The effect of chemicals on somatic homologous recombination in the rice blast fungus: its possible application for detection of mycotoxins

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Abstract

We previously established a detection/selection system for somatic homologous recombination (HR), which is one of the genetic modification mechanisms in eukaryotes. Because HR is stimulated by the protein synthesis inhibitor blasticidin S, it is presumed that HR in Pyricularia oryzae can be induced by various chemical stresses. To evaluate the effects of chemical stresses on the frequency of HR, several chemical agents were applied to P. oryzae and HR were detected using our detection system. Three well-known DNA-damaging agents—methyl methanesulfonate, bleocin, and methyl viologen—considerably increased the frequency of HR. Adding the amino acid synthesis inhibitor bialaphos, or the protein synthesis inhibitor T-2 toxin, to the medium also significantly increased the frequency of HR. These results suggest that the increased frequency of HR caused by inhibitors of the primary metabolic pathway reflect destabilization of the genome by chemical stressors. Taken together, these findings suggest that the HR detection system may become one of the most useful biological assays for detecting mycotoxins.

Somatic homologous recombination (HR) plays an important role in the DNA double-strand break (DSB) repair. However, HR can be mutagenic if the template for repair is similar, but not identical, to the broken sequence (ectopic HR)¹⁻². In human cells, ectopic HR events drive genetic disorders through the genome rearrangement³. In plants, the frequencies of somatic HR are increased by abiotic and biotic stress factors, and HR may be one of the mechanisms for introducing genetic variations that enable organisms to adapt and respond to the stress environment⁴⁻⁵.

Infection of food crops with toxigenic fungi can result in the contamination of infected grain with mycotoxins. The risk of contamination with mycotoxins has been recognized for decades. However, preventive measures remain costly and inadequate⁶. We previously reported construction of a detection/selection system for exploring ectopic HR at the somatic cell level of Pyricularia oryzae using nonfunctional yellow fluorescence protein (YFP) and blasticidin S deaminase (BSD) fusion genes⁷. The system could detect ectopic HR events between two substrate genes by YFP fluorescence and blasticidin S (BS)-resistance via...
restoration of functional YFP::BSD. Using this HR detection/selection system, we have shown that the onset of ectopic HR was stimulated by treatment with BS, an inhibitor of protein synthesis in both prokaryotes and eukaryotes\(^{10-12}\). In the current study, the effects of various chemical agents on the frequency of ectopic HR were evaluated. We also discuss the applicability of the HR detection/selection system for detecting mycotoxins.

Conidia from TR9 were suspended in distilled water to a final density of \(1 \times 10^5\) conidia/mL. A 100 \(\mu\)L aliquot of the TR9 conidial suspension was then plated onto a PSA plate containing one of the following chemicals: bialaphos (0.5 and 1.0 \(\mu\)g/mL), T-2 toxin (1.0 and 3.0 \(\mu\)g/mL), methyl methanesulfonate (MMS; 0.008 and 0.01%), bleocin (0.1 and 0.5 \(\mu\)g/mL), or methyl viologen (MV; 0.25 and 0.5 \(\mu\)M). Four days after plating, the frequency of HR was determined as the percentage of total colonies in which YFP fluorescence was observed under a MZFLIII fluorescence stereomicroscope (Leica Microsystems, Tokyo). The survival rates after treatments with chemical stressors were calculated as the number of colonies remaining on the plates after treatment with chemical stressors relative to the number of colonies on the plate without chemical stressors (Fig. 1).

![Diagram](image-url)
YFP fluorescence and BS resistance enabled us to select TR9 lines that have undergone ectopic HR during the asexual reproduction of *P. oryzae*. In previous reports, we confirmed that HR events occurred at a higher level following treatment with BS as compared with an untreated control. To evaluate the effect of other chemicals on the frequency of HR, we applied several chemical stress inducers instead of BS. Initially, the HR response to the DNA-damaging agents MMS, bleocin, and MV were examined because somatic HR plays an important role in the repair of DSBs in the genome of *P. oryzae*. Following treatment with MMS, bleocin, or MV, several hyphae in a portion of the active mycelium exhibited fluorescence (Fig. 2A-C). Treatments with these chemicals increased the percentage of colonies having hyphae with YFP fluorescence (HR frequencies) in a concentration-dependent manner, and simultaneously decreased the rate of colony formation (Fig. 2D-F). These results indicated that DSBs caused by exogenously applied DNA-damaging agents induced ectopic HR in the genome of *P. oryzae*.

TR9 was next treated with bialaphos, an amino acid synthesis inhibitor, or T-2 toxin, a protein synthesis inhibitor. Treatment with either agent resulted in some portions of the hyphae exhibiting YFP fluorescence (Fig. 3A, B). The frequencies of HR in the genome of *P. oryzae* were elevated even at very low concentrations of these agents (Fig. 2C, D). In contrast, there were no differences in the frequency of HR and the rate of colony formation after treatments with carproamid or tricyclazole, two melanin biosynthesis inhibitors, compared to an untreated control (data not shown). These results suggested that chemical stress mediated by metabolic inhibitors causes DSB damage and/or HR repair in the genome of *P. oryzae*.

In this study, we have demonstrated that high levels of somatic HR occur after treatments with both DNA-damaging agents and inhibitors of primary metabolic pathways. In fact, similar results were observed for the response of the plant genome to abiotic and biotic stresses. These results suggest that increases in the somatic HR frequency reflect instability imposed to the genome by inhibitors of primary metabolic pathways. Hence, the HR detection system may be used to evaluate the toxicity of trichothecenes other than T-2 toxin. It is of great interest to determine whether other important mycotoxins such as aflatoxin, fumonisins, ochratoxin, sterigmatocystin, and cyclochlorotin cause similar increases in the frequency of HR. A deeper understanding of the mechanisms by which non-DNA-damaging xenobiotics lead to activation of HR may contribute to the development of more sensitive assays for the detection of different mycotoxins.
Fig. 2. Increased somatic HR frequencies induced by treatments with methyl methanesulfonate (MMS), bleocin, or methyl viologen (MV). Conidia of the transformed Pyricularia oryzae line TR9 were plated onto PSA plates in the presence or absence of MMS, bleocin, or MV. Bright-field (BF), epifluorescence (YFP). A. YFP fluorescence of hyphae with MMS. B. YFP fluorescence of hyphae with bleocin. C. YFP fluorescence of hyphae with MV. D. HR frequency and colony formation rate obtained following treatment with MMS. E. HR frequency and colony formation rate obtained following treatment with bleocin. F. HR frequency and colony formation rate obtained following treatment with MV. Data are expressed as means ± SD from three independent experiments.
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