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Abstract

Seed Priming with Pectic-Oligosaccharides Improved Seed Germination and Growth of Chili †

Wascharin Udchumpisai *, Yuree Wandee, Ditpon Kotatha and Dudsadee Uttapap

Division of Biochemical Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkhuntien, Bangkok 10150, Thailand; yuree.wan@kmutt.ac.th (Y.W.); ditpon.kot@kmutt.ac.th (D.K.); dudsadee.utt@kmutt.ac.th (D.U.)

* Correspondence: udchumpisai.w@outlook.co.th; Tel.: +66-871666446

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Abstract: The aim of this study was to assess the impact of pectic-oligosaccharides (POSs) obtained from oxidative degradation of pomelo peel with H_2O_2 under alkaline conditions on seed germination and growth of chili using the seed priming technique. Two types of POSs (POS-I and POS-II) with different size distributions were prepared. Chili seeds were soaked in 500 ppm of POS solutions for 16 h at two temperatures, 30 and 50 °C, with moderate shaking, and then air-dried. The primed seeds were planted on wet filter paper in a Petri dish at 30 °C for 9 d and the effects of priming on germination and growth were observed. Priming of seeds with POS at 30 °C increased the germination percentage and vigor index at 9 d after sowing by 16.7–20.5% and 16.0–25.5%, respectively, whereas root and shoot length did not differ from the hydropriming. However, there were no significant differences in all growth parameters between POS-I and POS-II treatments. Seedling length and the vigor index of seeds primed with POS at 30 °C (29.3–31.0 mm, 2693.9–2914.0) were also significantly higher than those of non-primed seeds (22.9 mm, 2062). Priming the seeds at 50 °C had an adverse effect on seed germination, i.e., the germination percentage was reduced by 24.4–31.9% compared to seeds primed at 30 °C. It also resulted in a significant reduction in chili seed growth, possibly due to the inactivation of some enzymes in chili seeds.

Keywords: chili; germination; growth; oxidative degradation; pectic-oligosaccharides; pomelo; seed priming

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1. Introduction

Poor seedling growth and seedling vigor lead to poor planting and yield. In various crops, different seed treatment techniques are used to improve the germination and seedling vigor after harvest [1]. Seed priming is the conventional strategy for improving crop production and minimizing the negative effects against stress. Partial seed pre-hydration promotes membrane protein hydration and activates various metabolism processes, as well as early germination events in seeds. These activities stop after the re-drying of seeds [2,3]. Several priming technologies such as osmopriming, solid matrix priming, hormopriming, biopriming and chemical priming have been developed recently to increase the efficiency of germination and strength of seeds [2].

Plant biostimulators are natural compounds that can improve plant life processes without an adverse impact on principal natural plant pathways such as the regulation of carbon and nitrogen metabolism, the control of secondary metabolism, and the activation of plant enzyme production related to growth and detoxification, etc., [4,5]. Hu et al. [6] reported that soaking maize seeds in alginate-derived oligosaccharide solution for 15 h can increase root and shoot growth on day 7 up to 18% and 46%, compared to seeds soaked in water. Chitosan microparticles can enhance the germination and vigor index of tomato seeds and also improve the root and stem development [7]. The study of Nandhini

and Somasundaram [8] showed that priming maize seeds with lipo-chito oligosaccharide improved the tolerance of seeds to salinity, as indicated by the increasing germination stress tolerance index.

Pectic-oligosaccharides (POS) are oligosaccharides produced from the partial depolymerization of pectin using different techniques including chemical, hydrothermal, and enzymatic degradation. POS products include oligo-galacturonides (OGA), galacto-oligosaccharides (GalOS), rhamnogalacturonan-oligosaccharides (RGOS), and others [9–11]. Although there has been research on POS obtained from a variety of raw materials, such as orange and apple pomaces, there have been few studies on POS derived from pomelo peel. Moreover, the bioavailability of POS is mostly examined in food applications, such as prebiotics properties and antioxidant activity, etc., with less work being done on their usage in agriculture. In recent years, many researchers have found that OGAs affect the morphogenesis and organogenesis of various plants, such as inducing root formation, stimulating growth and improving robustness of the in vitro cultivated plants [12]. This study aimed to assess the impact of POSs derived from pomelo peel on chili seed germination and seedling growth by using the priming technique.

2. Materials and Methods

2.1. Materials

Chili seeds were prepared from dried chili purchased from a local market. Fruit peels of pomelo (*Citrus maxima Merr.*) cultivar Khao-Yai were collected from local vendor in Nakhon Pathom Province, Thailand. Galacturonic acid (GalA), digalacturonic acid and trigalacturonic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The H_2O_2 and other chemical reagents used were of analytical reagent grade.

2.2. Preparation of POS

The albedo part of pomelo peels was washed, chopped into small pieces and dried at 40 °C before being pulverized to powder with a particle size less than 200 µm. POSs were produced from the dried pomelo peel powder by using H_2O_2 under alkaline condition, as described by Wandee et al. [13], with minor modifications. Briefly, dried pomelo peel powder was hydrolyzed with 5% (*v*/*v*) H_2O_2 in 50 mM NaOH at 80 °C for 5 h with continuous stirring. The mixture was collected and centrifuged at 6136× *g* for 10 min. Absolute ethanol was added to the supernatant at a ratio of 1:1 (*v*/*v*) and kept overnight at 4 °C. The suspension was centrifuged at 6136× *g* for 20 min. The pellet was collected and washed 3–4 times with 95% ethanol to obtain POS-I, whereas another 2 vol of absolute ethanol was added to the supernatant and kept overnight at 4 °C. The precipitate was collected to obtain POS-II. The POS precipitates were dried in a hot air oven at 40 °C for 24 h. The dried POS was ground and sifted through a 200 µm sieve to obtain fine powders.

For the POS size distribution analysis, 20 µL of POS solution was injected into a Shimadzu HPLC system consisting of LC-20AD pump, RID-10A detector, two serially linked columns (both Shodex OHpak SB-802.5 HQ, 8 mm × 300 mm, Showa Denko K.K., Tokyo, Japan) with a specified guard column, and a computer with a data processing software program (CLASS-VP). An isocratic elution with 0.1 M $NaNO_3$ containing 0.01 M NaN_3 was carried out at 50 °C and a flow rate of 0.8 mL·min^{-1}. Galacturonic acid, digalacturonic acid and trigalacturonic acid were used as standards.

2.3. Imbibition Curve

Seeds were disinfected by soaking in 1% (*v*/*v*) sodium hypochlorite containing 0.2% (*v*/*v*) Tween 80 for 30 min and rinsed three times with sterilized water. The seeds were dried overnight in an oven at 37 °C. Five grams of sterilized seeds were soaked in 25 mL of sterilized water (or 500 ppm POS solution) for 72 h with shaking at 30 °C. At specified times, 0.3 g of soaked seeds were collected, weighed, and dried at 105 °C for 48 h. Three

replicates of each treatment were conducted, and the water content of seeds was calculated using the following equation:

$$\text{Water content } (\%) = \left(\frac{W_s - W_d}{W_d}\right) \times 100 \quad (1)$$

where W_s and W_d are the weights of soaked seeds and dried seeds, respectively.

2.4. Seed Priming Treatment

Five grams of disinfected seeds were fully immersed in 25 mL of priming media (water and 500 ppm POS solution) with shaking at two temperatures, 30 and 50 °C. Priming time was based on the early time of the stationary phase (phase II) of the imbibition curve. After priming, seeds were washed with sterile water, removed of excess water, and dried in an oven at 37 °C for 48 h. The dried primed seeds were kept at room temperature until use.

2.5. Determinations of Seed Germination and Seedling Characteristics

Seeds were germinated on a filter paper wetted with 5 mL of distilled water in Petri dishes and kept in the dark at 30 ± 2 °C. The seed growth was conducted over a nine-day period. Each seed soaking treatment was replicated three times with 50 seeds each. The approach developed by the Association of Official Seed Analysts (AOSA) [14] was used to count seed germination on a daily basis. A seed was deemed germinated when the radical length was at least 2 mm long. Other seed growth characteristics including percentage of germination and vigor index [15] were evaluated using the following equations.

$$\text{Percentage of germination } (\%G) = \frac{\text{Total no. of germinated seeds}}{\text{Total no. of initial seeds}} \times 100 \quad (2)$$

$$\text{Vigor index} = \%G \times \text{seedling length (cm)} \quad (3)$$

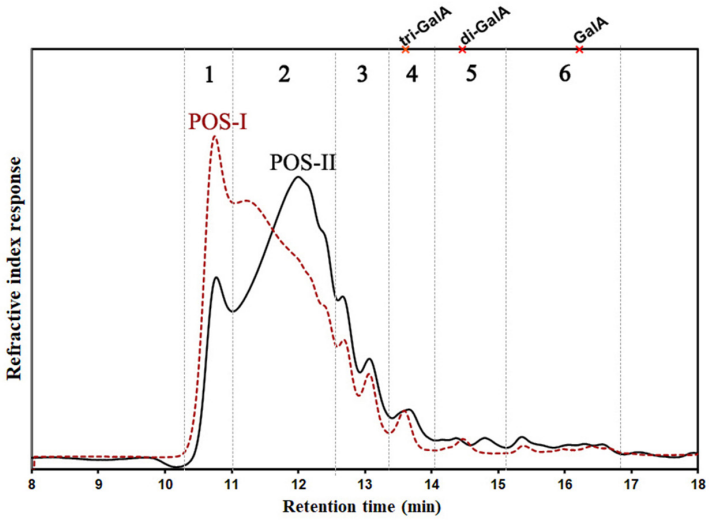
2.6. Statistical Analyses

The experimental data were analyzed using analysis of variance (ANOVA) and expressed as mean values ± standard deviations. A Tukey test was conducted to examine significant differences among experimental mean values ($p \leq 0.05$).

3. Results and Discussion

3.1. Size Distribution of POS

HPSEC chromatograms of POS-I and POS-II, and relative amount of POS species contained in each sample (% weight basis) are shown in Figure 1. The lower retention time indicates the larger molecular size of POS species. Although the same molecular size range was shown in both POS fractions, the relative area under the peaks of POS-I and POS-II was clearly different. A higher relative area under area #1 and a lower relative area under area #2 − #6 of POS-I indicated that POS-I contained a higher proportion of large-sized POS species than POS-II. The percent masses of POS species having a size larger than > DP5 of POS-I and POS-II were 81.9 and 73.8%, respectively.



Area No.	Relative area (%)					
	1	2	3	4	5	6
POS I	24.0	57.9	10.2	2.8	2.0	3.1
POS II	12.4	61.4	14.0	4.3	3.4	4.5

Figure 1. HPSEC chromatograms of POSs and relative amount of POS species in POS-I (dot line) and POS-II (solid line).

3.2. Imbibition Curve

Imbibition curves of chili seeds when soaked in water and POS solutions for 48 h are shown in Figure 2. Generally, the imbibition curve of the seeds can be divided into three stages; imbibition, metabolism activation and elongation [16]. For the imbibition stage, the seed imbibes water rapidly to activate enzyme activity. Water contents of chili seeds in all treatments jumped from 1.2% to 39.8–42.2% during the first 2 h.

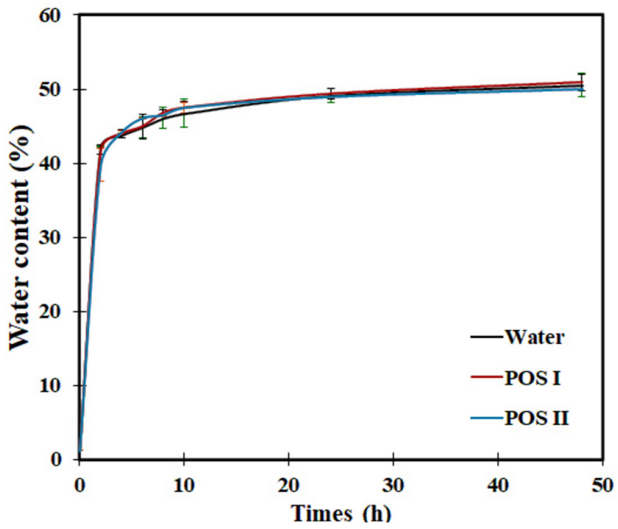


Figure 2. Imbibition curves of chili seeds when soaked in water, and 500 ppm of POS-I and POS-II solutions.

After 2 h, the seeds still further imbibed water but the rate of water imbibition decreased markedly. Water contents of seeds soaked in water and POS solutions were in the range of 42.2–50.5% and 39.8–50.9%, respectively. The metabolic processes of seeds were activated during this phase to prepare seeds for germination. However, the germination was not observed in during this period.

3.3. Germination and Seed Growth Parameters

Germination curves of chili seeds primed with different treatments are shown in Figure 3. The germination curve of chili seeds primed at 30 °C indicated that germination rates of seeds primed with POS solutions were significantly higher than that with hydropriming, especially after 6 d of incubation. Seeds primed with POS-I showed slightly higher germination percentage than those with POS-II. When compared to the non-primed seeds, seeds primed with POS solutions showed observably higher germination. Priming at 50 °C had adverse effect on seed germination, i.e., the germination percentage was reduced by 24.4–31.9% compared to seeds primed at 30 °C.

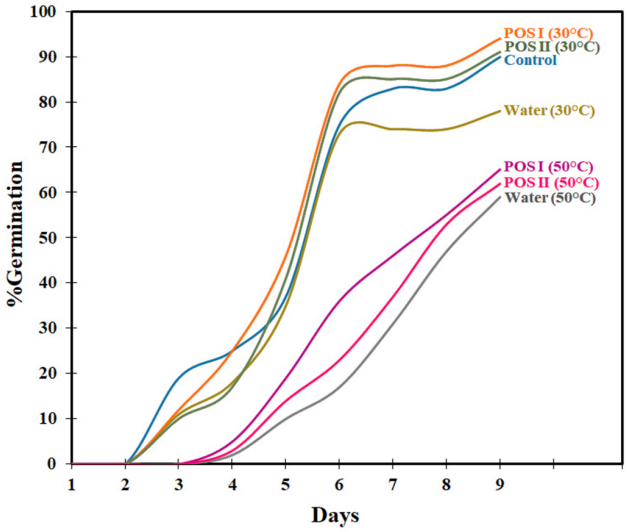


Figure 3. Germination curve (cumulative germination percentage vs. time, in d) of chili seeds after priming with different treatments.

The growth parameters of chili seedlings after incubating in Petri dish for 9 d are summarized in Table 1. The non-primed and hydroprimed seeds developed shoots 11.6 and 15.7 mm in length on average, respectively. Under the same cultivation environment, the seeds primed with POS-I and POS-II solutions demonstrated significantly longer shoots, 17.7 and 18.1 mm, respectively, while the root length in all treatments was not significantly different. POSs also increased the chili seedling length and vigor index by 27.9–35.4% and 30.6–41.3%, respectively, when compared to non-priming treatment. However, there were no significant differences in all growth parameters between POS-I and POS-II treatments. Priming the seeds at 50 °C resulted in a significant reduction in chili seed growth, possibly due to inactivation of some enzymes in chili seeds [17].

Table 1. Root length, shoot length, seedling length and vigor index of chili seeds primed with water, and 500 ppm of POS-I and POS-II solutions at 9 d after sowing compared to non-primed seeds (control).

Treatments	Root Length (mm)	Shoot Length (mm)	Seedling Length (mm)	Vigor Index
Non-priming (Control)	11.2 ^{BC}	11.6 ^{B}	22.9 ^{B}	2062.0 ^{C}
Priming at 30 °C				
Water	14.1 ^{A}	15.7 ^{A}	29.8 ^{A}	2322.0 ^{BC}
POS-I	13.3 ^{AB}	17.7 ^{A}	31.0 ^{A}	2914.0 ^{A}
POS-II	11.2 ^{BC}	18.1 ^{A}	29.3 ^{A}	2693.9 ^{AB}
Priming at 50 °C				
Water	6.0 ^{E}	6.2 ^{C}	12.2 ^{D}	720.0 ^{E}
POS-I	7.4 ^{DE}	6.8 ^{C}	14.2 ^{CD}	925.7 ^{DE}
POS-II	9.6 ^{CD}	9.4 ^{BC}	19.0 ^{BD}	1178.0 ^{D}

Means with different letters (^{A}, ^{B}, ...) within columns are significantly different at $p \leq 0.05$.

4. Conclusions

Pectic-oligosaccharides (POS) derived from pomelo peel can promote chili seed development, as evidenced by the increase in the germination percentage and vigor index of seeds after hydropriming treatment compared to non-primed seeds. Although the percentage of germination of seeds primed with POS-I was slightly higher than that of those primed with POS-II, there was no significant difference in chili seed growth parameters. Priming of chili seeds at a high temperature (50 °C) had adverse effects on seed germination and seedling growth.

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