A model transgenic cereal plant with detoxification activity for the estrogenic mycotoxin zearalenone

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Abstract

Zearalenone (ZEN) is an estrogenic mycotoxin produced by the necrotrophic cereal pathogen Fusarium graminearum. This mycotoxin is detoxified by ZHD101, a lactonohydrolase from Clonostachys rosea, or EGFP:ZHD101, its fusion to the C-terminus of an enhanced green fluorescence protein. We previously showed that egfp:zhd101 is efficiently expressed in T₀ leaves of rice. In this study, we assessed the feasibility of in planta detoxification of the mycotoxin using progeny. When protein extract from T₁ leaves was incubated with ZEN, the amount of the toxin decreased significantly as measured by HPLC. ZEN degradation activity was also detected in vivo in transgenic T₂ seeds. These results suggest that zhd101 can be exploited as an efficient and cost-effective system for protection of important cereals that are more susceptible to the pathogen (e.g., wheat and maize) from contamination with the estrogenic mycotoxin.

Introduction

Fusarium head blight (FHB) is a significant disease of small grain cereals (e.g., wheat, barley and maize) caused by Fusarium species such as F. culmorum and F. graminearum. In addition to the yield loss, Fusarium-infected grains are often contaminated with the mycotoxins trichothecenes (e.g., deoxynivalenol) and zearalenone (ZEN). Although several fungicides reduce the disease severity of FHB, mycotoxin contamination is not necessarily correlated with the visible disease symptoms (Edwards et al., 2001). For example, a number of field and greenhouse studies demonstrated that application of the strobilurin fungicide azoxystrobin increases the amount of deoxynivalenol in wheat grains (Simpson et al., 2001). In addition, sublethal concentrations of certain fungicides were reported to increase trichothecene production by Fusarium species (Magan et al., 2002).

To reduce the risks of mycotoxin contamination, transgenic inactivation using a detoxification gene is an alternative strategy (Karlovsky, 1999). Previously, we have isolated zhd101, a ZEN detoxifying gene, from a fungal strain of Clonostachys
rosea (Kakeya et al., 2002; Takahashi-Ando et al., 2002). Although the molar activity of ZHD101 was quite low and its optimal pH inadequate for living organisms (i.e., pH = 10.5) (Takahashi-Ando et al., 2004), the genetically modified (GM) microbes completely converted 2 μg/mL of ZEN to a cleaved product that is devoid of estrogenic activity (Takahashi-Ando et al., 2005). This result suggests the feasibility of the transgenic strategy for efficient decontamination of ZEN in important cereal crops.

The success of the detoxification approach for mycotoxins depends in part on the extent to which the GM plant-produced detoxification enzyme reaches its substrate mycotoxin. We previously showed that egfp:zhd101, a ZEN detoxification gene fused to the C-terminus of an enhanced green fluorescence protein gene (egfp), is efficiently expressed in T₀ leaves of rice. In this study, the ZEN degradation activity of the GM crops was characterized in grains using the seeds of the T₂ generation.

Materials, results and discussion

Transgene integration and inheritance

We previously obtained six T₀ rice plants expressing egfp:zhd101 on the basis of the bright-green EGFP fluorescence of regenerated shoots (Higa et al., 2003), the intensity of which is proportional to the ZEN detoxification activity (Takahashi-Ando et al., 2004). The transgenic rice plants flowered normally and produced viable seeds. In this communication, we focus on description of molecular and genetic analyses of one promising line, Z54.

The genomic DNA of T₀ line Z54 and its fluorescent T₁ progenies were examined by Southern blot analysis. The DNA (10 μg) was digested with PstI, whose recognition sequences are present in the polylinker upstream of the Act1 promoter cassette and in the coding region of zhd101 (between the BsrGI and NcoI sites) in pAct1-egfp:zhd101 (Higa et al., 2003), separated on an agarose gel, and transferred to a Nytran N membrane (Schleicher and Schuell, Dassel, Germany). As a probe, the entire coding region of egfp:zhd101 was labeled with digoxigenin (DIG) using the PCR DIG Probe Synthesis kit (Roche Applied Science GmbH, Manheim, Germany) with primers 5′-egfp:zhd101 (5′-ATGGTGAGCAAGGGCGAGGCTGTT-3′) and 3′-egfp: zhd101 (5′-TCAAGATGCTTC TGCGTAGTTC3′). Probe-target hybrids were detected using the DIG Nucleic Acid Detection kit (Roche). The Southern blot of the T₀ line and its T₁ progenies revealed the same hybridization patterns comprising of five bands (see Figure 1a), suggesting a multiple-copy integration of the transgene at a single locus in line Z54. The transgene showed a stable pattern of integration through the three generations investigated in this study (data not shown).

Expression of egfp:zhd101 was visually examined by monitoring the bright-green EGFP fluorescence under a Leica MZFLIII fluorescence stereomicroscope. For the observation of shoots, roots, and flowers, filter sets GFP-Plus (460–500 nm excitation and >510 nm barrier filter) were used. Expression of egfp:zhd101 in the transgenic lines could easily be confirmed by the presence of bright-green EGFP fluorescence, which was clearly visible in the pistil and stamen inside the flowering spikelets (Figure 1b).

Figure 1. Analyses of T₀ and T₁ rice plants transformed with egfp:zhd101. (a) Southern blot analysis of PstI-digested genomic DNA from wild-type and transgenic rice plants. Z54 is the name of the T₀ plant, and the extended names joined by hyphens represent T₁ progenies derived from the T₀ parent (e.g., Z54-8 implies a T₁ progeny “8” from the T₀ parent Z54). (b) Expression of egfp:zhd101 in flowering spikelets. The flowers of wild-type and T₁ line Z54-9 were dissected and observed under an epifluorescence stereomicroscope.