Synergistic effects of harpin and NaCl in determining soybean sprout quality under non-sterile conditions

Abstract: Salinity induces antioxidant accumulation but always enhances disease susceptibility in plants. However, the effects of NaCl in regulating disease infection in soybean sprouts have not been well studied under conditions that are not strictly aseptic. Here, the effects of harpin and NaCl in controlling disease development and antioxidant accumulation were investigated in soybean sprouts under non-sterile conditions. After storage for 0 and 7 days, NaCl-enhanced disease severity was drastically suppressed by application of either harpin or salicylic acid (SA), relative to controls. Moreover, the decrease in SA content due to salinity was partially restored by harpin. Interestingly, the combined treatment with both NaCl and harpin drastically enhanced the accumulation of antioxidants (e.g., ascorbic acid and phenolics) and the total antioxidant ability (evaluated by Fe³⁺ reducing power) in soybean sprouts, compared with individual NaCl and harpin treatments. Compared with individual treatments, the synergistic effects of NaCl and harpin in eliciting phenylalanine ammonia-lyase and polyphenol oxidase activities were also examined in postharvest soybean sprouts. The results suggest that harpin drastically improved the quality of salt-treated soybean sprouts by reducing disease severity and elevating antioxidant accumulation during storage under non-sterile conditions.

Keywords: bioactive metabolite, disease infection, elicitor, salinity, soybean sprout

1 Introduction

Plant sprouts are consumed worldwide due to their high nutritive value, ease of cultivation, and sustainable production [1]. For example, soybean sprouts are widely used in many kinds of foods such as soymilk and tofu [2]. Research has shown that germination produces high levels of secondary metabolites (e.g., polyphenols) in vegetables [3,4]. In general, these secondary metabolites are considered to be beneficial antioxidants, and they can vary dramatically in level and type during sprouting [5]. Interestingly, this
sprouting-induced secondary metabolite accumulation can be further enhanced by application of elicitors in vegetables [6]. For example, moderate salinity has long been used as a strategy to increase secondary metabolite biosynthesis [7–9]. However, stress due to salinity (e.g., NaCl) affects plant tolerance to biotic stress (e.g., fungi) by attenuating their immunity to pathogens, leading to downregulated defense gene expression and attenuation of defense signaling activation, as demonstrated for salicylic acid (SA) [10].

In general, contamination of sprouts by microbial pathogens is a constant problem due to the presence of pathogenic bacteria and fungi on seeds; and the germination and sprouting process provides optimal conditions for microbial growth and proliferation [11,12]. This makes the sprouts potential sources of foodborne infections and microbial toxins [13,14]. Thus, contamination of sprouts has become a worldwide food safety concern. To increase food safety, many methods (e.g., ultrasonication, blanching, and gamma irradiation) have been developed to prevent microbial pathogen proliferation in sprouts [13,14]. Moreover, elicitors such as harpin have also been used to control disease development in fruit during storage [15].

Harpins are a group of heat-stable, glycine-rice proteinaceous bacterial elicitors produced by Erwinia amylovora and other plant pathogenic bacteria. Harpins are now commonly used in various biological agricultural practices [16,17]. Interestingly, harpins can induce antioxidant accumulation in vegetables and fruits during storage [9,15,18]. Harpins can induce the hypersensitive response and enhance disease resistance [19,20]. In addition, they can stimulate phenylalanine ammonia-lyase (PAL, EC 4.3.1.5; a key enzyme in polyphenol biosynthesis) and polyphenol oxidase (PPO, EC 1.14.18.1; an enzyme that rapidly degrades polyphenols) activities, and enhance levels of phenolics in plants [17,18]. Furthermore, the SA signaling pathway is required for harpin-activated disease resistance in plants [20]. However, abscisic acid (ABA) signaling, which is always induced by salinity [21], is always antagonistic with SA signaling during plant–pathogen interactions [10].

Many recent reports have shown that treatment with two different chemicals consistently achieves better elicitation effects than individual treatment [22,23]. For example, applying SA and NaCl enhanced the formation of anticancer isothiocyanates in broccoli sprouts compared with individual treatment [24]. In the present study, soybean seeds and sprouts were treated with two different elicitors (harpin and NaCl), together and separately, and the effects were explored. We explored whether NaCl enhances or reduces disease development in soybean sprouts under non-sterile conditions and whether harpin suppresses disease infection in soybean sprouts treated with salt. The potential underlying mechanisms were also investigated. This study provides a simple, effective method for elevating the quality of postharvest soybean sprouts under not strictly aseptic conditions.

2 Materials and methods

2.1 Reagents

All reagents including ABA, SA, polyvinylpyrrolidone (PVP), 2,6-dichlorophenolindophenol (2,6-DPI), and l-phenylalanine used in experiments were of analytical grade and were purchased from Sigma-Aldrich Company (Steinheim, Germany).

2.2 Experimental design

Soybean seeds (Glycine max L. Yudou 16) were obtained from Luoyang Kechuang Seed Co. (Luoyang, China). Healthy seeds of uniform size were selected for use in subsequent experiments (a schematic flowchart of the study design is shown in Figure 1). Seeds were rinsed and then steeped in tap water at 25°C for 1 h. In subsequent elicitor treatments, soybean seeds were soaked in water containing 0 (control), 10, 30, or 100 mg L⁻¹ harpin protein (Messenger, Eden Bioscience Co., Bothell, WA) and 0 (control), 50, 100, or 200 mM NaCl; or NaCl (100 mM) and harpin (30 mg L⁻¹) for 0.5 h; then, they were transferred to fine sand moistened with tap water, and incubated in a dark chamber at 25°C for germination and sprouting. In addition, soybean seeds were soaked in SA (50 µM) or ABA (5 µM) solutions for 0.5 h before being transferred to fine sand moistened with tap water or salt solution (100 mM NaCl). After sowing for 1, 4, and 7 days, soybean sprouts were collected for determining the germination rate and sprout growth experiments. Moreover, soybean sprouts were stored at 25°C for 0 and 7 days and then collected for disease index assays. Similarly, soybean sprouts were stored at 25°C for 0 and 7 days, then flash-frozen in liquid N₂ and stored at ~−80°C for subsequent biochemical analyses of ascorbic acid levels, total phenolic accumulation, total antioxidant capacity, PAL and PPO activities, and ABA and SA contents. For all assays, each treatment consisted of three replicates of 100 seeds.
2.3 Seed germination, sprout length, and disease index assay

Soybean seeds with a radical length of 1 mm were defined as having germinated. The number of germinated seeds was counted daily, and the germination rate was calculated during the first 7 days after sowing. Sprout lengths (excluding roots) were measured using vernier callipers.

Disease severity was evaluated during soybean sprout growth and storage using the scoring system described previously by Knudsen et al. [25]: 0, healthy seedlings; 1, coleoptile and roots with slight browning; 2, coleoptile and roots with moderate browning; 3, coleoptile and roots with severe browning; and 4, dead seedlings. A change in disease severity was evaluated by comparing the disease state of harpin-treated sprouts with that of the water-treated controls.

2.4 ABA and SA assays

For ABA assays, sprouts were ground in 80% methanol (4°C) and the filtrate was evaporated under vacuum at 35°C. The residue was dissolved in acidic water (pH 3.0) to acidify the extract, which was mixed with an equal volume of cold diethyl ether. The ether phase was separated and dried over anhydrous Na₂SO₄ and the extract was incubated at 4°C for 12 h. Finally, the extract was filtered, the ether was evaporated, and the residue was dissolved in 50 mM Tris buffer (pH 7.8) for ABA assay [26]. An ELISA kit (Panrui Biotechnique Company, Shanghai, China) was used for ABA measurement.

SA content was determined as described previously [27] with some modifications. Sprout samples (200 mg) were ground in liquid N₂, extracted with 4 mL of 90% (v/v) methanol for 0.5 h, and centrifuged at 9,500 × g for 20 min at room temperature. Distilled water (50 mL) and 50 µM Na₂SO₄ buffer (100 mL, pH 7.0) were used for the separation of the collected supernatants on a diethylaminoethyl cellulose chromatography column. Trichloroacetic acid (pH 2.5; 5%) was used to adjust the eluant, which was then separated on a Sep-Pak C₁₈ column (5 mm, 250 mm × 4.6 mm; Waters, USA) in 80% methanol (v/v). The SA eluant was collected for HPLC analysis on an HP 1100 Series instrument (Agilent, USA).

2.5 Ascorbic acid, total phenolics, and total antioxidant capacity assays

The ascorbic acid content of soybean sprouts was determined using the 2,6-DPI titrimetric method [10]. Soybean sprout samples (5 g) were homogenized and mixed with 100 mL of 2% oxalic acid. The mixture was diluted to 500 mL with 2% oxalic acid and filtered. The filtered solution was titrated with 2,6-DPI solution (0.01%), and a pink color for 15 s indicated the endpoint.

The Folin–Ciocalteu reagent was used for determining the total phenolics of soybean sprouts [28]. Dry samples (10 g) were frozen in liquid N₂, ground to a powder, suspended in 1 L of methanol/water solution (70%, w/v), and
incubated at 25°C for 3 days in the dark. Extracts were filtered, and filtrates were collected and stored in a desicator at 4°C until used for total phenolics analysis. Next, 10 mL of crude extracts were mixed with 50 mL of the Folin–Ciocalteu reagent and incubated for 3 min in the dark, and 40 mL of 0.7 M sodium carbonate was added. After incubation at 25°C for 2 h in the dark, the absorbance was monitored at 760 nm.

Sprout samples (10 g) were flash-frozen in liquid N2 ground to a powder, suspended in 1 L of ethanol/water (7:3, v/v) solution, and incubated in the dark at 4°C for 72 h. Filtrates of the extracts were collected and stored at 4°C for measurement of total antioxidant capacity, which was evaluated using the ferric-reducing ability of plasma (FRAP) assay [29]. The FRAP reagent was prepared freshly that contained 20 mM FeCl3 solution, 10 mM 2,4,6-tripyridyl triazine solution in 40 mM HCl, and acetate buffer (pH 3.6) in 1:1:10 (v/v) proportions. Different amounts of extract filtrates (10–100 µL) were mixed with 1.5 mL of the FRAP reagent, incubated for 10 min, and the absorbance of the reaction mixture was measured at 593 nm.

2.6 Defense enzyme assays

Fresh samples of soybean sprouts (5 g) were ground with 50 mL of chilled sodium borate buffer (0.1 M, pH 8.7). The homogenate was collected and centrifuged at 16,000 × g for 20 min at 4°C, and the supernatant was collected for PAL assay. Enzyme activities were measured as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm [30].

Fresh samples of soybean sprouts (2 g) were ground with 8 mL of sodium phosphate buffer (50 mM, pH 5.8) containing 0.2 g of PVP. Extracts were homogenized, centrifuged at 12,000 × g for 30 min at 4°C, and supernatants were collected for PPO assay. Next, 0.1 mL of supernatant was added to the reaction mixture consisting of 2 mL of sodium phosphate buffer (50 mM; pH 5.8) and 0.5 mL of catechol (500 mM) at 25°C. After 5 min, the absorbance at 420 nm was measured every 15 s for 2 min [31]. One unit (U) of PPO activity was defined as an increase of 0.01 absorbance unit per minute at 25°C.

The soluble protein concentration was measured with the Bradford method [32] using bovine serum albumin as the standard.

2.7 Data analysis

All experiments were performed using a completely randomized design, and there were three replicates for each treatment. All data were analyzed using Duncan’s multiple range test (p < 0.05) using SPSS 13.0 software (IBM Cop., Armonk, NY, USA).

3 Results and analysis

3.1 Seed germination and sprout growth

Compared with the control, both NaCl and harpin profoundly affected soybean seed germination and sprout growth (Figure 2). For example, treatment with 100 mM NaCl decreased the germination rate by ~16, ~6, and ~5% at 1, 4, and 7 days after sowing, respectively, compared with the untreated control (Figure 2a; p < 0.05). In contrast, treatment with 30 mg L−1 harpin increased the germination rate by ~29, ~5, and ~3% at 1, 4, and 7 days after sowing, respectively, compared with the control (Figure 2b; p < 0.05). Similarly, 100 mM NaCl treatment decreased the sprout length by approximately 14, 7, and 6% at 1, 4, and 7 days after sowing, respectively, compared with the control (Figure 2c; p < 0.05). However, the 30 mg L−1 harpin treatment enhanced the sprout length by approximately 53, 38, and 44% at 1, 4, and 7 days after sowing, respectively, compared to the control (Figure 2c; p < 0.05). Moreover, high concentrations of NaCl were found to inhibit seed germination and sprout growth (Figure 2a and c). In contrast, treatments with high concentrations of harpin further enhanced seed germination and sprout growth (Figure 2a and d). For example, 200 mM NaCl reduced the germination rate and sprout growth by approximately 30 and 38%, respectively, compared with the control at 4 days after sowing (Figure 2a and c; p < 0.05). Treatment with 100 mg L−1 harpin enhanced the germination rate and sprout growth by approximately 38 and 60%, respectively, 1 day after sowing compared to the control (Figure 2b and d; p < 0.05).

3.2 Disease development in soybean sprouts

Compared with the control, NaCl + harpin treatments drastically affected postharvest disease development in soybean sprouts during storage (Table 1). Treatment with 100 mM NaCl profoundly increased the disease index by approximately 123 and 176% compared to the control after storage for 0 and 7 days, respectively (Table 1, p < 0.05). In contrast, treatment with 30 mg L−1 harpin reduced the disease index by approximately 54 and 57% compared to the control at 0 and 7 days after harvest, respectively (Table 1, p < 0.05). Similarly, treatment with 50 µM SA decreased the
disease index by approximately 35 and 30% compared to the control after 0 and 7 days of storage, respectively (Table 1, \( p < 0.05 \)). However, treatment with 5 \( \mu \text{M} \) ABA increased the disease index by approximately 96 and 83% compared to the control after storage for 0 and 7 days, respectively (Table 1, \( p < 0.05 \)). Compared with the NaCl treatment, the combined treatment with NaCl + harpin reduced the disease index by approximately 64 and 79% in soybean sprouts after storage for 0 and 7 days, respectively (Table 1, \( p < 0.05 \)). Similarily, treatment with NaCl + SA reduced the disease index by approximately 59 and 69% in soybean sprouts after storage for 0 and 7 days, respectively, compared to the salt-only treatment (Table 1, \( p < 0.05 \)). Moreover, the NaCl + ABA treatment increased the disease index by approximately 34 and 44% in soybean sprouts after storage for 0 and 7 days, respectively, compared to the NaCl treatment alone (Table 1, \( p < 0.05 \)).

### 3.3 Effects of harpin and NaCl on ABA and SA contents

The effects of NaCl, harpin, and their combination on the contents of ABA and SA in soybean sprouts were investigated during storage (Table 2). Compared with the control,

**Table 1: Disease index of soybean sprouts**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0-day post-harvest</th>
<th>7-day post-harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.6 ± 0.4(^a)</td>
<td>9.7 ± 0.3(^d)</td>
</tr>
<tr>
<td>NaCl</td>
<td>5.8 ± 0.5(^b)</td>
<td>26.8 ± 1.7(^a)</td>
</tr>
<tr>
<td>Harpin</td>
<td>1.2 ± 0.3(^c)</td>
<td>4.2 ± 0.4(^g)</td>
</tr>
<tr>
<td>SA</td>
<td>1.7 ± 0.1(^c)</td>
<td>6.8 ± 0.3(^e)</td>
</tr>
<tr>
<td>ABA</td>
<td>5.1 ± 0.4(^d)</td>
<td>22.3 ± 1.3(^f)</td>
</tr>
<tr>
<td>NaCl + harpin</td>
<td>2.1 ± 0.2(^b)</td>
<td>5.7 ± 0.6(^f)</td>
</tr>
<tr>
<td>NaCl + SA</td>
<td>2.4 ± 0.3(^b)</td>
<td>8.4 ± 0.5(^e)</td>
</tr>
<tr>
<td>NaCl + ABA</td>
<td>7.8 ± 0.8(^b)</td>
<td>38.5 ± 2.2(^e)</td>
</tr>
</tbody>
</table>

The effects of water (control), NaCl (100 mM), SA (50 \( \mu \text{M} \)), ABA (5 \( \mu \text{M} \)), harpin (30 mg L\(^{-1}\)), NaCl + harpin, NaCl + SA, and NaCl + ABA treatments on the disease index (%) of soybean sprouts at 0 and 7 days post-harvest. Mean values associated with the same letter (superscript) are not significantly different (\( n = 3 \); \( p < 0.05 \)).

**Table 2: ABA and SA content**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ABA content</th>
<th>0 day</th>
<th>7 day</th>
<th>SA content</th>
<th>0 day</th>
<th>7 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.9 ± 1.1(^a)</td>
<td>7.8 ± 0.6(^a)</td>
<td>2.6 ± 0.2(^a)</td>
<td>1.9 ± 0.1(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>35.8 ± 1.3(^a)</td>
<td>19.3 ± 1.5(^c)</td>
<td>1.7 ± 0.2(^d)</td>
<td>1.3 ± 0.1(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harpin</td>
<td>9.1 ± 0.8(^a)</td>
<td>5.6 ± 0.4(^d)</td>
<td>5.3 ± 0.3(^b)</td>
<td>3.5 ± 0.2(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl + harpin</td>
<td>23.1 ± 1.8(^b)</td>
<td>14.1 ± 1.2(^d)</td>
<td>3.3 ± 0.2(^b)</td>
<td>2.6 ± 0.2(^d)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Effects of harpin (30 mg L\(^{-1}\)) and NaCl (100 mM) on ABA (ng g\(^{-1}\) dry weight) and SA (\( \mu \text{g} \) g\(^{-1}\) dry weight) levels in soybean sprouts at 0 and 7 days post-harvest. Mean values associated with the same letter (superscript) are not significantly different for each phytohormone (\( n = 3 \); \( p < 0.05 \)).
treatment with NaCl and NaCl + harpin increased ABA levels by ~158 and ~66%, respectively, in soybean sprouts after storage for 0 days (Table 2; p < 0.05). In contrast, harpin reduced the ABA content by ~35 and ~28% in soybean sprouts after storage for 0 and 7 days, respectively, compared to the control (Table 2; p < 0.05). Similarly, NaCl decreased SA content by ~35 and ~32% in soybean sprouts stored for 0 and 7 days, respectively, compared to the control (Table 2; p < 0.05). However, harpin and NaCl + harpin increased SA by ~130 and ~27%, respectively, in soybean sprouts after storage for 0 days (Table 2; p < 0.05).

3.4 Antioxidant accumulation and enzyme activities

Treatment with NaCl, harpin, and the combination increased ascorbic acid accumulation, total phenolic content, and total antioxidant capacity in soybean sprouts during storage (Figure 3). For example, treatment with NaCl, harpin, and NaCl + harpin increased ascorbic acid content by ~110, ~33, and ~156%, respectively, in soybean sprouts compared to the control after storage for 0 days (Figure 3a; p < 0.05). Similarly, NaCl, harpin, and NaCl + harpin treatments increased the total phenolic contents by ~51, ~36, and ~81%, respectively, in soybean sprouts compared to the control after storage for 0 day (Figure 3b; p < 0.05). Moreover, the total antioxidant capacity increased by ~57, ~29 and ~104% after NaCl, harpin, and NaCl + harpin treatments, respectively, compared with controls after storage for 7 days (Figure 3c; p < 0.05).

NaCl, harpin, and NaCl + harpin treatments increased PAL and PPO activities in soybean sprouts to different extents during storage (Figure 3d and e). Moreover, the harpin + salt treatment increased PAL activity by ~24 and ~34%, respectively, compared to the salt-treated soybean sprouts at 0 and 7 days of storage (Figure 3d; p < 0.05). Compared with controls,

Figure 3: Antioxidant and enzyme activities. The effects of water (control), NaCl (100 mM), harpin (30 mg L⁻¹), and NaCl + harpin treatments on ascorbic acid accumulation (a), total phenolics content (b), and total antioxidant capacity (c), as well as the activities of PAL (d) and PPO (e) in soybean sprouts at 0 and 7 days post-harvest. Bars represent the standard deviation of the mean (n = 3); mean values associated with the same letter are not significantly different (p < 0.05).
NaCl, harpin, and NaCl + harpin treatments increased PPO activity by ~14%, ~25%, and ~42%, respectively, in soybean sprouts after storage for 0 days (Figure 3e; \( p < 0.05 \)). Similar trends were also observed for PAL and PPO activities after storage for 7 days (Figure 3d and e; \( p < 0.05 \)).

4 Discussion

In general, eustress or positive stress (e.g., moderate salinity) can drastically enhance the levels of bioactive ingredients and the quality characteristics of sprouts [8,22,33]. Here, 100 mM NaCl and 30 mg L\(^{-1}\) harpin could drastically promote soybean seed germination and sprout growth compared with control and high-concentration samples (Figure 2). Thus, 100 mM NaCl and 30 mg L\(^{-1}\) harpin were used as elicitors in subsequent experiments.

Elicitation is an established strategy for enriching the bioactive composition of sprouts [6]. However, most previous studies on seed germination and sprouting were performed under sterile conditions (e.g., seeds were disinfected before germination). In the present study, we explored whether NaCl treatment affects the disease infection and antioxidant accumulation of postharvest soybean sprouts under non-sterile conditions. As shown in Table 1, the results showed that NaCl treatment alone increased disease development in soybean sprouts under non-sterile conditions. However, this NaCl-enhanced disease severity was dramatically suppressed by harpin and SA (Table 1). Moreover, the effect of NaCl on disease severity was aggravated in the NaCl + ABA treatment in soybean sprouts during storage (Table 1). It is well-known that salt can induce biosynthesis and accumulation of ABA in plants [21]. Moreover, harpin can induce SA accumulation in plants [34]. In a further study, the effects of harpin, NaCl, and harpin + NaCl treatments on ABA and SA levels in soybean sprouts were also investigated (Table 2). The results showed that NaCl treatment increased the ABA content but decreased SA accumulation in soybean sprouts (Table 2). However, the salt-increased ABA accumulation was partly attenuated by harpin (Table 2). Accordingly, the increased SA level and decreased ABA accumulation due to harpin treatment would both contribute to the enhanced pathogen resistance in NaCl-treated soybean sprouts during storage under non-sterile conditions (Table 1). This suggests that the reduced disease resistance caused by salt treatment can be partly attributed to the inhibition of SA, which is required for most pathogen resistance in plants [9]. Typically, abiotic stress (e.g., salinity) negatively affects plant susceptibility to disease [35]. With few exceptions, ABA plays a negative role in regulating disease resistance in plants by interfering with biotic stress signaling, which is regulated by SA, jasmonic acid, and ethylene [10]. This can also partly explain why NaCl treatment can increase disease severity in soybean sprouts under non-sterile conditions.

Abiotic and biotic elicitors have been applied to sprouts to increase secondary metabolite accumulation and biological activities [36]. For example, applying salinity (e.g., NaCl) improves antioxidant accumulation in vegetable sprouts [8,33,37]. Moreover, studies have also shown that harpin application can induce antioxidant accumulation in vegetables and fruits during storage [9,15,18,38]. However, enhanced disease resistance in plants may also attenuate abiotic stress tolerance [10,39], which is closely associated with the antioxidant defense system [40]. We, therefore, explored whether the harpin-enhanced disease resistance attenuated antioxidant metabolite accumulation and antioxidant capacity in NaCl-treated soybean sprouts under not strictly aseptic conditions. The results showed that NaCl + harpin treatment drastically increased ascorbic acid accumulation, total phenolic content, and total antioxidant capacity (as determined by Fe\(^{3+}\) reducing power) compared to the individual NaCl and harpin treatments (Figure 3a–c). This result is partially consistent with the results of a previous study showing that applying SA enhanced bioactive compound accumulation in Giant Juncao under saline conditions [41]. Moreover, SA can increase antioxidant nutrient accumulation in sprouts and fruits during storage [42–44]. Therefore, SA, which can be induced by harpin [20], has multiple functions in plants, under both optimal and environmental stress conditions [45]. This suggests that the increased antioxidant accumulation and antioxidant capacity caused by harpin treatment could be partly attributed to this elicitor-induced SA under saline conditions.

Phenolics perform multiple functions in plant defense responses to abiotic and biotic stresses [46]. Our results showed that both harpin and NaCl could induce phenolics accumulation in postharvest soybean sprouts (Figure 3b). Previous studies showed that PAL is a key enzyme for phenolic biosynthesis [30], while PPO plays a key role in the rapid degradation of polyphenols [31]. Our results showed that harpin increases the accumulation of phenolic compounds, which is closely associated with pathogen resistance (Table 1) in soybean sprouts treated with NaCl (Figure 3b). How does harpin regulate the activities of two defense enzymes in soybean sprouts after exposure to salinity? Our results showed that harpin increased PAL and PPO activities in NaCl-treated soybean sprouts (Figure 3d and e). Similarly, a published study also showed that harpin induced PAL and PPO activities in fruits and
vegetables [9,18]. These results suggest that harpin-induced disease resistance can also be partly attributed to the increase in the accumulation of phenolics and phenolics-related enzyme activities of soybean sprouts treated with NaCl.

In general, microbial proliferation on soybean sprouts can be due to many factors of pre- and post-harvest contamination such as seeds, germination medium, and soaking water, as well as during storage of the seedlings [12]. In this experiment, we used non-sterile soybean seeds, a germination medium (fine sand), and tap water. It is possible that the results would have been somewhat better if sterilized seeds, germination medium, and water had been used. Thus, we have developed a new and effective method based on a combined harpin and NaCl treatment for improving soybean sprout quality by reducing disease severity and increasing the accumulation of antioxidants. This method would favor the production of high-quality soybean sprouts even under not strictly sterile conditions.

5 Conclusions

Several interesting conclusions can be drawn from the results of our study. First, treatment with 100 mM NaCl drastically increased the ascorbic acid accumulation, total phenolic content, and total antioxidant capacity of soybean sprouts, coupled with an increase in the disease severity under non-sterile conditions. Second, treatment with 30 mg L\(^{-1}\) harpin also increased the ascorbic acid accumulation, total phenolic content, and total antioxidant capacity but with reduced disease development. Third, the salt-induced antioxidant accumulation in soybean sprouts can be further increased by harpin treatment, and harpin treatment reduced the salt-increased disease severity in postharvest soybean sprouts under not strictly aseptic conditions. Finally, harpin treatment increased the activities of two defense enzymes (PAL and PPO) and SA accumulation in soybean sprouts under saline conditions.

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