

Systematically Assigning Gene Functions in Soybean Employing RNAi Technology

Submitted by Zhanyuan Zhang, University of Missouri

Zhang: zhangzh@missouri.edu

IMBA Project 2007-4
Project Description

Introduction

This project is a continuation of IMBA project 2006-3.

Specific Objectives

1. Develop and construct dsRNA expression cassettes which allow efficient silencing of soybean genes.
2. Construct plant transformation vectors that enable efficient RNAi technology and a high-throughput cloning process.
3. Develop transgenic soybean lines showing RNAi..
4. Characterize transgenic soybean lines to evaluate the silencing efficiency of various RNAi constructs.

Objectives 1 and 2 were addressed under IMBA project 2006-3. This project will concentrate on Objectives 3 and 4.

Procedure for Objective 3

The researcher will use an improved, efficient *Agrobacterium*-mediated transformation system, developed in his lab, for producing transgenic soybean lines (Zhang et al., U.S. probationary patent is filed). This system employs a cell-cycle gene expression cassette as key elements for enhancing T-DNA transfer. This system was derived from his previously published protocol employing public soybean genotype as starting materials (Zeng et al, 2004).

Public soybean genotypes, especially Williams 82 and Jack, are the primary soybean genotypes used to derive soybean ESTs. Therefore, use of these genotypes as transformation materials will assure a high degree of sequence homology between soybean endogenous genes to be silenced and the reverted repeats of dsRNA we choose from soybean ESTs. This high degree of homology will assure a high probability of achieving efficient RNAi.

Procedure for Objective 4

All primary (T) transformants and some of their progeny (T2) will be analyzed using PCR which will be further confirmed by Southern blot (Southern, 1975). RT-PCR and/or a modified Northern blot analysis (Zhang et al., 1998) will be used to analyze down regulation of target genes. PCR will confirm the organization of the expression cassettes integrated into the soybean genome. Southern blot will confirm transgene integration and copy number per genome and confirm PCR detection of the presence of transgenes. RT-PCR or Northern blot will estimate RNAi efficacy. The researcher will determine if there is any correlation between the degrees of gene silencing caused by dsRNA and the number of endogenous gene duplicates to be silenced.

For detecting short interference RNA (siRNA, 21-24bp) present in RNAi lines, a modified Northern blot analysis will be used which was devised to detect siRNA effectively (Hamilton et al., 2002).

Phenotypic analyses will be conducted on both primary (T1) and progeny (T2) RNAi lines. Specific assay approaches are designed to analyze the phenotypes. For example, morphological observations will be made on flower organs and hairy roots, respectively. Five to ten progeny (T2) plants derived from each T1 line displaying efficient RNAi will be analyzed. This progeny analysis will allow the researcher to evaluate transgene inheritance, stability of efficient RNAi, and any correlation between RNAi and transgene dosages. Data for quantitative traits will be analyzed statistically at a 0.05% significant level using Duncan Multiple Range Tests.

Impact

Genome-wide functional assignment of soybean genes employing RNAi technology and ESTs will validate useful genes that will be valuable resources immediately available for genetic improvement of soybean for value-added traits. The information obtained from this investigation will be relevant and useful to explore gene functions in other crop species as well.