

Arabidopsis thalianaにおけるガンマ線照射誘導過酸化水素蓄積と脂質過酸化の軽減にカタラーゼCAT2は関与しない

誌名	食品照射
ISSN	03871975
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発行元	日本食品照射研究協議会
巻/号	48巻1号
巻号補足	
掲載ページ	p. 38-41
発行年月	2013年9月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター

Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council
Secretariat



[Note]

Catalase, CAT2, is not Involved in Mitigation of Gamma Irradiation-induced H₂O₂ Accumulation or Lipid Peroxidation in *Arabidopsis thaliana*

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Summary

In *Arabidopsis* wild-type (Col-0 ecotype) and *cat2* mutant plants, gamma radiation induced H₂O₂ accumulation and lipid peroxidation at 10 kGy but not at 0.1 to 1 kGy. There were no significant differences in H₂O₂ accumulation and lipid peroxidation between the wild type and the *cat2* mutant. Hence, CAT2 may not be a key enzyme to protect gamma irradiation-induced damage.

Key words: Gamma radiation, catalase, hydrogen peroxide accumulation, lipid peroxidation

Introduction

Gamma irradiation affects growth and development due to cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissues of plants.¹⁾ Gamma irradiation induces H₂O₂ accumulation and lipid peroxidation.²⁾ Catalase is one of antioxidant enzymes to scavenge H₂O₂ and catalase

activities are changed by irradiation.³⁾ Hence, it is thought that increasing activity of catalases is favorable to alleviation of damage caused by irradiation.⁴⁾ However, it remains to be clarified roles of catalases in irradiated plants. We examined effects of gamma irradiation on an *Arabidopsis* catalase-deficient mutant, *cat2*, to elucidate roles of catalases in responses to gamma irradiation.

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Materials and Methods

Arabidopsis wild-type (Col-0, Columbia ecotype) and *cat2* mutant plants were grown as described previously.⁵⁾ The catalase activities in *cat2* mutants were 50% lower than those in wild type.⁶⁾ *Arabidopsis* Genome Initiative numbers for the genes discussed in this article are as follows: *CAT1*, AT1G20630; *CAT2*, AT4G35090; *CAT3*, AT1G20620. Rosette leaves were exposed to gamma rays from a ¹³⁷Cs source in an irradiator (CS-500C, Yoshizawa Namari Kogyo Co. Ltd., Osaka, Japan). The dose rate was 0.1 kGy/h as measured with a Fricke dosimeter. Hydrogen peroxide ac-

cumulation in rosette leaves was detected by 3,3'-diaminobenzidine with some modifications according to previously described.⁷⁾ Lipid peroxidation was evaluated using thiobarbituric acid according to previously described.⁸⁾ The significance of differences between mean values was assessed using one way analysis of variance (ANOVA) with Tukey's test. Differences were considered significant for *p* values of < 0.05.

Results and Discussion

Hydrogen peroxide accumulation was not significantly increased by irradiation at 0.1 kGy or 1 kGy but was significantly increased by irradiation at 10 kGy

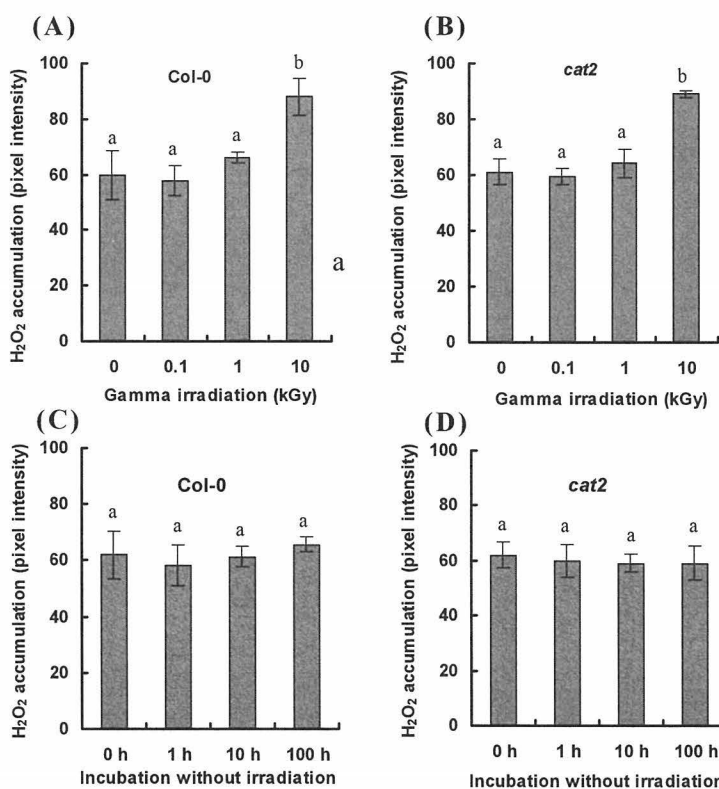


Fig. 1. Accumulation of H₂O₂ (A, B, C, and D) in *Arabidopsis* Rosette Leaves Irradiated by Gamma Rays.

A-B) Rosette leaves of wild-type and *cat2* mutant plants were irradiated with gamma rays at 0 kGy, 0.1 kGy, 1 kGy, and 10 kGy; C-D) Rosette leaves of wild-type and *cat2* mutant plants were incubated for 0 h, 1h, 10h and 100h. Averages for three independent experiments are shown. Error bars represent standard deviations. Values indicated by the same letter do not differ significantly at the 5% level, as determined by ANOVA with Tukey's test.

(Fig. 1A and B). There were no significant differences in H_2O_2 accumulation between the wild type and the *cat2* mutant (Fig. 1). Hence, scavenging of H_2O_2 may not be attributed to CAT2. Incubation in dark at room temperature for up to 100 h without irradiation did not affect H_2O_2 accumulation (Fig. 1C and D).

Lipid peroxidation level was not significantly increased by irradiation at 0.1 kGy or 1 kGy but was significantly increased by irradiation at 10 kGy (Fig. 2A and B). There were no significant differences in lipid peroxidation level between the wild type and the *cat2*

mutant (Fig. 2). Incubation in dark at room temperature for up to 100 h without irradiation did not affect lipid peroxidation level (Fig. 2C and D).

CAT2 is one of three catalase isoforms in *Arabidopsis thaliana* and is more dominant than CAT1 and CAT3⁹⁾ and CAT2 rather than CAT1 or CAT3 more considerably contributes to the oxidative stress tolerance.^{9,10)} However, this study suggests that CAT2 is not a key enzyme to mitigate H_2O_2 accumulation and lipid peroxidation induced by gamma irradiation in *Arabidopsis*.

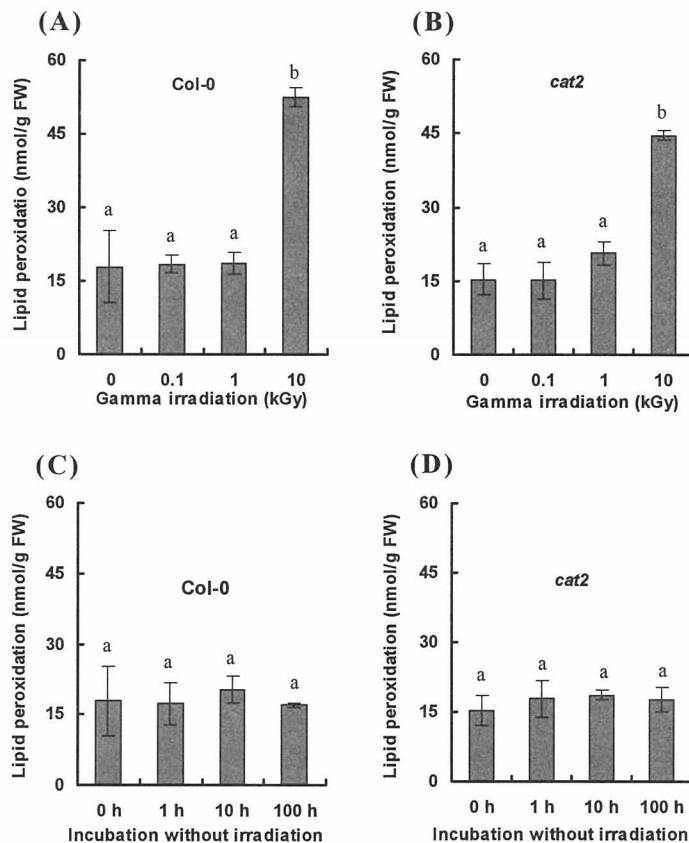


Fig. 2. Lipid Peroxidation (A, B, C, and D) in *Arabidopsis* Rosette Leaves Irradiated by Gamma Rays. A-B) Rosette leaves of wild-type and *cat2* mutant plants were irradiated with gamma rays at 0 kGy, 0.1 kGy, 1 kGy, and 10 kGy; C-D) Rosette leaves of wild-type and *cat2* mutant plants were incubated for 0 h, 1h, 10h and 100h. Averages for three independent experiments are shown. Error bars represent standard deviations. Values indicated by the same letter do not differ significantly at the 5% level, as determined by ANOVA with Tukey's test.

We recently have presented that there was no significant differences in susceptibility to gamma irradiation between *Arabidopsis* wild type and other catalase deficient mutants, *cat3* and *cat1 cat3*.¹¹⁾ Hence, catalases may not be key enzymes to protect cellular damage induced by gamma irradiation in *Arabidopsis*.

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和文要旨

シロイヌナズナ野生株 (Col-0) 及び *cat2* 変異体への 10 kGy のガンマ線照射は、H₂O₂ 蓄積及び脂質過酸化生成を誘起したが、0.1 kGy と 1 kGy のガンマ線照射は、誘起しなかった。また、野生株と変異体との間で、H₂O₂ 蓄積及び脂質過酸化に有意な差はなかった。以上の結果より、カタラーゼ CAT2 は、ガンマ線によって誘起される損傷を軽減するための重要な酵素ではないことが示唆された。

キーワード：ガンマ線照射, カタラーゼ, 過酸化水素蓄積, 脂質過酸化

(2013 年 7 月 29 日受理)