7 Suppression of Zn Stress on Barley by Irradiated Chitosan

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Abstract

Chitosan was irradiated up to 1000 kGy in solid state. Irradiation of chitosan caused the reduction of molecular weight. The molecular weight of the chitosan reduced from *ca.* 4×10^5 to *ca.* 6×10^3 by irradiation at 1000 kGy. For the barley growth promotion, irradiated chitosan showed the significant effect and 1000 kGy irradiated chitosan improved 20 % of growth. Using the positron emitting tracer imaging system (PETIS), the effect of chitosan on uptake and transportation of ^{62}Zn in barley were investigated. It was found that the transportation of Zn from root to shoot and the damage of plant by Zn were suppressed with irradiated chitosan.

Keywords: Radiation degradation, Chitosan, Growth promotion, Suppression of Zn stress, Positron emitting tracer imaging system (PETIS)

1.Introduction

Polysaccharides such as chitin / chitosan, alginate and carrageenan are widely used in food processing to increase viscosity, and as emulsifiers in medicine and cosmetic fields [1]. Recently, oligosaccharide derived from depolymerization of alginates by enzyme was reported to have novel features such as stimulation of growth of *Bifidiobacteria*,

promotion of germination and shoot elongation of plants [2, 3, 4, 5]. Oligochitosan degraded by chitosanase, has been also reported to enhance for antimicrobial activity [6]. These oligosaccharides can be prepared by acid hydrolysis or enzymatic degradation of polysaccharides [7, 8, 9, 10]. On the other hand, radiation technique can provide a useful tool for degradation of polysaccharides. It has been well known that polysaccharides such as cellulose can be degraded due to scission of glycosidic bond by radiation [11, 12]. We have been studied on radiation effect of these polysaccharides and its biological activities for plants. They were easily degraded by irradiation and induced various kinds of biological activities such as promotion of plant growth, anti-bacterial activity, and so on. Irradiated alginate has been proved to promote growth of rice and barley through our cooperative study with Vietnam Atomic Energy Commission. Furthermore, we found that chitosan irradiated in 1% solution (0.5M acetic acid) at 100 kGy not only recovered seedlings growth damaged by heavy metals but also reduced vanadium levels in seedlings [13]. In this study, suppression of zinc (Zn) stress on barley by irradiated chitosan was investigated.

2. Experimental

2.1. Materials and irradiation

Plant of barley (*Hordeum vulgare*) was used for experiment. Chitosan (degree of deacetylation = 80%) used in this work were purchased from Katokichi Bio Co. Ltd., Japan. This material in solid (less than 200-mesh powder) was sealed in a glass tube with the air atmosphere, and then irradiated. The irradiation was carried out using gamma rays from Co-60 sources with a dose rate of 10 kGy/hr.

2.2. Analytical procedures

Molecular weight of irradiated chitosan was measured by gel permeation

chromatography (SEC-MALLS; Shodex Co., Ltd., Japan). The instrument was equipped with Shodex Asahipak GF-7MHQ and elution at room temperature (27°C) with 1/3M acetic acid + 0.1 M sodium acetate aqueous solution with a flow rate of 0.6 mL/min. The eluent was monitored by a Shodex RI-71 differential refractometer and a Wyatt DAWN DSP and polymer concentration was *ca.* 0.1%(w/v).

2.3. Toxicity test of Zn on plant growth

Germinated seeds were sown on plastic nets floating on nutrient solution of Hyponex® at 1/1000 in controlled room (25 °C). Zinc (Zn) stresses were applied at 1.0 – 2.0 mM (Zn as $ZnSO_4$) on 3 day old seedlings with 30 plants per each treatment. After 14 days growing, each 20 seedlings were collected, washed gently, air dried and finally dried in oven at 105 °C for 24 hr, and weighed

2.4. Production of ^{62}Zn

^{62}Zn was produced from the reaction ^{63}Cu (p, 2n) ^{62}Zn by bombarding Cu plate with 0.3 mA of 30 MeV H^+ particles from TIARA AVF cyclotron for 90 min. This Cu plate was dissolvied in nitric acid, and then added hydrochloric acid and pure water. The solution was purified through anion exchange column, and ^{62}Zn solution produced *ca.* 5.55 MBq in 7mL of 5mM HCl solution.

2.5. Analysis of ^{62}Zn transport in plants

The solution containing *ca.* 2.7 MBq/mL of ^{62}Zn was fed to the root of plants and then the plants were dipped in water for 18 hr during the image analysis of tracer activity by using positron emitting tracer imaging system (PETIS) [14].

3. Result and Discussion

3.1. Irradiation effect of chitosan

Chitosan, polymer of glucosamine, irradiation effect of chitosan has been investigated. Figure 1 shows the relationship between number-average molecular weight ($\overline{Mn}$) and the irradiation dose. It can be seen that $\overline{Mn}$ of chitosan in solid state decreased remarkably by irradiation. The molecular weight of the chitosan reduced from *ca.* 4×10^5 to *ca.* 6×10^3 by irradiation at 1000 kGy. This degradation behavior of chitosan is almost the same as that of alginate [15].

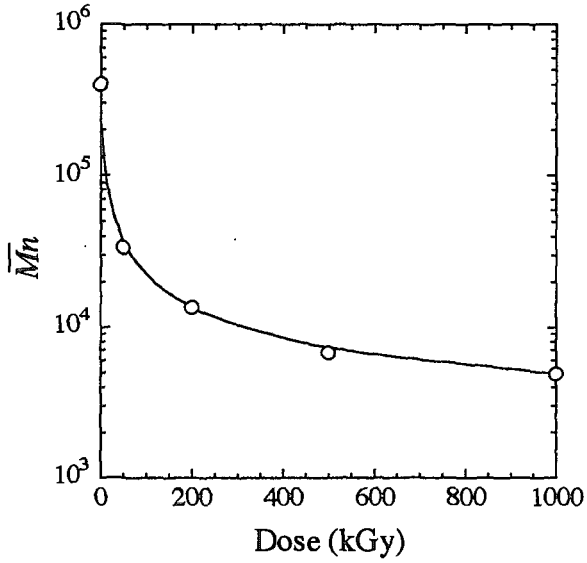

Fig. 1 Change in molecular weight of chitosan in solid state by irradiation. Molecular weight was measured by GPC.

3.2 Effect of irradiated chitosan on growth of barley

Chitosan affects the growth of barely plant. Figure 2 shows the effect of irradiation dose of chitosan on barely growth. Degraded chitosan obtained from higher dose (up to 1000 kGy) shows a stronger effect on the increase of dry matter of barely seedlings. It is expected that a certain molecular weight of degraded chitosan ($\overline{Mn}$ *ca.* 6×10^3 at 1000 kGy) is effective for plants growth.

The growth of barely at various concentrations of irradiated chitosan is shown in Fig. 3. The results show that the optimium concentration of degraded chitosan is 20 ppm.

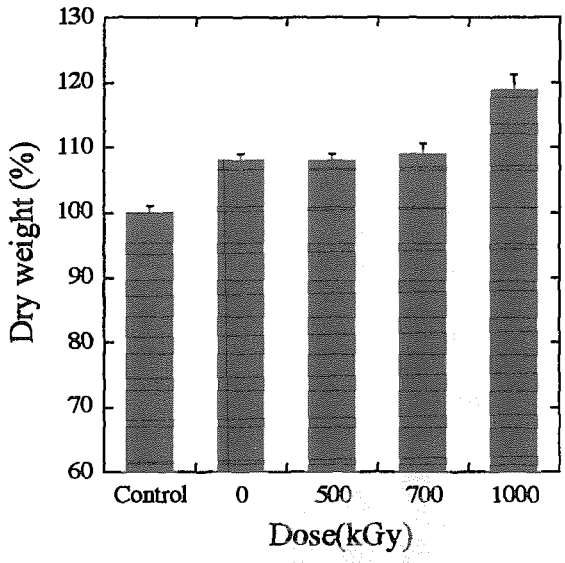

Fig. 2 Effect of irradiation dose for chitosan on barley growth. Barley was cultivated for 14 days in hydroponic solution with 20 ppm chitosan irradiated in solid state.

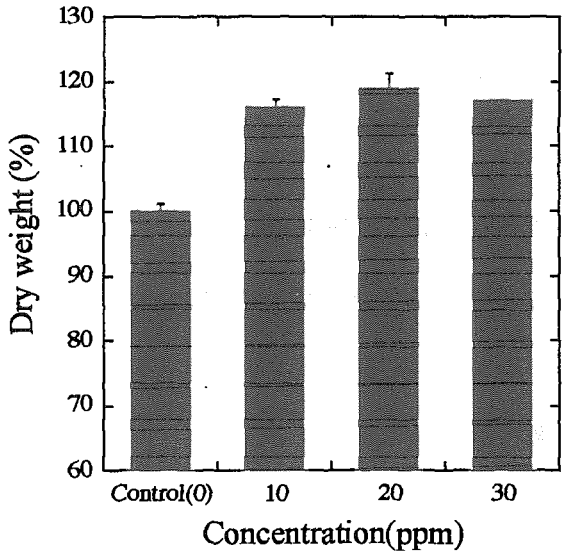

Fig. 3 Concentration effect of irradiated chitosan on barley growth. Barley was cultivated for 14 days in hydroponic solution with chitosan irradiated at 1000kGy in solid state.

Using the chitosan irradiated at 1000 kGy with the concentration of 20 ppm, effect of irradiated chitosan on barley growth under Zn stress was tested. Figure. 4 shows the damage of barley by Zn (left) and the suppression of Zn damage on barley with irradiated chitosan. The growth of barley decreased with increasing concentration of $ZnSO_4$. With 2.0 mM $ZnSO_4$, significant damage (45%) was observed but it was slightly smaller recoverd (65%) with 20 ppm of irradiated chitosan.

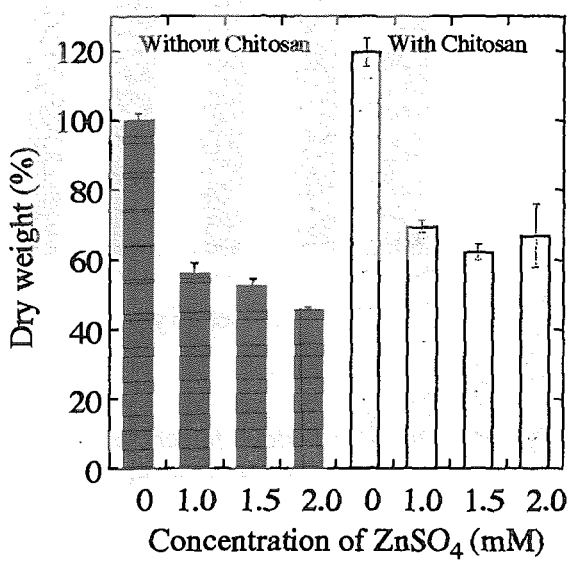

Fig. 4 Effect of irradiated chitosan (1000kGy, 20ppm) on barley growth under zinc stress at different concentrations.

3.3 Uptake and translocation of Zn in Plants

The recovery of metal damage when treated with irradiated chitosan at 1000kGy suggest that chitosan under irradiation can regulate transportation of Zn. Figure. 5 shows the ^{62}Zn imaging of barley leaves using PETIS. The results revealed that the plant treated with 20 ppm irradiated chitosan transported Zn very weakly into the shoot compared to without chitosan. The results show that the radiation degraded chitosan are effective to suppress the uptake and translocation of Zn into the shoots and suppress the heavy metal damage on the growth of plants.

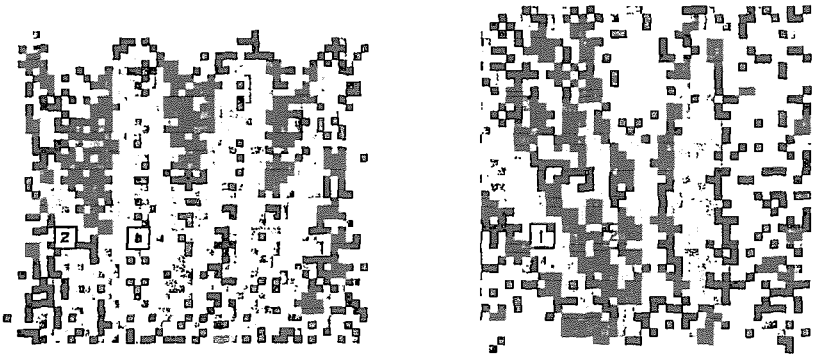

Fig. 5 PETIS images of ^{62}Zn in barley leaves with or without irradiated chitosan(1000kGy, 20ppm).

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References

[1] Tosa, T., Sato, T., Mori, T., Yamamoto, K., Takata, I., Nishida, Y. and Chibata, I., *Biotech. Bioeng.*, 21, 1697 (1979).

[2] Akiyama, H., Endo, T., Nakakita, R., Murata, K., Yonemoto, Y. and Okayama, K., *Biosci. Biotech. Biochem.*, 56, 335 (1992).

[3] Yonemoto, Y., Tanaka, H., Yamashita, T., Kibatake, N., Ishida, Y., Kimura, A. and Murata, K., *J. Ferment Bioeng.*, 75, 68 (1993).

[4] Tomoda, Y., Umemura, K. and Adachi, T., *Biosci. Biotech. Biochem.*, 58, 203 (1994).

[5] Natsume, M., Kamo, Y., Hirayama, M. and Adachi, J., *Carbohydr. Res.*, 258, 187 (1994).

[6] Kendra, D. F. and Hadwiger, L. A., *Exp. Mycol.*, 8, 276 (1984).

[7] Haug, A., Larsen, B. and Smidsrod, O., *Acta. Chem. Scand.*, 20, 183 (1966).

[8] Tsujino, I. and Saito, T., *Nature* , 192, 970 (1961).

[9] Nakada, H. I. and Sweeny, P. C., *J. Biol. Chem.*, 242, 845 (1967).

[10] Shimokawa, T., Yoshida, S., Takeuchi, T., Murata, K., Ishii, T. and Kusakabe, A., *Biosci. Biotech. Biochem.*, 60,1532 (1996).

[11] Woods, R. T., Pikaev, A. K., Applied radiation chemistry: radiation processing, New York, John Wiley & Sons, 1994. p. 341.

[12] Leonhardt, J., Arnold, G., Baer, M., Langguth, H., Gey, M. and Huebert, S., *Radiat. Phys. Chem.*, 25, 899 (1985).

[13] Tham, L. X., Nagasawa, N., Matsuhashi, S., Ishioka, N. S., Ito, T. and Kume, T., *Radiat. Phys. Chem.*, in press.

[14] Kume, T., Matsuhashi, S., Shimazu, M., Ito, H., Fujimura, T., Adachi, K., Uchida, H., Shigeta, N., Matsuoka, H., Osa, A. and Sekine, T., *Appl. Radiat. Isot.*, 48, 1035 (1997).

[15] Nagasawa, N., Mitomo, H., Yoshii, F. and Kume, T., *Polym. Degrad. Stab.*, 69, 279 (2000).