Expression in Rice Genes that Inactivate *Fusarium Mycotoxins*

Sun-Hee Woo*, Arisa Nishiyama-Higa[1], Makoto Kimura[1], Se-Young Kim, Hong-Sig Kim[2], Sang-Il Park[2], Seok-Young Son[2], Young-Mok Park, Seung-Keun Jong[2], Isamu Yamaguchi[1], Yong-Gu Cho[2]

Proteome analysis team, Korean Basic Science Institute, Daejeon 305-333, Korea
[1]Lab. of Remediation Research, Plant Science Center, The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako, Saitama, 351-0198 Japan
[2]Department of Agronomy, Chungbuk National University, Cheongju, 361-763, Korea

Objectives

As a first step to generate transgenic crops with a mycotoxin degrading activity, we transformed the model monocot, *Oryza sativa*, with this vector and obtained a couple of transgenic rice plants.

Materials and Methods

For expression of the trichothecene resistance gene, two vectors were constructed (Fig. 1A: top and middle): (1) *pAct1-Tri101*, in which the resistance gene is placed under constitutive and powerful rice *Act1* promoter-intron and (2) *pAct1-spTri101*, which contains an *N*-terminal signal peptide sequence of a wheat acidic beta-1,3-glucanse gene (Glb3; AF112967) fused to 5' end of *Tri101*. Total RNA was isolated with the RNeasy Plant Mini kit (Qiagen). Oligo(dT) primed cDNA was synthesized with the Superscript First Strand Synthesis System (Invitrogen) and used for reverse transcription (RT)-PCR.

Results and Discussion

Trichothecenes are a group of sesquiterpenoid secondary metabolites that inhibit protein synthesis in eukaryotes. They are produced by phytopathogenic *Fusarium* species (*e.g.*, *F. graminearum*) that cause head blight (FHB) of wheat, barley, and maize. The fungus also produces another mycotoxin, zearalenone, that shows potent oestrogenicity in humans and livestock. Since the mold-infected cereal plants accumulate much trichothecenes and zearalenone in grains, FHB poses threats of mycotoxin contamination of agricultural products. Trichothecene 3-*O*-acetyltransferase (encoded by *Tri101*) inactivates the virulence factor of the cereal pathogen *Fusarium graminearum*. Zearalenone hydrolase (encoded by *zhd101*) detoxifies the oestrogenic mycotoxin produced by the same pathogen. These genes were introduced intoa model monocotyledon rice plant to evaluate their usefulness for decontamination of mycotoxins. The strong and constitutive rice *Act1* promoter did not cause accumulation of TRI101 protein in transgenic rice plants. In contrast, the same promoter was suitable for transgenic production of ZHD101 protein; so far, five promising T0 plants have been generated. Low transgenic expression of *Tri101*was suggested to be increased by addition of an ? enhancer sequence upstream of the start codon.

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* Corresponding author: Sun-Hee Woo, TEL:042-865-3424, E-mail:woosh322@kbsi.re.kr

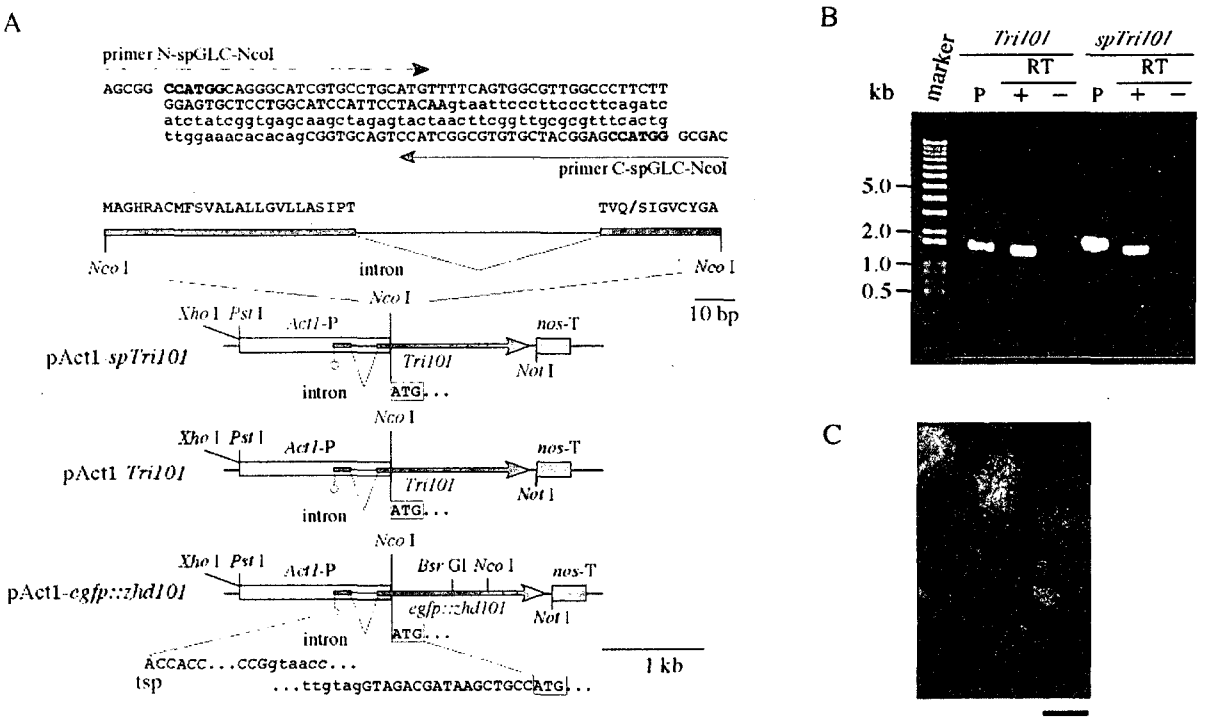

Fig. 1. Vectors used for the mycotoxin inactivation and their transient expression in wheat Calli.

(A) Trichothecene resistance and zearalenone detoxifying vectors. Rice *Act1* promoter-first intron segments (*empty box*) are labeled as "*Act1-P*" and nopaline synthase terminator (*closed box*) as "*nos-T*". The *Act1* non-coding first exon/intron sequence enhances transcription of the fused downstream gene in monocotyledonous systems. Open circles indicate the reported transcription start point (*tsp*) of the *Act1* gene.9) Translation start codons are boxed. Top: Structure of pAct1-*spTri101*. Genomic DNA sequence of a signal peptide from wheat acidic -glucanase (*Glb3*)10) is shown above the vector map. Large and small letters (which corresponds to thick and thin lines) represent exons and introns, respectively. The translated signal peptide sequence is shown above the thick lines and the processing site is indicated by a slash. Primers N-spGLC-NcoI and C-spGLC-NcoI (marked by arrows) were used for amplification of the *Glb3* signal sequence from wheat genomic DNA (*i.e.*, the signal peptide portion contains an intron derived from *Glb3*). *Nco* I recognition sequences (shown in *bold letters*) were created on both sides of the amplified fragment. This PCR product was inserted at the *Nco* I site of pAct1-*Tri101*. Middle: Structure of pAct1-*Tri101*. The internal *Nco* I site of *Tri101* was eliminated by a synonymous A to T mutation at position 296 (AB000874) by site directed mutagenesis. Bottom: Structure of pAct1-*egfp::zhd101*. The *Bsr* GI-*Not* I segment of pEGFP-1 (Clontech) downstream of *egfp* was replaced with a synthetic *Srf* I polylinker comprising of 5'-GTACAGGCCCGGGCCGC-3' and 5'-GGCCGCGGCCCGGGCCT-3' to generate pEGFP-SrfI. Vent DNA polymerase (New England Biolabs) was used for amplification of *zhd101* with primers GFP-zhd-5' (5'-ATGCGCACTCGCAGCAC-AATCTCG-3') and GFP-zhd-3' (5'-TGTACCGTTCAAAGATGCTTCTGC-3'), and the amplified fragment was inserted at the *Srf* I site of pEGFP-SrfI in the correct orientation to generate pEGFP-*zhd101*. *Tri101* in pAct1-*Tri101* was replaced with the *Nco* I-*Not* I fragment of *egfp::zhd101* fusion in pEGFP-*zhd101*. (B) Transient expression of *Tri101* and *spTri101* in wheat callus cells as assessed by RT-PCR. Two g of vector was precipitated on 0.6 mg of gold particles and used for each bombardment. Total RNA was isolated two days after the bombardment and the synthesized cDNA was used for RT-PCR. "P" indicates amplification directly from the bombarded vector. DNA size markers (1kb Plus DNA Ladder, Invitrogen) are indicated on the left. (C) Transient expression of *egfp::zhd101* in the bombarded calli monitored by fluorescence stereomicroscope (Leica MZ12). Filter sets GFP-Plus (460-500 nm excitation, 505 nm emission, and > 510 nm emission barrier) were used.