigan,  
Mgr. Intellectual Property Licensing and Management  
Email: [amy.cigan@pioneer.com](mailto:amy.cigan@pioneer.com)

Lawrence Berkeley National Laboratory  
One Cyclotron Rd. MS. 70A3317  
Berkeley, CA 94720  
Attn: Tamas Torok, Ph.D.  
Email: [ttorok@lbl.gov](mailto:ttorok@lbl.gov)

### 2. Title of the Project

**“Novel enabling technologies of gene isolation and plant transformation for improved crop protection”**

### 3. Summary of the specific research and project accomplishments

Meeting the needs of agricultural producers requires the continued development of improved transgenic crop protection products. The completed project focused on developing novel enabling technologies of gene discovery and plant transformation to facilitate the generation of such products. The project had three main components:

- Investigated native chloroplast promoters from different species by testing their ability to drive the expression of a reporter gene in heterologous systems;
- Tested promoter-less vector constructs for the lox-mediated expression of transgenes;
- Screened diverse plant species for the detection of insecticidal, fungicidal, and antiviral activities.

To perform the scheduled screening the following main tasks were carried out: scale-up of growing plant material; developed sample preparation protocols for accumulation and storage of plant tissues; grew, accumulated, and freeze-dried plant tissues; optimized subsequent protein extraction protocols; searched for insect, fungal, and

viral species that satisfied the screening conditions; developed and performed protocols for antifungal, antiviral, and insecticide tests.

All stages planned for the 2-year project were performed under the Work Schedule. The scientific results obtained in the course of experiments represent the basis for further investigations in this field of research.

#### 4. Deliverables

The completed CRADA built upon a DOE/NNSA-funded joint research project between LBNL and two institutes in Ukraine; the Institute of Cell Biology and Genetic Engineering (ICBGE) in Kyiv and the Ukrainian Anti-Plague Research Institute (UAPRI) in Odesa. Scientific results obtained in the project were transferred to Pioneer Hi-Bred International for further investigation and application in crop protection. All tasks and milestones were jointly established and progress reviewed between LBNL, the two Ukrainian institutes, and Pioneer during site visits and monthly SKYPE sessions. LBNL and Pioneer jointly approved the delivered reports, including the project's Final Report.

<b>Deliverable Achieved</b>	<b>Party (LBNL, Participant, Both)</b>	<b>Delivered to Other Party?</b>
Constructed promoter-less vectors for crop species	both	yes
Supplied protocols for protoplast isolation and proliferation from soybean and canola	both	yes
Tested promoter-less constructs that yielded results with a second reporter gene	both	yes
Supplied protocols for chloroplast expression regulation	both	yes
Transformed crop species with promoter-less vectors	both	yes
Isolated promoters from crop species and made new constructs	both	yes
Developed protocols for transgenes in plastid genomes	both	yes
Propagated 2,000 tissue samples for protein extraction and activity screening	both	yes
Prepared a Final Report	both	yes

**5. Identify publications or presentations at conferences directly related to the CRADA?**

V Lozitsky, A Fedchuk, T Grydina, L Mudryk, L Shitikova, L Socheslo; VIRUSCYDAL ACTIVITY OF CALLUS EXTRACTS FROM TOBACCO PLANTS – Antiviral Research, 2011. vol.90, A57-58.

V. Lozitsky, A. Fedchuk, T. Grydina, L. Mudryk, L. Shitikova, L. Socheslo, N. Kuchuk, V. Belokurova, T. Torok; ANTI-INFLUENZA ACTION of CALLUSES from TOBACCO-PLANTS - <http://www.themacraegroup.com/2012-symposia.php> , poster presentations, P41.

V. Lozitsky, A. Fedchuk, T. Grydina, L. Mudrik, L. Socheslo, N. Kuchuk, V. Belokurova, T. Torok; ANTIVIRAL ACTION of CALLUS EXTRACTS and PROTEOLYTIC INHIBITORS of PLANT ORIGIN - [http://www.isar-icar.com/resource/resmgr/docs/final\\_program\\_2012.pdf](http://www.isar-icar.com/resource/resmgr/docs/final_program_2012.pdf)

**6. List of Subject Inventions and software developed under the CRADA:**

N/A

**7. A final abstract suitable for public release:**

Meeting the needs of agricultural producers requires the continued development of improved transgenic crop protection products. The completed project focused on developing novel enabling technologies of gene discovery and plant transformation to facilitate the generation of such products.

We scaled-up the methods of growing plant material; developed sample preparation protocols for accumulation and storage of plant tissues; grew, accumulated, and freeze-dried plant tissues; optimized subsequent protein extraction protocols; searched for insect, fungal, and viral species that satisfied the screening conditions; developed and performed protocols for antifungal, antiviral, and insecticide tests.

All stages planned for the 2-year project were performed under the Work Schedule. The scientific results obtained in the course of experiments represent the basis for further investigations in this field of research.

**8. Benefits to DOE, LBNL, Participant and/or the U.S. economy.**

Even the most developed countries lose about 20% of their agricultural crops to pests. The novel enabling technologies developed in this project will be used to generate transgenic crop plants that themselves will produce crop protecting natural products.

**Benefit to DOE**

The completed CRADA evolved from a DOE/NNSA-funded non-proliferation program joint research project between LBNL and two institutes of interest in Ukraine. DOE/NNSA (NN-24) runs the Global Initiatives for Proliferation Prevention (GIPP) program. The program re-trains former Soviet weapons of mass destruction (WMD)

workers and introduces them to peaceful projects and the international scientific community. A mission-relevant proposal (LBNL-T2-0221-UA) was submitted to the GIPP program and passed its review process. Inclusion of Pioneer Hi-Bred International, a DuPont company was the follow-up and the basis for this CRADA.

**Benefit to Participant**

Pioneer Hi-Bred International, a DuPont company, is the worldwide leader of seed production for major crop plants. The company is also dedicated to R&D in protecting crop plants from pests. The novel enabling technologies developed in this project benefit Pioneer since they have first access to any new IP.

**Benefit to US Taxpayer**

Even the most developed agriculture such as the US agriculture loses about 20% of the crop to pests and diseases. Leading seed and agricultural companies constantly improve their seeds and technologies to lower these losses. Collaborating with Ukrainian researchers, who have pioneered numerous protocols for the generation of transgenic plants benefits the US taxpayer by lowering the costs of these novel enabling technologies and replacing the use of synthetic chemicals in crop protection, while the projects supports DOE mission and contributes to US national security.

**9. Financial Contributions to the CRADA:**

DOE Funding to LBNL	\$186,000
Participant Funding to LBNL	\$0
Participant In-Kind Contribution Value	\$690,000
Total of all Contributions	\$1,340,000

## **DISCLAIMER**

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

DE-AC02-05CH11231