

P86. 기내 방사선 돌연변이유기에 의한 Azetidine-2-Carboxylic Acid 저항성
세포주 선발 및 RAPD 분석

한국원자력연구소:현도윤,이인석,김동섭,신인철,이영일*

고려대학교 식량자원학과:서용원,김재윤

**Selection of Azetidine-2-Carboxylic Acid Resistant Cell Lines by *in vitro*
Mutagenesis and RAPD Analysis of Rice**

D. Y. Hyun, I. S. Lee, D. S. Kim, I. C. Shin, Y. W. Seo¹⁾, J. Y. Kim¹⁾, Y. I. Lee*

Korea Atomic Energy Research Institute

1) Department of Agronomy Korea University

Objectives

The objective of this study was to select resistant callus to azetidine-2-carboxylic acid (AZC), a proline analog, by *in vitro* mutagenesis and to obtain materials overproducing proline content or representing salt tolerance.

Materials and Methods

1. Plant material : Donganbyeon
2. Callus induction medium : N₆ basal medium with 2 ppm 2,4-D
3. Mutagen and selection : After irradiation with 30, 50, 70, 90, and 120 Gy gamma rays, resistant calli were selected from callus induction medium containing 4 mM AZC
4. Regeneration medium : MS medium with 0.2 ppm IAA and 3 ppm kinetin
5. Proline content assay : Bates *et. al.* method (1973)
6. RAPD analysis : Use of thirty reproducible Operon random primers

Results

- When calli were inoculated on the medium with AZC, survival rate and fresh weight of calli were decreased as increasing AZC concentration.
- On the base of LD₅₀, the optimum AZC concentrations for the selection of AZC resistant cell lines were 3-4 mM concentration.
- As increasing gamma-ray dosages, the fresh weight of calli was decreased and LD₅₀ dosage was 70 Gy.
- Proline contents of resistant cell lines were increased from 1.2 at 50 Gy to 2.3 times at 120 Gy than those of control lines.
- Reproducible 30 primers were selected to test the RAPD analysis.
- Amplified products of about 500 bp fragment with the OPH-06 primer were generated in only resistant cell lines.

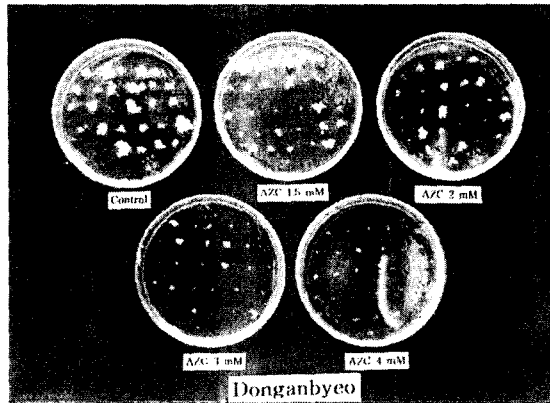


Fig 1. Determination of optimum concentration for selection of AZC resistant callus.

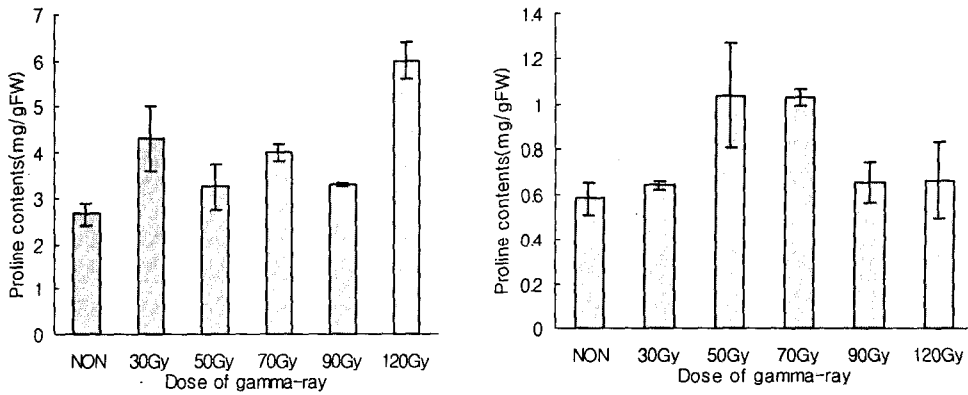


Fig 2. Proline contents of 4 mM AZC resistant calli and regenerants. Resistant calli (left) and regenerants induced from selected calli (right).

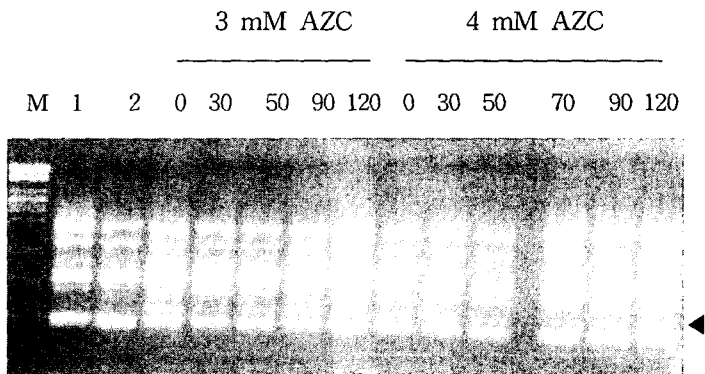


Fig 3. RAPD pattern of resistant callus obtained with primer OPH-06. M: size marker, lane 1: control plant, lane 2: control callus, lane 3-7: selected calli on 3 mM AZC with irradiation, lane 8-13: selected calli on 4 mM AZC with irradiation. Number given was radiation dose (Gy).