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Identification of Radiation Inducible Genes and DNA Damage Using Random Primers and Comet Assay

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Objectives

To isolate radiation(γ -ray, ⁶⁰Co) inducible cDNA from pepper seedlings which were subjected to various doses of γ -ray stress, and to identify physical modification of nuclear DNA by enhanced radiation stress through RT-PCT and RAPD analysis using random oligonucleotide primers.

Materials and Methods

- 1. Plant material: Rice, pepper seedlings and suspension cells
- Methods: Gene expression by RT-PCR method using pepper seedlings and cultured cells, and DNA damage detection by RAPD method using sixty oligonucleotide primers, comet assay (single cell gel electrophoresis)

Results and Discussion

In RT-PCR, four primers showed different banding patterns in PCR products between treatment and control cDNA. Especially, PCR product of DPA-C11 primer was absent from control cDNA, but highly amplified in treated cDNA. In comet assay, radiation treated cells showed longer tail moment than that of control cell suggesting that radiation stress causes the damage of nuclei DNA in the plant cells. In PCR with random primers, there was no significant differences between control and treated (50 Gy) DNA based on the banding patterns using genomic DNA of pepper suspension cell. Variation of PCR products among individual plants in rice, which is second generation of radiation, was observed. Further analysis are conducting to see if the variation is the radiation effect or not.

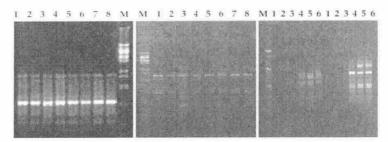


Figure 1. PCR with genomic DNA of pepper (left), rice (middle) and RT-PCR (left).

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