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Effects of Chitosan on Production and Rot Control of Soybean Sprouts

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ABSTRACT

The practicality of utilizing chitosan as a natural antimicrobial compound to reduce soybean sprout rot was tested. Domestic and imported soybean seeds were soaked for 6 hours in solutions containing different levels of chitosan and acetic acid (glacial), and cultured at 25℃ for 6 days. In case of domestic seeds, soaking with 1,000ppm chitosan increased germination percentage, hypocotyl thickness, total length, and fresh weight of sprouts by 4%, 5%, 2%, and 1%, respectively. The total sprout yield was increased by chitosan in a concentration-dependent manner in that 1,000ppm chitosan resulted in 8% increment of total yield (7.47kg sprouts/kg seed). Chitosan significantly reduced sprout rot percentage to 7.0% compared to control (13.8%), and consequently enhanced marketable sprout yield by 39%. Compared to domestic seeds, the imported soybean seeds exhibited very low germination percentage regardless of chitosan treatments. Chitosan, nevertheless, consistently induced yield increment and rot decrement in imported soybean sprouts. Although 100ppm acetic acid was effective in reducing sprout rot percentage down to 11.8%, its yield-increasing effects were not as prominent as chitosan. In conclusion, soaking soybean seeds with chitosan seems to be a practical method to enhance the efficiency of soybean sprout production.

Keywords: acetic acid, chitosan, rot, soybean sprouts.

The soybean sprout is a traditional Korean food of high popularity due to its high nutritional value and low price. The soybean sprout production has been commercialized and recently became an important agricultural business. The food safety of soybean sprouts, however, endangered by illegal application of pesticides to reduce sprout rot and pesticide residue in sprouts still remains as a social concern. Sprout rot is a major factor limiting soybean sprout production. The outbreak of sprout rot during culture has been reported to be caused by microbes such as *Fusarium, Pseudomonas,* and *Erwinia* (Myung, 1987; Oh & Park, 1996; Park et al., 1997). The high temperature and high humidity conditions during sprout cultivation, the exudates from growing sprouts, the mixture of ungerminated or low viability seeds, as well as the application of plant growth regulators to enhance growth rate of sprouts (Park et al., 1995; Suh et al., 1995a; Park et al., 1986) readily provide feasible conditions for microbial growth and resultant sprout rot. Despite of continuous researches on sprout rot, practical and safe methods to replace pesticide application in preventing sprout rot has not been established well. In case of using imported soybean seeds of low germinability, sprout rot becomes an even more serious problem. The imported seeds rarely provide enough information on cultivar, postharvest handling conditions and storage duration. Soybean seeds are known to rapidly lose their germinability in proportion to storage duration (Suh et al., 1995b). Considering long-distant shipping conditions, the imported seeds are very likely to lose their germinability, which in turn provides higher chance of microbe-induced sprout rot. Most of soybean sprout studies, however, have been conducted with Korean domestic seeds and sprout rot studies for imported seeds are very limited.

The chitosan, derived from chitin of crabs or crustacean shell, is a polymer of 2-amino-2-deoxy-D-glucose (glucosamine). Chitosan is accepted as a natural food additive and healthy diet based upon its high food safety. Consequently there are increasing number of reports on application of chitosan for medicinal and food industrial purpose (Lee et al., 1995; Chung et al., 1996). Chitosan is also known to hold antimicrobial function against phytopathogens such as *Fusarium solani* and *F. axysporum* cepae via forming ionic bridges between positively charged amino group of chitosan and negatively charged sites of microbe cell wall (Jeon et al., 1996; Jo, 1989).

This study was conducted to test the practicality of using chitosan as a safe natural antimicrobial compound to prevent soybean sprout rot.

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MATERIALS AND METHODS

Plant materials

Korean domestic soybean (*Glycine max* L. cv. 'Junjory') seeds and imported soybean se eds were purchased from the Korean Bean Sprout Association and stored at 5℃ prior to experimental use. The domestic seeds were harvested in a previous year, while no informations on cultivar name and harvest year regarding imported seeds were available.

Soybean sprout culture

Soybean seeds were soaked for 4 to 6 hours and transferred into a culture container. Two different size of culture containers were used for these experiments: a commercial soybean culture box (25 × 25 × 25 cm) for yield and growth test, and a miniature size (∅15 × 30 cm) culture box for sprout rot test. Culture containers were placed in a plastic house covered with three layers of black PE film (thickness 0.05 mm) and two layers of insulation sheet (thickness 1.0 cm) and one layer of flexible styrofoam sheet (thickness 1.0 cm). Soaked soybean seeds were cultivated for 6 to 7 days under a top-irrigation system commercially used for soybean sprout production. The irrigation was given for 3 minutes every 4 hours with underground water. The average temperature regime inside the culture house during experiments was 20℃ to 25℃.

Chitosan and acetic acid treatment

Different concentrations of chitosan (0 as a control, 100, 500, and 1,000 ppm) and acetic acid (glacial; 10, 50, and 100 ppm) were treated during seed soaking. For comparison, thiram (tetramethylthiuram disulfide), a seed-sterilizing fungicide was also used. For thiram treatment, 1 kg of soybean seeds were soaked in a control solution for 3 hours and dried for 30 min at room temperature, and homogeneously mixed with 1.4g of thiram prior to transferring to culture containers. The molecular weight of chitosan used in these experiments ranged from 5,000 to 10,000.

Sprout growth, yield, marketable yield, and rot measurements

The growth parameters such as germination percentage, hypocotyl thickness, hypocotyl length, root length, as well as sprout rot percentage were quantified with harvest soybean sprouts. The total yield was expressed as the percentage of harvested sprout weight relative to dry seed weight. The marketable yield percentage was expressed as the percentage of marketable sprout weight relative to total sprout weight harvested. The rot percentage was calculated as the percentage of rot sprout numbers relative to total seed numbers and the term 'rot sprout' was defined as sprouts showing two or more dark rot symptoms in either hypocotyl or cotyledon. All experiments were replicated at least three times and each replication consisted of at least three container subreplications. The growth parameters were obtained from over 40 representative plant measurements.

RESULTS AND DISCUSSION

Uptake of soaking solution

The uptake of soaking solutions containing different levels of chitosan and acetic acids was expressed as seed weight increment percentage relative to soaked dry seed weight during soaking. In case of domestic seeds, 1,000 ppm chitosan decreased soaking solution uptake of domestic seeds by 2% although such decrease caused no significant impacts on subsequent growth of sprouts (Fig. 1). Significantly reduced soaking solution uptake shown in thiram treatment was due to the differences in thiram treatment process, during which shorter period of soaking was conducted. Compared to imported seeds, domestic seeds exhibited higher uptake of soaking solution.

Seed germination

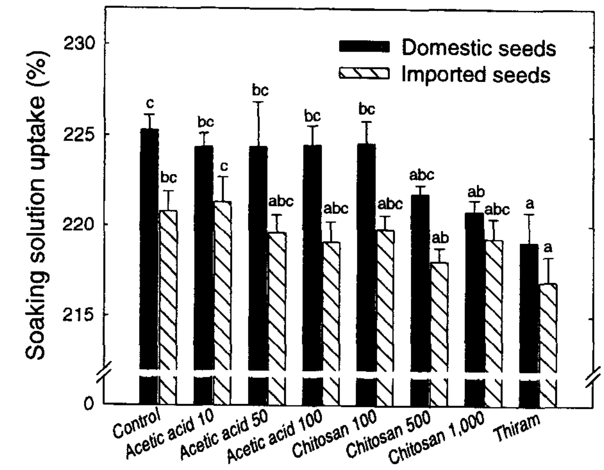

Fig 1. Effects of chitosan and acetic acid on soaking solution uptake of domestic and imported soybean seeds. Treatment concentrations are in ppm unit and same letters within either domestic or imported seeds indicate no significant difference at 5% level by Duncan's multiple range test.

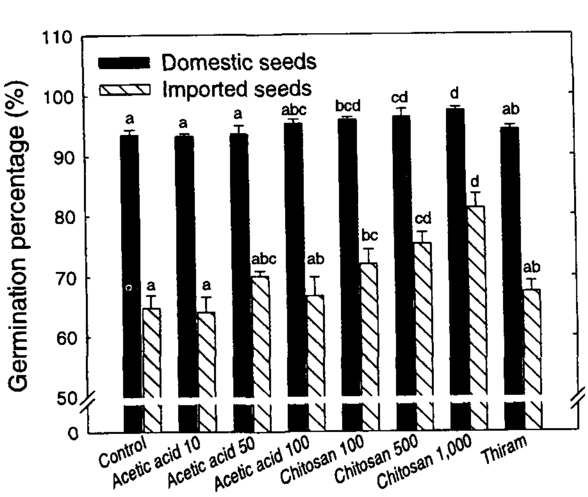

Fig 2. Effects of chitosan and acetic acid on germination percentage of domestic and imported soybean seeds. Treat ment concentrations are in ppm unit and same letters within either domestic or imported seeds indicate no significant difference at 5% level by Duncan's multiple range test.

Soaking seeds with chitosan significantly increased germination percentage in proportion to chitosan concentration in both domestic and imported soybean seeds (Fig. 2). Germination-enhancing effects of chitosan could be detected from as low as 100ppm. In case of domestic seeds, 100, 500, and 1,000 ppm chitosan increased germination percentage from 93.5% (control) to 95.9, 96.3, and 97.4%, respectively. Germination-enhancing effects of chitosan was more prominent in imported seeds, in that 1,000 ppm chitosan increased seed germination up to 81.2% compared to control (64.8%). This might in part due to the fact that imported soybean seeds exhibited very poor germination compared to domestic seeds. Neither acetic acids nor thiram affected germination of soybean seeds. Although the mechanism how chitosan can enhance soybean seed germination still remains in question, these results implies that soaking soybean seeds with chitosan may practically enhance the germination, the initial process of sprout production. The fact that imported seeds exhibited very poor germination percentage indicated that using non-domestic soybean seeds may significantly cause sprout rot problems.

Sprout growth

Soaking domestic soybean seeds with 1,000 ppm chitosan significantly increased hypocotyl thickness by 0.1 mm, corresponding to 5% of control (Table 1), which indicated the possibility of higher customers preference of chitosan-treated sprouts over thinner control sprouts. Same concentration of chitosan, however, decreased hypocotyl length of domestic soybean sprouts by 0.6 cm and increased root length by 1 cm, resulting in no significant total sprout length changes. Reduced hypocotyl length and enhanced root length as affected by chitosan may serve as an adverse quality factor in market. However, considering that root length is strongly affected and can be controlled by irrigation methods, additional studies on irrigation intervals and duration combined with chitosan application may reveal effective methods for chitosan application. In case of imported seeds, 1,000 ppm chitosan increased root length, but decreased hypocotyl thickness. Acetic acid up to 100 ppm showed no prominent concentration-dependent changes in growth of domestic soybean seeds.

Total yield and marketable sprout percentage

The total sprout yield as expressed by fresh weight

Table 1. Effects of chitosan and acetic acid on growth of soybean sprouts.

	Domestic Seeds				Imported Seeds			
	Hypocotyl thickness (mm)	Hypocotyl length (cm)	Root length (cm)	Total length (cm)	Hypocotyl thickness (mm)	Hypocotyl length (cm)	Root length (cm)	Total length (cm)
Control	2.09^{ab†}	10.63^{cd}	10.61^{a}	21.24^{ab}	2.07^{b}	10.30^{abc}	8.42^{ab}	18.72^{ab}
Acetic acid 10 ppm	2.13^{abc}	10.60^{bcd}	11.34^{ab}	21.94^{bc}	2.01^{ab}	10.15^{a}	7.72^{a}	17.87^{a}
Acetic acid 50 ppm	2.06^{a}	10.93^{d}	11.70^{b}	22.63^{abcd}	2.05^{ab}	10.56^{abc}	9.08^{bc}	19.64^{bc}
Acetic acid 100 ppm	2.15^{bcd}	10.29^{abc}	10.57^{a}	20.86^{c}	2.05^{ab}	10.72^{c}	9.35^{cd}	20.06^{cd}
Chitosan 100 ppm	2.21^{d}	10.36^{abc}	11.22^{ab}	21.58^{a}	2.00^{ab}	10.60^{bc}	9.49^{cd}	20.09^{cd}
Chitosan 500 ppm	2.12^{abc}	10.18^{a}	11.37^{ab}	21.55^{ab}	1.98^{a}	10.72^{c}	10.10^{cd}	20.81^{d}
Chitosan 1,000 ppm	2.19^{cd}	10.03^{a}	11.65^{b}	21.69^{ab}	1.98^{a}	10.62^{bc}	9.88^{d}	20.50^{cd}
Thiram	2.11^{abc}	10.21^{ab}	11.20^{ab}	21.41^{ab}	2.04^{ab}	10.18^{ab}	9.26^{cd}	19.45^{bc}

† Means within column followed by same letters are not significantly different at 5% level by Duncan's multiple range test.

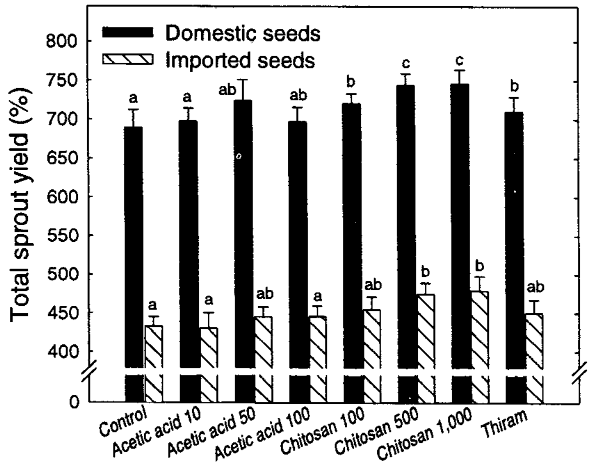

Fig 3. Effects of chitosan and acetic acid on total yield of soybean sprouts. Treatment concentrations are in ppm unit and same letters within either domestic or imported seeds indicate no significant difference at 5% level by Duncan's multiple range test.

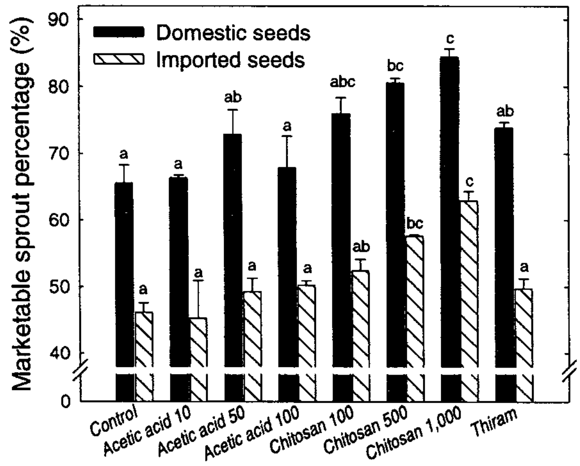

Fig 4. Effects of chitosan and acetic acid on marketable yield percentage of soybean sprouts. Treatment concentrations are in ppm unit and same letters within either domestic or imported seeds indicate no significant difference at 5% level by Duncan's multiple range test.

increment percentage relative to soaked seed dry weight was significantly increased by chitosan in both domestic and imported seeds (Fig. 3). In case of domestic seeds yield-increasing effects of chitosan could be detected from as low as 100 ppm resulting in total yield of 72.1%, 74.4%, 74.6% in 100, 500, and 1,000 ppm, respectively, compared to control (68.9%). Domestic seeds exhibited very low total yield compared to imported seeds in that only 433% of total yield could be achieved in control, which was 63% of domestic seeds. Yield-increasing effects of chitosan could be detected over 500 ppm chitosan in imported seeds.

The marketable sprout percentage expressed as fresh weight percentage of highly marketable sprouts relative to total amount of harvested sprouts was even more significantly increased by chitosan in both domestic and imported seeds (Fig. 4). In case of domestic seeds, 500 ppm and 1,000 ppm chitosan significantly enhanced marketable sprout perecntage up to 81% and 85%, respectively compared to control (65%). Considering no significant differences between chitosan 500 ppm and 1,000 ppm in their effects on increasing yield and marketability, it is likely that 500 ppm chitosan soaking is preferable in soybean sprout culture. Usually most of soybean sprouts are circulated and sold in market as harvested in culture containers. However, film-packed sprouts in 300g or 500g unit is currently increasing in markets to meet customers' preference for clean and safe sprouts. By definition, marketable sprout percentage excludes the portion of rot, under-developed, or abnormally-shaped sprouts among harvested sprouts. When harvested sprouts are selected, weighed, and packed in a plastic film for market circulation, it is the marketable sprout yield not total yield that determines final product quantity. The marketable sprout yield of domestic seeds as calculated by multiplying total sprout yield by marketable sprout percentage was 45.1% and 63.1% in control and 1,000 ppm chitosan, respectively. These results indicate that yield-increasing effects of chitosan become even more prominent in film-packed circulation case (40% yield increment) than in culture container circulation case (8% yield increment). Soaking seeds with 50 ppm acetic acid showed slight but non-significant increase in total sprout yield.

Sprout rot

Soyean sprout rot was significantly reduced by soaking seeds with 1,000 ppm chitosan (Fig. 5), suggesting high possibility of using chitosan as a natural antimicrobial compound in soybean sprout production. In case of domestic seeds, chitosan decreased sprout rot percentage in a concentration-dependent manner in that 100, 500, and 1,000 ppm chitosan exhibited 9.3, 8.9, and 7.0% sprout rot, respectively, while control showed 13.8% sprout rot. Regardless of chitosan treatments, imported seeds exhibited sprout rot over 40%, which was significantly higher than domestic seeds. These results indicated that using low-cost but high-risk imported seed of unknown postharvest conditions may readily induce severe sprout rot problems.

In conclusing, soaking soybean seeds with 500 to

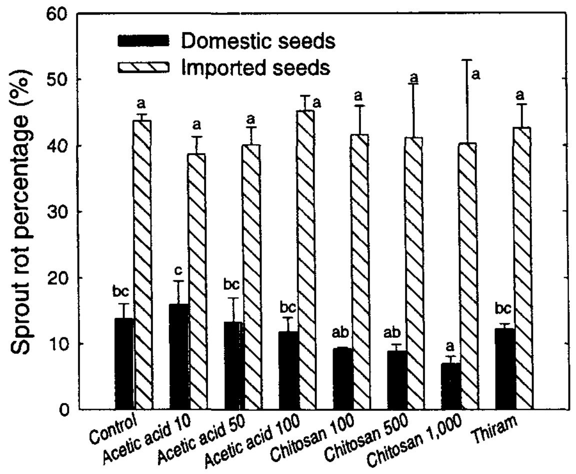

Fig 5. Effects of chitosan and acetic acids on rot percentage of soybean sprouts. Treatment concentrations are in ppm unit and same letters within either domestic or imported seeds indicate no significant difference at 5% level by Duncan's multiple range test.

1,000 ppm chitosan seems a practical method to enhance the productivity of soybean sprout in both yield and sprout rot factors.

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