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Preparation of Oligoalginate Plant Growth Promoter by γ Irradiation of Alginate Solution Containing Hydrogen Peroxide

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ABSTRACT: Degraded alginate compounds with molecular weights of 7−26, 40−77, or 11−26 kDa were obtained by γ irradiation, hydrogen peroxide (5% H_2O_2) treatment, or a combination treatment involving ionizing radiation and H_2O_2, respectively. The 14 kDa oligoalginate, prepared by the combined method, promoted the growth of mustard greens and lettuce at an optimal concentration of 75 mg/L. The growth promotion effects of the oligoalginate prepared by γ irradiation in the presence of H_2O_2 were statistically equivalent to those of the oligoalginate prepared by γ irradiation only. The combination of γ irradiation and H_2O_2 reduced the required irradiation dosage by a factor of 9 relative to the oligoalginate produced by γ irradiation only. The combination treatment (γ irradiation/H_2O_2) may be carried out on a large scale at low cost to produce oligoalginate for use as a plant growth promoter in agricultural industries.

KEYWORDS: *Degradation, γ irradiation, hydrogen peroxide, oligoalginate*

INTRODUCTION

Alginate, a block polymer of mannuronic and guluronic acids attached through random 1,4 glycosidic linkages, is extracted from the cell walls of marine molluscs, bacteria, and brown algae and has been widely used in the pharmaceutical, cosmetic, biotechnology, and agricultural industries.[1−5] Recent studies reported that alginate degradation products provide an important bioactive polysaccharide with several novel features that can be useful in the agricultural sciences. Oligoalginate, an alginate degradation product, was found to have plant growth promotion effects that can be useful in the agricultural fields.[6−13] For instance, oligoalginate increased the leaf and tuber weights of Japanese radishes by 114 and 324%, respectively,[13] induced the growth of longer shoots of rice, grains, and tobacco,[8] increased the germination rate of barley, and increased the rate of root growth in barley seedlings.[14] Oligoalginate also triggers phytoalexin induction and can enhance the activities of several enzymes, such as alcohol dehydrogenase, lactate dehydrogenase, 5′-phosphodiesterase, and chitinase.[6,14,15] Previous studies examined alginate degradation by enzymes or radiation, which yielded a weight-average molecular weight (M_w) of 14 kDa, that strongly promoted plant growth in rice, peanuts, carrots,[10,16] barley, soybeans,[17] and *in vitro* flowering plants.[12] The conventional methods for producing oligoalginate have involved enzymes (alginate lyase)[8,9,14] or chemical agents.[18] Radiation processes have been explored recently for the degradation of alginates because such degradation reactions may be conducted at room temperature, the products can be used without purification, and the processes may be reliably controlled for use in large-scale applications. One drawback to the process is that the irradiation dose required for the preparation of oligoalginates used as a plant growth promoter is high (at least 75 kGy),[10,12,17] which presents challenges for large-scale applications. The purpose of the present study was to investigate the feasibility of producing plant growth promoters via a low-cost production method by treating alginate solutions with a combination of γ ray radiation and H_2O_2.

MATERIALS AND METHODS

Chemicals and Vegetables. The alginate powder used in this study was supplied by Katokichi Chemical Co., Ltd., Japan. Other chemicals, including H_2O_2, KBr, and $NaNO_3$, were supplied by Sigma-Aldrich Co. (St. Louis, MO). The vegetable varieties used for testing were mustard greens (*Brassica juncea* var. *rugosa*) and lettuce (*Lacuta sativa lollo rossa*).

Degradation of Alginate. Alginate degradation by radiation was conducted as follows: the alginate was incubated overnight in water at room temperature to induce hydration and swelling and then was stirred for 5 h to obtain 4% (w/v) solutions. The alginate solutions were irradiated using γ rays from a Co-60 source at doses up to 200 kGy with a dose rate of 10 kGy/h for degradation. Degradation by H_2O_2 was conducted using a 4% alginate solution containing 5% H_2O_2, and the reaction was permitted to proceed for a maximum reaction time of 96 h at room temperature. Alginate degradation via a combination of irradiation and H_2O_2 was conducted by dissolving 4 g of alginate in 100 mL of distilled water containing 0.5% H_2O_2. The mixtures were then exposed to a γ Co-60 source for irradiation at doses of 4−16 kGy.

Molecular-Weight Determination. The M_w values of the degraded alginates were measured by gel permeation chromatography

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(GPC) using a model CO-8020 (Tosho Co., Ltd., Japan) equipped with three TSKgel PW_{XL} columns (300 × 7.8 mm) in series, $G6000PW_{XL}$, $G3000PW_{XL}$, $G2500PW_{XL}$ (Tosho Co., Ltd., Japan), combined with a TSK guard column PW_{XL} (40 × 6.0 mm). The compounds were eluted at 40 °C using 0.1 mol/L sodium nitrate solution at a flow rate of 0.5 mL/min. The eluent was monitored using a RI-8020 differential refractometer at 40 °C, and the alginate concentration was 0.1% (w/v). Polyethylene glycol (PEG) with a M_w from 2×10^2 to 6×10^3 and eight pullulan samples with M_w values from 5.9×10^3 to 7.9×10^5 (Wako Co., Ltd., Japan) were used as standards.

Fourier Transform Infrared (FTIR) Spectrometry. The FTIR spectra of samples were collected in KBr pellet form using a Spectrum One FTIR spectrometer 8100 (Perkin-Elmer Co., Ltd., Waltham, MA). All spectra were recorded at ambient temperature over the range of 4000−450 cm^{-1} at a resolution of 4 cm^{-1} over 128 scans. The KBr pellets were prepared from well-dried mixtures of 3 mg of sample and 100 mg of KBr, as described previously.[12]

Growth Promotion Test. The germination effects were tested by sowing the seeds of *Lactuca* and *Brassica* in Petri dishes containing coconut coin supplemented with irradiated alginates, and the germination rate was determined after 4 days.

The effects of the degraded alginate on growth promotion were evaluated using 10 seedling plants (14-day-old) of each variety of lettuce and mustard green. A seedling plant was cultivated in 1000 mL of solution containing 0.1% hyponex and degraded alginate. The controls were performed under identical conditions without the degraded alginate supplement. All cultures were cultivated in a standardized greenhouse at the Saigon Thuy Canh Corporation. The shoot height, root length, fresh biomass (root and shoot), and dried matter content (root and shoot) were determined after 28 days of cultivation. The degree of growth promotion was calculated according to the following formula: (growth promotion degree, %) = [100 × (evaluation of the treated bed)]/(evaluation of the untreated bed).

Statistical Analysis. The triplicate experiments were conducted using three blocks for each treatment. Data were statistically analyzed using the analysis of variance (ANOVA) test. The means were compared using the least significant difference (LSD) at a 5% probability level, and the standard deviations were calculated.

RESULTS AND DISCUSSION

Change in M_w of the Alginates Prepared by Each Degradation Method. The γ-irradiated degradation of the alginate samples produced a decrease in the alginate M_w with an increasing irradiation dose (Figure 1A). The M_w of the alginate rapidly decreased at irradiation doses up to 50 kGy. The M_w of the alginate subsequently decreased more gradually as the irradiation dose increased. A dose of 200 kGy provided an alginate sample with a rather low M_w of 7 kDa. The results agreed well with previous reports.[10,12,17,19]

Yang et al.[18] used a 5% (v/v) H_2O_2 solution to degrade alginate and induce formation of oligoalginate within 1−4 h at 95 °C. Their results revealed that the reducing end of most oligomers prepared by H_2O_2 degradation at high temperatures yielded a carboxyl group. Our experiments used the same concentration of H_2O_2 to degrade alginate, but the reaction was carried out at room temperature to minimize the structural changes introduced in the degraded product. The alginate M_w was reduced rapidly during a reaction time of 4 h, and then the M_w decreased slowly from 8 to 24 h (Figure 1B). A M_w of 41 kDa was obtained after 24 h of reaction. The M_w did not thereafter change significantly, even after 96 h of reaction time (40 kDa). The preparation of oligoalginates with a M_w below 20 kDa and without structural modifications to the oligomers remains a challenge.

Ionizing radiation combined with oxidizing agents, such as hydrogen peroxide, potassium persulfate, and ammonium

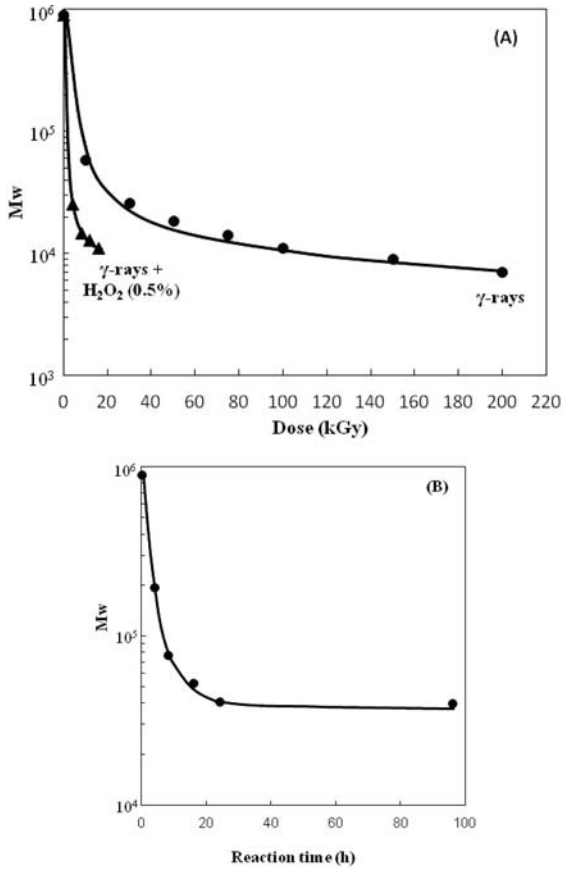

Figure 1. Change in the M_w of the alginate as a function of the (A) irradiation dose and (B) in the presence of 5% H_2O_2 with various reaction times. Alginate degradation was conducted in a 4% (w/v) solution, and the M_w of the product was determined by GPC using three TSKgel PW_{XL} columns ($G6000PW_{XL}$, $G3000PW_{XL}$, and $G2500PW_{XL}$), using a sodium nitrate solution (0.1 mol/L) as the solvent.

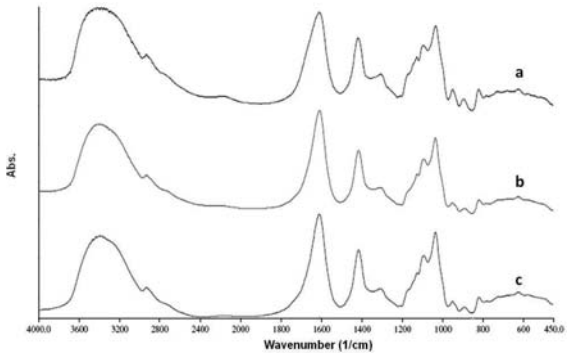

Figure 2. FTIR spectra of the degraded alginate: (a) without irradiation, (b) sample irradiated at 75 kGy, and (c) sample degraded by γ irradiation at 8 kGy in the presence of H_2O_2 (0.5%). The spectra were collected using a Spectrum One FTIR spectrometer 8100 type (Perkin-Elmer Co., Ltd., Waltham, MA), with the sample in a KBr pellet (3 mg of sample and 100 mg of KBr) at ambient temperature at a resolution of 4 cm^{-1} over 128 scans.

persulfate, act synergistically to degrade alginate[20] and chitosan,[21] thereby permitting a reduction in the radiation dose used to degrade polysaccharides. In our experiment, a 4% alginate solution containing 0.5% H_2O_2 was degraded by γ irradiation. The results revealed that the addition of H_2O_2 led

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Table 1. Assignment of the Main IR Absorption Bands of the Degraded Alginates

wavenumber (cm^{-1})	intensity/shape	assignment
3360−3380	very strong broad	O−H stretching
2930−2980	strong shoulder	C−H stretching
1617−1835	very strong sharp	COO^- stretching (asymmetric)
1414−1507	strong sharp	COO^- stretching (asymmetric)
1299−1356	strong shoulder	C−O stretching
1122−1156	weak shoulder	C−O stretching and C−C stretching
1091−1117	strong shoulder	C−C stretching and C−O stretching
1032−1071	strong sharp	C−O−C stretching
944−970	weak sharp	C−O stretching and C−C−H stretching
885−915	weak shoulder	C−O stretching, C−C−H stretching, and C−O bending

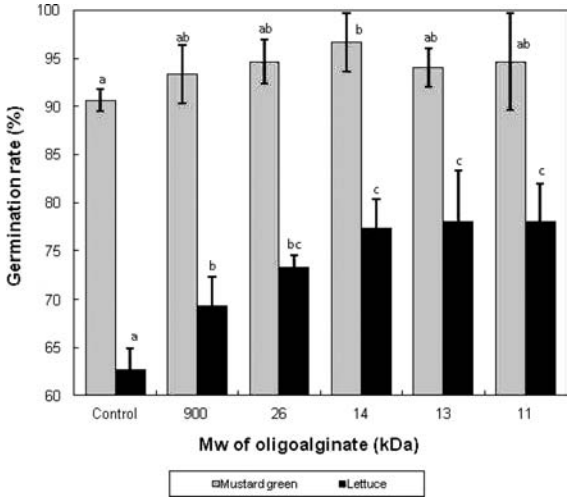

Figure 3. M_w of the oligoalginate affected the germination rate of the vegetable seeds. The seeds were sown in Petri dishes containing coconut coin supplemented with irradiated alginates, and the germination rate was determined after 4 days. Bars with the same letter are not significantly different ($p > 0.05$).

to a rapid decrease in the M_w of the alginate product at a dose of 4 kGy and the M_w of the degraded alginate irradiated at 8 kGy was 14 kDa (Figure 1A). The M_w of the product was close to that of alginate irradiated at 75 kGy without H_2O_2 treatment. Clearly, the addition of 0.5% H_2O_2 to the alginate solution reduced the required irradiation dose by 90%.

FTIR Analysis. FTIR analysis is a useful method for elucidating the structural changes present in a degraded alginate. The infrared (IR) spectra of the degraded alginate samples are shown in Figure 2, and the main assignments of the absorption bands are listed in Table 1. The results indicated comparable sample structures resulting from degradation by irradiation at 75 kGy in a 4% solution or irradiation at 8 kGy in combination with H_2O_2.

Plant Growth Promotion Effects of the Oligoalginate. *Effects of the Molecular Weight.* The enzymatically degraded alginate product stimulated the rate of barley seed germination, grain growth, root elongation in rice, and increase in the leaf and tuber biomass of Japanese radish.[8,9,13,14] The oligoalginate growth promotion tests were performed by examining the germination of mustard greens and lettuce seeds. The germination rates of both mustard greens and lettuce seeds in a medium supplemented with 11−26 kDa oligoalginate products prepared by irradiation in the presence of H_2O_2 were significantly higher than the untreated control

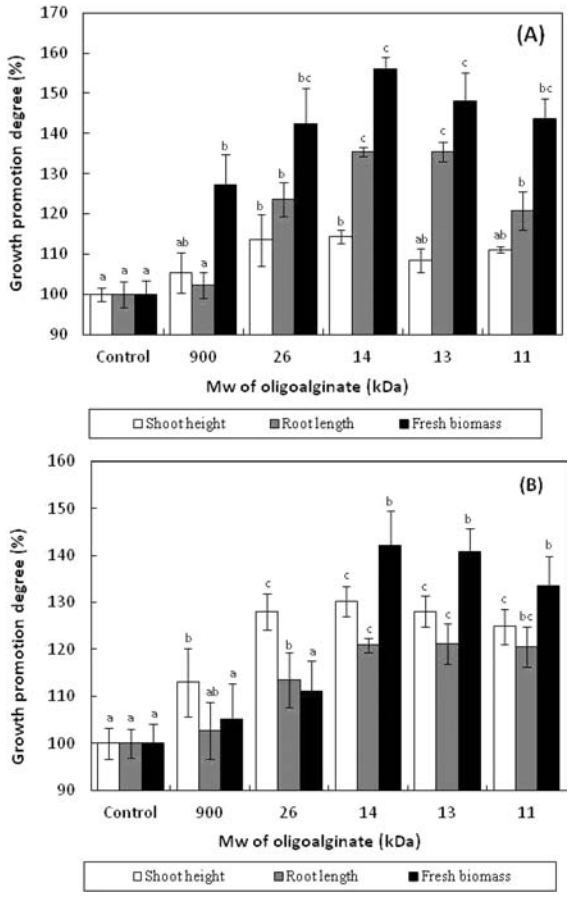

Figure 4. Growth of (A) mustard greens and (B) lettuce as a function of the M_w of oligoalginate. The 14-day-old seedling plants were cultivated in a hyponex solution (0.1%) containing the degraded alginates with various M_w. The control samples were prepared under identical conditions but without the supplemental degraded alginate. Data were collected after cultivation for 28 days. The bars with the same letter are not significantly different ($p > 0.05$).

(Figure 3). The highest germination rate was obtained using the 14 kDa oligoalginate.

Our previous studies showed that the oligoalginate prepared by irradiation promoted the growth and development of crop plants (rice, peanuts, barley, and soybeans)[10,17] and flowering plants (chrysanthemum, lisianthus, and limonium).[18] The results of the present study indicated that the oligoalginate prepared by irradiation at 4−16 kGy, in combination with 0.5% H_2O_2 (M_w = 11−24 kDa), stimulated the growth and development of mustard greens (Figure 4A) and lettuce (Figure 4B) relative to the control (untreated) or the treatment

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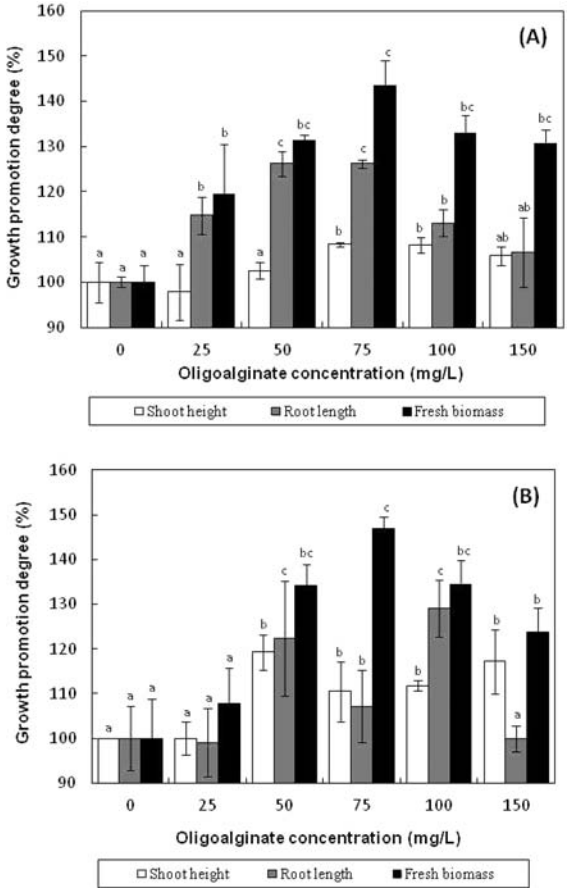

Figure 5. Effects of the oligoalginate concentration on the growth of (A) mustard greens and (B) lettuce. The 14-day-old seedling plants were cultivated in a hyponex solution (0.1%) containing 14 kDa degraded alginate. The controls were prepared under identical conditions but without the supplemental degraded alginate. Data were collected after cultivation for 28 days. Bars with the same letter are not significantly different ($p > 0.05$).

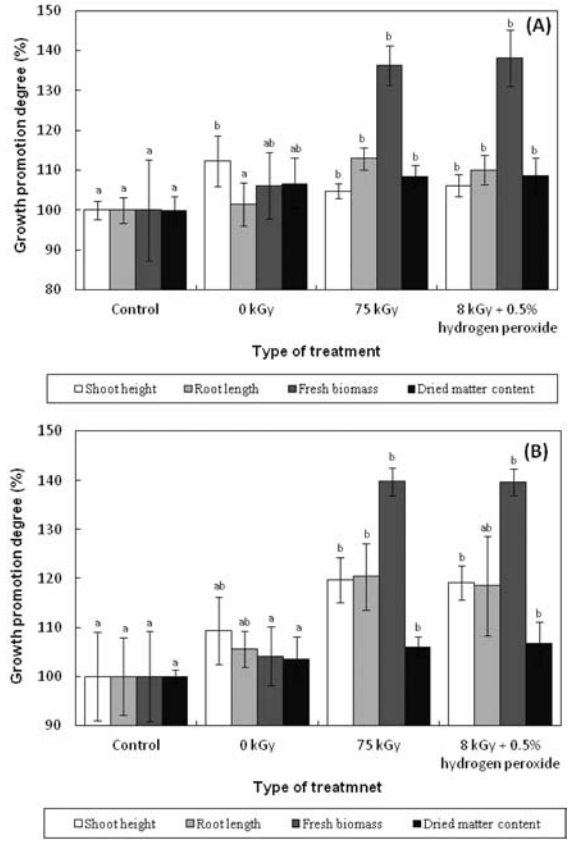

Figure 6. Growth promotion of the oligoalginates prepared by γ irradiation or the combined treatment on (A) mustard greens and (B) lettuce. The 14-day-old seedling plants were cultivated in a hyponex solution (0.1%) supplemented with alginate degraded by radiation or radiation in the presence of 0.5% H_2O_2. The controls were prepared under identical conditions but without the supplemental degraded alginate. Data were collected after cultivation for 28 days. Bars with the same letter are not significantly different ($p > 0.05$).

involving non-irradiated alginate (M_w = 900 kDa). The greatest stimulation of vegetables was obtained by treatment with 14 kDa oligoalginate. Relative to the control, the presence of oligoalginate increased the mustard greens shoot height, root length, and fresh biomass by 14, 35, and 56%, respectively. The lettuce shoot height, root length, and fresh biomass were increased by 30, 21, and 42%, respectively.

Effects of the Concentration. The 14 kDa oligoalginate was found to optimally promote the growth of the tested vegetables. In the present study, 0−150 mg/L oligoalginate concentrations were tested to identify the optimal test concentration. Mustard greens treated with oligoalginate displayed a significant increase in the root length and fresh biomass over the range of 50−100 mg/L, whereas the shoot height increased over the range of 75−100 mg/L (Figure 5A). The greatest promotion of shoot height (8% increase), root length (26% increase), and fresh biomass (44% increase) was obtained using 75 mg/L oligoalginate. Concentrations of 50−100 mg/L were found to be suitable for promoting the growth of lettuce and increasing the fresh biomass gain (47% increase) compared to the control (see Figure 5B). The results agreed well with previous studies of rice, peanuts, barley, and soybeans.[10,16,17]

Effects of the Oligoalginate Prepared by γ Irradiation (75 kGy) and γ Irradiation/H_2O_2 (8 kGy). The oligoalginate prepared by irradiation displayed a strongly stimulating effect on the growth and development of rice, peanuts, carrots,[10,16] barley, soybeans,[17] and *in vitro* flowering plants.[12] In this experiment, the vegetable growth promotion effects of the 14.4 kDa oligoalginate prepared by irradiation at 75 kGy were compared to the 14.7 kDa oligoalginate prepared by irradiation at 8 kGy in the presence of 0.5% H_2O_2. Figures 6A and 7A indicate that the fresh biomass of mustard greens treated with oligoalginate prepared by irradiation at 75 kGy increased by 38% relative to the untreated control. This effect was comparable to the effects of the oligoalginate prepared by irradiation at 8 kGy in combination with 0.5% H_2O_2 (a 36% increase). The two oligoalginate products increased the shoot height, root length, and dried matter content of the mustard greens to a similar extent. On the other hand, the fresh biomass, shoot height, root length, and dried matter content of the lettuce treated with the oligoalginate products prepared by either method were higher than those treated with the non-irradiated alginate and the control (see Figures 6B and 7B); however, the values resulting from treatment with oligoalginate prepared by irradiation at 75 kGy or irradiation of a 4% alginate solution at 8 kGy in the presence of 0.5% H_2O_2 were indistinguishable. Thus, the oligoalginate prepared by the combined treatment (γ ray/H_2O_2) shows potential as an

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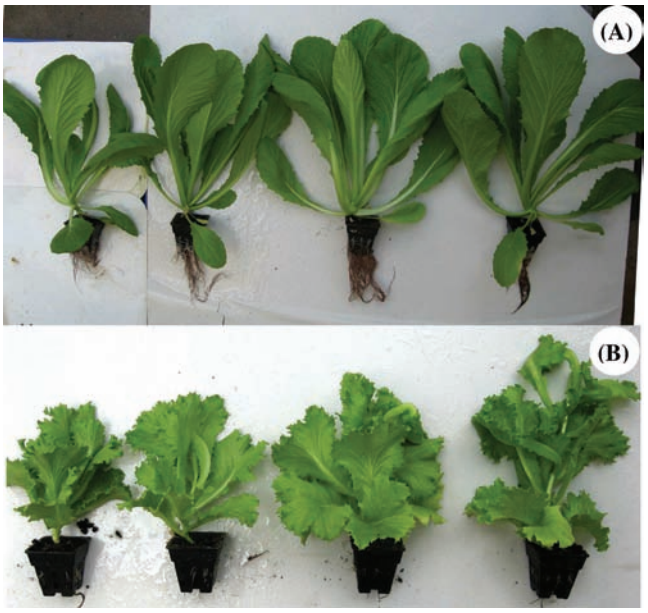

Figure 7. Photographs of (A) mustard greens and (B) lettuce grown in the presence of oligoalginates prepared by the different methods. From left to right: control plants (without oligoalginate), plants treated with native alginate, plants treated with oligoalginate prepared by γ irradiation/H_2O_2 (8 kGy), and plants treated with oligoalginate prepared by γ irradiation only (75 kGy). The plants were cultivated for 28 days in a 0.1% hyponex solution supplemented with the degraded alginate. The controls were performed under identical conditions but without the supplemental degraded alginate.

environmentally friendly plant growth promoter in agricultural industries.

The oligoalginates described here were prepared by γ irradiation of an alginate solution containing H_2O_2. The addition of H_2O_2 (0.5%) to a 4% alginate solution reduced the radiation dose required to achieve growth-enhancing oligoalginates from 75 to 8 kGy. The oligoalginates prepared by the combined treatment promoted the growth of vegetables to the same extent as the oligoalginate prepared by the irradiation method only. The oligoalginate plant growth promoter may potentially be used in agricultural applications because of the economical costs of production.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

ANOVA, analysis of variance; LSD, least significant difference; FTIR, Fourier transform infrared; M_w, weight-average molecular weight

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