

Systematically Assigning Gene Functions in Soybean Employing RNAi Technology

Submitted by Zhanyuan Zhang, University of Missouri

Zhang: zhangzh@missouri.edu

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Introduction

Soybean is used as a model for studies on seed physiology and biochemistry as well as plant-microbial interactions. RNA interference technology, simply called RNAi, has been shown to be an efficient tool to assign gene functions systematically in various organisms and has promise for soybean genomics. The RNAi approach, when coupled with Gateway cloning technology and efficient soybean transformation process, would allow systematic functional assignment of soybean genes. This project is a continuation of IMBA project 2006-3.

Objectives

The specific objectives of this project year are: 1) Develop transgenic soybean events expressing RNAi and 2) Characterize RNAi soybean events to evaluate silencing efficiency.

Procedures

Additional transgenic soybean events were developed for expressing RNAi of the candidate genes discussed in previous report. These events were subjected to herbicide leaf-painting assay, PCR and Southern blot analysis. The PCR results were consistent with Southern blot in detecting the presence of transgenes in primary (T0) transgenic plants. Based on this consistency, we used PCR screening as a robust and cost-effective way of confirming the integrity of the RNAi cassette including the presence of two inverted repeats, intron-spacer, promoter and terminator. Events with such integrity were further analyzed by qRT-PCR to detect the level of target mRNA. Phenotypic observations were made for those events which showed developmental abnormality due to the disruption of the target transcripts.

Impact

Large-scale functional analysis of soybean genes employing RNAi technology and ESTs will verify the functions of numerous useful genes whose deployment will help analyze the functions of other genes or pathways as well as improve soybean traits. However, only when effective RNAi is developed, can this technology become practically useful. To date we have evaluated the impact of MARs, the lengths of inverted repeats, and sequence homology of intron-spacer on RNAi efficacy. The information obtained from this project year has been critical for achieving effective RNAi.

Publications

Flores T, Karpova O, Su X, Zeng P, Bilyeu K, Sleper D, Nguyen H, Zhang Z. 2008 Silencing of GmFAD3 gene by siRNA leads to low α -linolenic acids (18:3) of fad3-mutant phenotype in soybean [*Glycine max* (L.) Merr.] Transgenic Research (online first: DOI 10.1007/s11248-008-9167-6).

Karpova, Olga V.; Kennon Angela; Su, Xiujuan; Zhou, Liwen; Zhao, Changzeng; and Zhanyuan Zhang. 2008. Optimization of a high-throughput transformation vector for gene silencing in soybean. Soy, July 2008. Indianapolis, IN.