

ASRF Research Project Annual Report 2006
(Year 1)

Project Title: *Molecular Mechanisms of Seed Germination and Seedling Establishment*

Principal Investigators: Hiro Nonogaki, PI, Department of Horticulture, Oregon State University; Ruth Martin, Co-PI, USDA-ARS, National Forage Seed Production Research Center, Corvallis, Oregon

Overall Objectives:

Elucidate the molecular mechanisms of seed germination and seedling establishment to understand the early events in plant production. Identify and characterize key transcription factors and other regulatory factors, which play potentially important roles in seed dormancy, germination and seedling establishment in Arabidopsis. The information obtained from Arabidopsis will be transferred to tomato, a model species for seed germination research. For production and quality control of high performance seeds, it is essential to gain a better understanding of the mechanisms controlling seed development. This basic research project aims to provide information for the future of seed biology and business.

Specific Objectives:

Year 1: Identify multiple genes associated with seed dormancy, germination and seedling establishment from Arabidopsis enhancer-trap lines

Year 2: *Characterize expression patterns and biological functions of seed-associated genes in Arabidopsis*

Year 3: *Transfer the information obtained from Arabidopsis to tomato seeds*

Research Accomplishments:

The *Seed-GUS-Expression* enhancer-trap library that we donated to Arabidopsis Biological Resource Center has been used to identify seed dormancy, germination- and seedling establishment-associated genes. We have conducted genome-walking PCR to locate the T-DNA insertions in lines showing reporter gene expression in germinating and germinated seeds. The PCR products amplified by genome-walking PCR were sequenced and compared to the Arabidopsis genomic sequence to identify the position of the T-DNA inserts. The candidate genes whose enhancers were trapped were identified using the *SIGnAL T-DNA Express Arabidopsis Gene Mapping Tool* (<http://signal.salk.edu/cgi-bin/tdnaexpress>) (see examples in Fig.1).

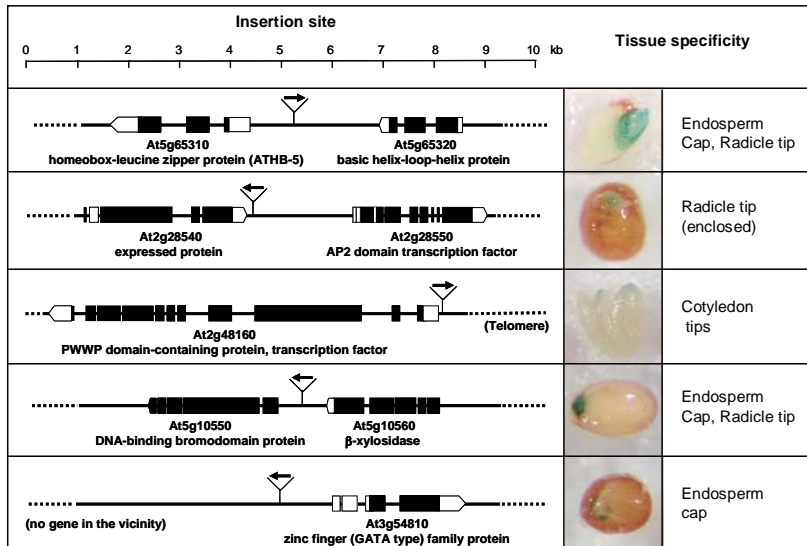
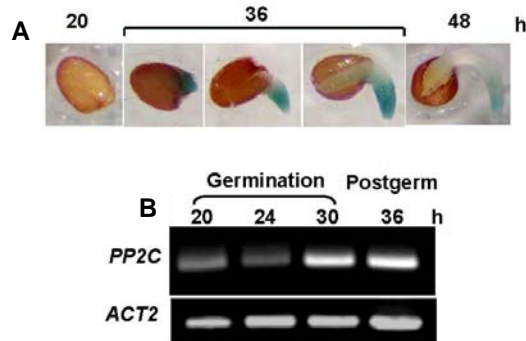


Figure 1. Identification of seed genes. Examples of the T-DNA insertion sites identified in the *Seed-GUS-Expression* lines by genome-walking PCR are shown. Photographs indicate tissue-specific reporter gene (*GUS*: β-glucuronidase) expression patterns in seeds. Genes responsible for reporter gene expression were identified by using the *SIGnAL T-DNA Express Arabidopsis Gene Mapping Tool* database.

For those lines that had genes successfully identified by genome-walking PCR, T-DNA insertion sites were verified using a T-DNA specific primer and a second primer designed based on the genomic sequence flanking the insertion locus. The presence of the T-DNA insertions at the corresponding positions of the genomic DNA in these lines was confirmed. The expression of the candidate gene in wild-type *Arabidopsis* seeds was analyzed by RT-PCR (see example in Fig. 2). We are currently extracting genomic DNA from other lines in the *Seed-GUS-Expression* population and are conducting genome-walking PCR to identify other genes important in seed development.

Figure 2. Expression of the protein phosphatase 2 C (PP2C) gene identified in the TMH1 enhancer-trap line. **A.** GUS reporter gene expression in the TMH1 enhancer-trap seeds. **B.** Expression analysis of the PP2C gene during and following germination of wild-type seeds as detected by reverse transcription (RT)-PCR. *ACTIN2* (*ACT2*) gene is used as control.



Educational contribution:

As planned in the original proposal, the budget has been used to support Po-Pu Liu, a Ph. D. student in the Department of Horticulture, Oregon State University who is taking a lead role in the enhancer-trap project. In addition to the research accomplishments mentioned above, the ASRF fund is also contributing to the career development of this young scientist. Po-Pu Liu will be taking his preliminary examination to become a Ph. D. candidate later this month. He has been awarded an American Society of Plant Biologists (ASPB) travel grant and invited to give an oral presentation at the ASPB meeting in August, 2006 in Boston. He will be presenting results from this ASRF-funded project at this meeting. The ASRF support will be acknowledged during his slide presentation.

Promotion of the ASRF Project in the International Seed Community:

We donated the *Seed-GUS-Expression* library to ABRC to provide these useful research tools to the international seed research community. ABRC has produced and made seed available to the research community. To promote this activity further, the PIs, Hiro Nonogaki and Ruth Martin attended the 8th International Workshop on Seeds (Brisbane, Australia). In addition to the invited talks at the meeting, they also organized a special session to introduce the *Seed-GUS-Expression* library and provided technical support to seed researchers in the community. This has promoted international collaboration for the research using these materials. Multiple seed groups including Dr Henk Hilhorst's Lab, Wageningen University, The Netherlands; Dr. Gerhard Leubner's Lab, University of Freiburg, Germany; and Dr. Oscar Lorenzo's Lab, University of Salamanca, Spain are using the enhancer-trap lines. The PI, Hiro Nonogaki, has been invited to the University of Angers, France for three weeks (June 2-24, 2006) to further develop collaborative research projects. Thus, this ASRF project is also contributing to international collaborations.

Publications:

- Liu P-P, Koizuka N, Martin RC, Nonogaki H. 2005. The *BME3* (*Blue Micropylar End 3*) GATA zinc finger transcription factor is a positive regulator of *Arabidopsis* seed germination. *The Plant Journal*, 44: 960-971
- Liu P-P, Koizuka N, Homrichhausen TM, Hewitt JR, Martin RC, Nonogaki H. 2005. Large scale screening of *Arabidopsis* enhancer-trap lines for seed germination-associated genes. *The Plant Journal* 41: 936-944
- Liu P-P, Martin RC, Hewitt JR, Koizuka N, Homrichhausen T, Nonogaki H. 2006. A '*Seed-GUS-Expression*' enhancer-trap library for germination research. In S. Adkins, S. Ashmore, S. Navie, eds, *The Biology of Seeds: Recent Research Advances* (tentative title), CAB International, Wallingford, UK (accepted)