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学位論文の題目	Roles of catalases in response to gamma irradiation in *Arabidopsis thaliana* （シロイヌナズナのガンマ線照射への応答におけるカタラーゼの役割）
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学位論文内容の要旨

Introduction

Gamma rays are a high-energy form of electromagnetic radiation. Gamma radiation penetrates living tissue and can damage all important cellular components both through direct ionization and through generating ROS due to water radiolysis and induce oxidative damage. Radiation-induced oxidative stress was evaluated by three independent approaches; DNA damage, lipid peroxidation and protein oxidation. Gamma irradiation induces DNA damage and increases hydrogen peroxide accumulation and lipid peroxidation level. Catalase is one of antioxidant enzymes to scavenge H_2O_2 and irradiation changes catalase activities. Hence, it is thought that increasing activity of catalases is favorable to alleviation of damage caused by irradiation. The Arabidopsis genome contains three CAT genes, *CAT1, CAT2, CAT3,* which are differentially expressed and can form up to six different isozymes. However, it remains to be clarified the roles of catalases in irradiated plants.

Objectives and methods

I investigated the functions of catalases in Arabidopsis in response to gamma irradiation using Arabidopsis wild type and *cat2*, *cat3-1*, *cat1 cat3*. Catalase activities in the rosette leaves were measured by spectrophotometer. Comet images were digitally captured and then analyzed with image analysis software (CASP, CaspLab.com). The ratio of the tail area of the comet to the sum of the tail area and the head area of the comet was calculated as DNA damage. Hydrogen peroxide accumulation was measured with 3,3'-diaminobenzidine, and lipid peroxidation was evaluated using thiobarbituric acid in the rosette leaves.

Results and discussion

Gamma irradiation at 0.1 kGy but not at 1 kGy increased catalase activities and irradiation at 10 kGy decreased catalase activities in the wild type, *cat3-1*, and *cat1 cat3* mutants. Irradiation at doses of 10 kGy but not at 0.1 kGy and 1 kGy significantly elevated DNA damage in the wild type, *cat3-1*, and *cat1 cat3* mutants. H_2O_2 accumulation was increased by irradiation at 10 kGy but not at 0.1 kGy or 1 kGy in the wild type, *cat3-1*, and *cat1 cat3* mutants. Lipid peroxidation increased by irradiation at 10 kGy but not at 0.1 kGy or 1 kGy in the wild type, *cat3-1*, and *cat1 cat3* mutants. There were no significant differences in catalase activities, DNA damage, H_2O_2 accumulation, and lipid peroxidation among the wild type and both mutants at doses of 10 kGy gamma irradiation. These results suggest that catalases, CAT1 and CAT3, did not alleviate gamma irradiation-induced DNA damage, H_2O_2 accumulation, or lipid peroxidation in *Arabidopsis thaliana.* H_2O_2 accumulation significantly increased by irradiation at 10 kGy but not at 0.1 kGy or 1 kGy in the wild type and *cat2* mutants. Lipid peroxidation levels significantly increased by irradiation at 10 kGy but not at 0.1 kGy or 1 kGy in the wild type and *cat2* mutants. There were no significant differences in H_2O_2 accumulation and lipid peroxidation levels between the wild type and *cat2* mutants at dose of 10 kGy gamma irradiation. These results indicate that catalase, CAT2, did not mitigate gamma irradiation-induced extracellular H_2O_2 accumulation and lipid peroxidation in whole leaves of *Arabidopsis thaliana*.

論文審査結果の要旨

植物の放射線に対する応答において，特に耐性においてカタラーゼ活性が重要であると考えられてきた。しかし，カタラーゼの細胞内の局在等から放射線耐性に本当に重要であるか疑問が残されていた。

本学位論文では，カタラーゼ欠損シロイヌナズナ変異体を用いて，そのガンマ線への応答を調べた。ガンマ線照射によって，カタラーゼ欠損変異体も野生株と同様に，カタラーゼ活性に大きな変化があった。しかし，カタラーゼ欠損変異体と野生株との間に，応答（活性酸素種産生，脂質過酸化，DNA断片化等）に有意な差はなかった。

以上の結果から，ガンマ線照射への応答において，カタラーゼは重要な活性酸素種消去酵素として機能していないことを明らかにした。

本研究内容は，学術的な価値のみならず，食品照射実践のための基礎となるものである。従って，本審査委員会は本論文が博士（学術）の学位論文に値すると判断した。