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Seaweed extract reduces foliar fungal diseases on carrot

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ABSTRACT

Greenhouse-grown carrot plants were sprayed with an extract (0.2%) of the seaweed *Ascophyllum nodosum* (SW) and then inoculated 6 h later with the fungal pathogens *Alternaria radicina* and *Botrytis cinerea*. Additional applications of SW were made 10 and 20 d after inoculation. Treated plants showed significantly reduced disease severity at 10 and 25 d after inoculation compared to control plants sprayed with water. SW was more effective than salicylic acid (SA) (100 μM) in reducing infection. Activity of certain defence-related enzymes, including peroxidase (PO), polyphenoloxidase, phenylalanine ammonia lyase, chitinase and β-1,3-glucanase, were significantly increased in plants treated with SW and SA compared to the control 12 h after treatment. The treated plants also had higher transcript levels of pathogenesis-related protein I (*PR-1*), chitinase, lipid transfer protein (*Ltp*), phenylalanine ammonia lyase (*Pal*), chalcone synthase, non-expressing pathogenesis-related protein (*NPR-1*) and pathogenesis-related protein 5 (*PR-5*) genes compared to control plants. These results show that SW enhances disease resistance in carrot, likely through induction of defence genes or proteins.

1. Introduction

Plants possess many adaptive defence mechanisms to counteract pathogen or insect attack. Following appropriate induction, plants are capable of mounting an enhanced defence capability, commonly referred to as induced resistance (Ton et al., 2006). Enhanced resistance to diseases in plants, both locally and systemically, can also be achieved by treatment with different agents, such as virulent or avirulent pathogens, cell wall fragments, synthetic chemicals and plant extracts (Walters et al., 2005). This type of induced resistance is not absolute but results in reduced disease incidence and damage due to fungal, bacterial and viral pathogens (Hammerschmidt, 1999). At the cellular level, the plant defence responses include an oxidative burst leading to cell death, changes in cell wall composition, synthesis of antimicrobial compounds such as phytoalexins, activation of defence genes and priming of host cells (Kuc, 2006). Systemic acquired resistance can also be induced by salicylic acid (SA) and related compounds, including benzo (1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester (BTH), 2,6-dichloro-isonicotinic acid and DL-3-amino-*n*-butyric acid (BABA), chitosan, oligosaccharides such as β-1,3-glucans, glucomannans and oligogalacturanides, and natural products containing these compounds (Walters et al., 2005). There are numerous reports of enhanced resistance in crops to various pathogens following application of commercial elicitor formulations containing acibenzolar-*S*-methyl, BTH, BABA and preparations containing carbohydrate elicitor molecules (Vallad and Goodman, 2004). These compounds are an environmentally friendly means of disease control (Vidhyasekaran, 1997) and could be utilized in organic farming and for vegetable cropping systems where application of synthetic fungicides or chemicals needs to be avoided.

Carrot (*Daucus carota* L.) is a root crop which is grown worldwide and fungal pathogens pose a major constraint to carrot production in the field and during storage (Ammirato, 1986). Several fungal diseases affect the carrot foliage, causing spotting and blighting, and losses due to these diseases can be severe. Modern-day mechanical harvesting necessitates the requirement of healthy foliage wherein roots are pulled out by their tops (Rubatsky et al., 1999). There are several species of *Alternaria* which infect carrot, including *A. dauci*, *A. radicina*, *A. caroteiincultae* and *A. petroselini* (Farrar et al., 2004). Alternaria black rot caused by *A. radicina* affects both the foliage and roots in the field and also post-harvest and is prevalent in most carrot-growing regions of the world (Simmons, 1995). Under optimal conditions, the pathogen causes severe foliar blight apart from crown infection. It is a serious pathogen of seed crops because infection may lead to significant seed losses (Beresniewicz and Duczmal, 1994). *Botrytis cinerea* infects senescent plant tissue and can also enter through wounds, causing disease on both foliage and roots at pre- and post-harvest stages. The diseased tissue becomes light brown and water-soaked, and as the lesions expand, they turn dark brown and the fungus sporulates profusely (Sharman and Heale, 1977).

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Disease control methods in carrot usually involve fungicide applications (Davis and Raid, 2002). While induced resistance has been extensively studied and reported as a means of disease control in other crops, there are only two previous reports for carrot. Chris et al. (2004) observed reduced infection by *Sclerotinia* on carrot roots treated with either chitosan hydrolysate or high molecular weight chitosan. Mercier and Kuc (1996) demonstrated the possibility of inducing systemic disease resistance in carrot leaves by using *Cercospora carotae* as the agent for both induction and challenge. Infection by *C. carotae* was reported to reduce the susceptibility of plants to subsequent infections by other foliar pathogens later in the growing season.

Ascophyllum nodosum is a temperate aquatic marine plant found in the Atlantic and Arctic seas and the plant has been widely studied for its properties, which include plant growth promotion and use in animal feed (Colapietra and Alexander, 2006). The seaweed is commercially available and some reports have indicated enhanced plant yield and health in different crops following application, although the mechanisms of action have not been determined (Norrie et al., 2002; Colapietra and Alexander, 2006). To study the potential elicitor and disease suppressive activities of the seaweed extract (SW), we utilized a commercial SW spray formulation (containing *A. nodosum* aqueous extract). For this study, we selected two carrot foliar fungal pathogens, namely *A. radicina* and *B. cinerea*, which are known to infect carrot and grow and sporulate well under laboratory conditions. Carrot plants treated with SW were assessed for enhanced resistance to *Alternaria* and *Botrytis* foliar blights, and the potential mechanism of disease resistance was studied.

2. Materials and methods

2.1. Plant material and treatments

Seeds of the carrot line High Carotene Mass (provided by Dr. Phil Simon, University of Wisconsin, USA) were surface sterilized in 10% Chlorox solution (containing 5.25% NaOCl) for 10 min and rinsed in sterile water and planted in pots (12 cm diam) containing Sunshine planting mix (Sun Gro Horticulture, Vancouver, BC, Canada). The pots were placed in a greenhouse at 22–28 °C, 70–85% relative humidity, 600–1000 $\mu mol\, m^{-2}\, s^{-1}$ light intensity and a 12 h photoperiod. After 8 weeks, the plants were sprayed with SW (0.2%) (containing protein/amino acids ~3–5%, lipid 1%, alginic acid 12–18%, fucose-containing polymers 12–15%, mannitol 5–6%, other carbohydrates 10–15%) (Acadian Seaplants Limited, Dartmoth, NS, Canada), or SA (Sigma) (100 μM), or distilled water (control) using a micro sprayer to run-off. Treatments were repeated at 10 and 20 d after inoculation. For a fungicide control, chlorothalonil-50% (Syngenta Canada, Guelph, Ontario, Canada) was sprayed at $2\, g\, l^{-1}$ concentration to run-off.

For protein and total RNA extraction, leaf samples were collected at 0, 12, 24, 48, 72 and 96 h after treatment and immediately frozen in liquid nitrogen and stored at −80 °C.

2.2. Effect of SW and SA treatments on disease incidence

Pure cultures of the fungal pathogens *A. radicina* (provided by Dr. Barry M. Pryor, University of Arizona, Tucson, USA) and *B. cinerea* isolated from infected carrot leaves were maintained on V8 and PDA media, respectively. To study the effect of treatments on disease development, the treated carrot plants (6 h after treatment) were inoculated with a conidial suspension (1×10^6 conidia ml^{-1}) of *A. radicina* or *B. cinerea* using an atomizer and incubated in a humid chamber for 72 h. The plants were then transferred to a greenhouse and grown under the conditions described earlier. For the fungicide treatment, chlorothalonil was sprayed 7 d after inoculation. Combinations of fungicide plus elicitor treatments were also made by applying chlorothalonil at 10 d instead of an elicitor treatment followed by an elicitor application at 20 d.

Plants were scored for disease severity 10 d after inoculation (dai) and again at 25 d using a 6-point disease rating scale based on the percentage of leaf area infected (1 = 0%; 2 = 1–10%; 3 = 11–25%; 4 = 26–40%; 5 = 41–55%; 6 = >56%) (Jayaraj and Punja, 2007). Percent disease index was calculated as (sum of disease ratings of individual leaves/total number of leaves) × (100/maximum rating). The experiment was conducted three times with 25 replicate pots per treatment. Dry plant biomass was recorded at the end of each experiment.

2.3. Effect of treatments on gene expression

Total RNA was extracted from 300 mg of carrot leaf tissues from pooled samples collected from SW, SA and water-treated plants using Trizol reagent (Invitrogen) as per the manufacturer's protocol. RNA (10 μg) was run on a 1.4% (w/v) agarose formaldehyde gel and transferred overnight onto membrane (Hybond N+, Amersham). The blots were probed with [α-^{32}P] dCTP-labelled probes of carrot-pathogenesis-related protein-1 (*PR-1*; AB127984.1), chitinase (U52848), lipid transfer protein (*Ltp*; M64746), phenylalanine ammonia-lyase 1 (*Pal*; AB089813), chalcone synthase-2 (AJ006779), Arabidopsis *NPR-1* (NM_105102) and *PR-5* (NM_106161) genes (Jayaraj and Punja, 2007).

2.4. Enzyme assays

For the extraction of total proteins, leaf samples taken from SW, SA and water-treated plants were frozen in liquid nitrogen and homogenized with a pestle and mortar. The fine frozen powder was suspended in 0.1 M phosphate buffer, pH 6.5, containing 0.5 mM phenylmethyl sulfonyl fluoride and centrifuged at 10,000*g* for 15 min at 4 °C. Protein content of crude extracts was determined by the bicinchoninic acid microtitre plate assay kit (Pierce, Rockford, IL, USA).

The activity of peroxidases (POs) was assayed spectrophotometrically by using pyrogallol as a substrate. The PO activity was expressed as changes in absorbance $s^{-1}\, g^{-1}$ fresh weight of tissue. Polyphenoloxidase (PPO) activity was assayed using catechol as a substrate and the enzyme activity was expressed as catechol equivalents. Phenylalanine ammonia lyase (PAL) activity was assessed spectrophotometrically by assaying the rate of conversion of L-phenylalanine to *trans*-cinnamic acid at 290 nm. The amount of *trans*-cinnamic acid synthesized was calculated using its absorption coefficient of $9630\, \mu mol\, s^{-1}\, g^{-1}$. Enzyme activity was expressed as nmol *trans*-cinnamic acid/mg protein (Rahman and Punja, 2005). Chitinase activity was determined using *n*-acetyl glucosamine (NAG) as a substrate and activity was expressed as NAG units (Singh et al., 1999). Glucanase activity was assessed using laminarin as a substrate and β-1,3-glucanase activity was expressed as μmol glucose equivalents (Wood and Bhat, 1988). Lipoxigenase activity was estimated employing linoleic acid as a substrate and activity was expressed as linoleic acid equivalents (Alexrod et al., 1981).

2.5. Effect of treatments on biochemical changes

2.5.1. Estimation of phenolic content

Elicitor-treated and control leaves (1 g) from replicate plants were homogenized in 10 ml of 80% methanol and agitated for 15 min at 70 °C. One millilitre of the methanolic extract was added to 5 ml of distilled water and 250 μl of Folin Ciocalteau reagent (1 N) and the solution was kept at 25 °C. After 3 min incubation, 1 ml of saturated solution of Na_2CO_3 and 1 ml of distilled water were added and the reaction mixture was further incubated for 1 h at 25 °C. The absorption of the developed blue color was measured using a spectrophotometer at 725 nm. The total phenolic content was calculated based on standards prepared with phenol and expressed as phenol equivalents g^{-1} fresh weight (Rahman and Punja, 2005).

Table 1
Effect of seaweed extract, salicylic acid and fungicide on the incidence of *Alternaria* and *Botrytis* blights on carrot

Treatments[a]	Percent disease index (PDI)[b]			
	Alternaria		*Botrytis*	
	10 d	25 d	10 d	25 d
SW[c]	37.3 (37.62)	33.52 (35.37)	42.23 (40.53)	37.05 (37.49)
SA	53.2 (46.83)	48.22 (43.98)	54.5 (47.58)	51.04 (45.59)
SW+F	24.21 (29.47)	19.0 (25.84)	28.8 (32.46)	21.52 (27.64)
SA+F	46.12 (42.77)	42.37 (40.8)	47.5 (43.54)	42.53 (40.7)
Fungicide[d]	50.53 (45.3)	37.4 (37.7)	52.52(46.45)	41.14 (39.9)
Control	69.7 (56.6)	76.8 (61.23)	72.17 (58.17)	79.53(63.17)
LSD ($P = 0.05$)		1.34		2.07

[a] Elicitors were applied 6 h before inoculation and again 10 and 20 d after inoculation and disease severity was rated 10 and 25 d after inoculation.
[b] Mean percent disease index was calculated based on the percentage of leaf area affected using a 6 (1–6)-point disease rating scale. Values in parenthesis were angular transformed values.
[c] SW—seaweed extract; SA—salicylic acid; F—fungicide (chlorothalonil).
[d] Fungicide application was made 7 d after inoculation.

2.5.2. Estimation of 6-methoxymellin

Leaf samples from elicitor-treated and control plants were extracted for 6-methoxymellin (6-ME) content according to Lafuente et al. (1996). Extraction of 6-ME was performed for 12 h at ambient temperature using spectrophotometric-grade hexane. The solution was decanted and then re-extracted with an equal volume of 80% ethanol. Absorbance of the ethanol layer was measured at 267 nm and 6-ME was calculated using a molar absorptivity of 14,800.

2.5.3. Staining for H_2O_2 production

Production of hydrogen peroxide from reactive oxygen species (ROS) following treatment with SW, SA or water was visualized by staining the leaves with 3,3′-diaminobenzidine solution (1 mg ml^{-1}, pH 3.8) for 3 h. Tissues were decolorized by boiling

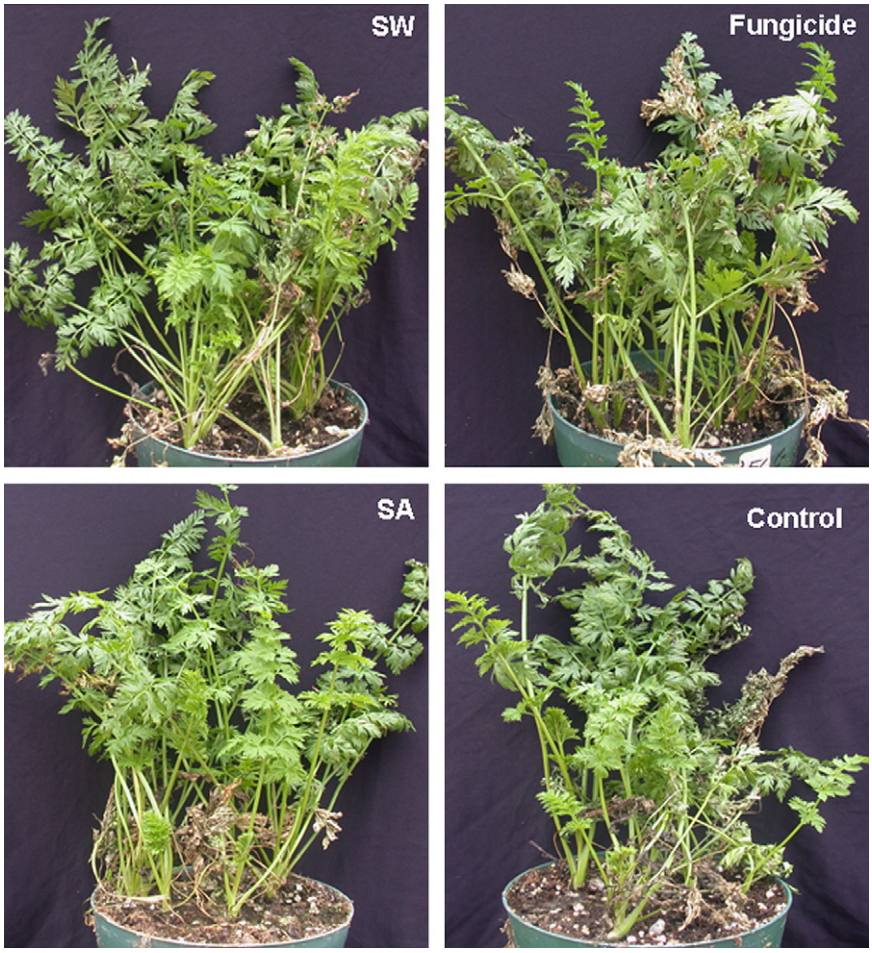

Fig. 1. Effect of seaweed extract and salicylic acid treatment on the incidence of *Alternaria* blight and growth of plants. Photographs were taken 25 d after inoculation. Treatments were made three times (0, 10 and 20 d). Fungal inoculation was made 10 h after the first treatment. Plants were placed in a humid chamber for 72 h and then maintained in a greenhouse.

in 95% ethanol for 20 min and viewed in the light microscope (Ganesan and Thomas, 2001).

2.7. Tissue nutrient analysis

Dried leaf tissues from elicitor-treated and control plants were ground to a powder in a blender. Two grams (dry wt) of the sample were analyzed for macronutrients (total nitrogen, phosphorus, potassium, calcium, magnesium, sodium, sulfur) and micronutrients (zinc, copper, iron, manganese, boron, molybdenum) using standard methods of analyses conducted by a commercial analytical lab (Norwest Laboratories, Calgary, Canada).

2.8. Statistical analysis

All experiments were repeated at least twice with a minimum of three replicates per treatment and appropriate controls. The greenhouse experiments were conducted three times with 25 replicate pots. All data were analyzed for significant differences by analysis of variance with means separation using the least significant difference (LSD) ($P = 0.05$) in Fisher's protected LSD test, employing Statistical Analysis System (SAS) computer program (SAS System Version 7, 1998, SAS Institute, Cary, NC, USA).

3. Results

3.1. Effect of treatments on disease incidence

One application of SW and SA to carrot plants significantly reduced disease development due to *Alternaria* and *Botrytis* 10 d after inoculation in all greenhouse trials conducted (Table 1). Additional applications of SW and SA made at 10 and 20 d after inoculation reduced disease incidence further. Two applications of SW reduced *Alternaria* (Fig. 1) and *Botrytis* infection by up to 57% and 53.5%, respectively, while applications of SA reduced disease by 37% and 35.2%, respectively. In SW-treated plants, severe leaf symptoms such as blighting or shrivelling were not observed when compared to SA or the water control. One spray of chlorotholonil alternated with SW reduced disease symptom expression the most. SW was not phytotoxic to carrot plants at the concentration used. There was also a significant increase in biomass yield of plants sprayed with SW. The mean dry plant biomass was 1.08, 0.75, 0.85 and 0.61 (g plant^{-1}) for SW, SA, fungicide and water-control plants, respectively. Nutrient (N, P, K, Mg, Na, S, Zn, Bo, Mn, Cu, Fe, Mo) analyses of treated and control plants revealed no significant differences in nutrient levels and profiles (data not shown).

3.2. Effect of treatments on gene expression

Carrot plants treated with SW and SA showed enhanced transcript levels of *PR-1*, chitinase, *Ltp*, *Chs-2*, *NPR-1*, *Pal* and *PR-5* genes compared to the water control (Fig. 2). Enhanced transcripts were observed from 12 up to 72 h and started to decline at 96 h. When compared to SA, SW induced a stable and sustained accumulation of the above gene transcripts.

Treatment with SW and SA caused significantly enhanced activities of chitinase, β-1,3-glucanase, PO, polyphenoloxidase, PAL and lipoxygenase compared to the water controls. The increase was pronounced at early time intervals (from 12 h) and declined at later time intervals (72 and 96 h). β-1,3-Glucanase activity was highest in SW-treated plants up to 48 h and decreased with time (Fig. 3a). Chitinase activity was the highest

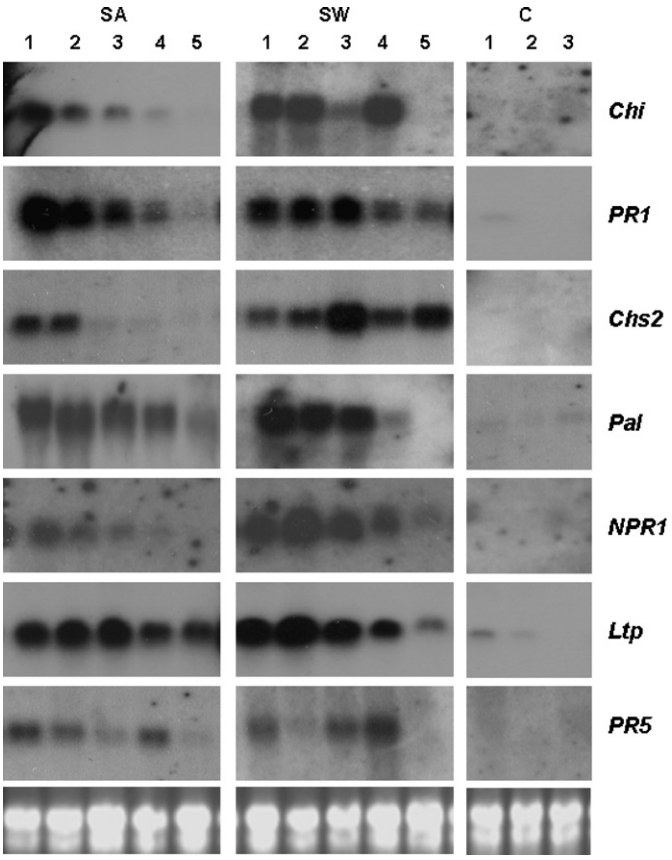

Fig. 2. Northern blot showing accumulation of transcripts of chitinase (*Chi*), pathogenesis-related protein 1 (*PR-1*), chalcone synthase (*Chs*), phenyalanine ammonia lyase (*Pal*), *NPR-1*, lipid transfer protein (*Ltp*) and pathogenesis-related protein 5 (*PR-5*) genes in carrot leaves following elicitor treatment. 10 μg of total RNA was used; bottom panel indicates RNA loading controls. SA- salicylic acid; SW- Seaweed extract; C- water control; Numbers 1–5 refer to 12, 24, 48, 72 and 96 h after spraying.

in SW-treated plants (Fig. 3b) at 72 h followed by SA (24 h). PO and PPO activities were higher in SW-treated plants up to 48 h (Fig. 3c, d). A higher activity of PAL was observed in SA-treated plants up to 48 h compared to SW (Fig. Fig. 3e). There was also a significant increase in lipoxygenase activity in SW-treated plants compared to SA, with the highest activity at 24 h after treatment (Fig. 3f).

3.3. Effect of treatments on biochemical changes

Total phenolic content in SW- and SA-treated plants was higher compared to water controls at 24 h (Fig. 3g). A carrot phytoalexin, 6-ME, was higher in SW- and SA-treated plants compared to the water control (Fig. 3h). The highest levels were detected in SW-treated plants at 48 h compared to SA or control.

Hydrogen peroxide accumulation in leaf tissues was assessed by DAB staining. Plants treated with SW and SA showed enhanced accumulation of H_2O_2 as indicated by dark brown patches in the interveinal area of leaves (Fig. 4). Interveinal browning was seen as early as 6 h in all treated leaves, and brown patches were greater in number in SW-treated plants compared to control leaves, which had very few or no brown spots.

4. Discussion

Carrot plants sprayed with SW showed less disease due to *Alternaria* and *Botrytis* compared to SA and the control. Molecular

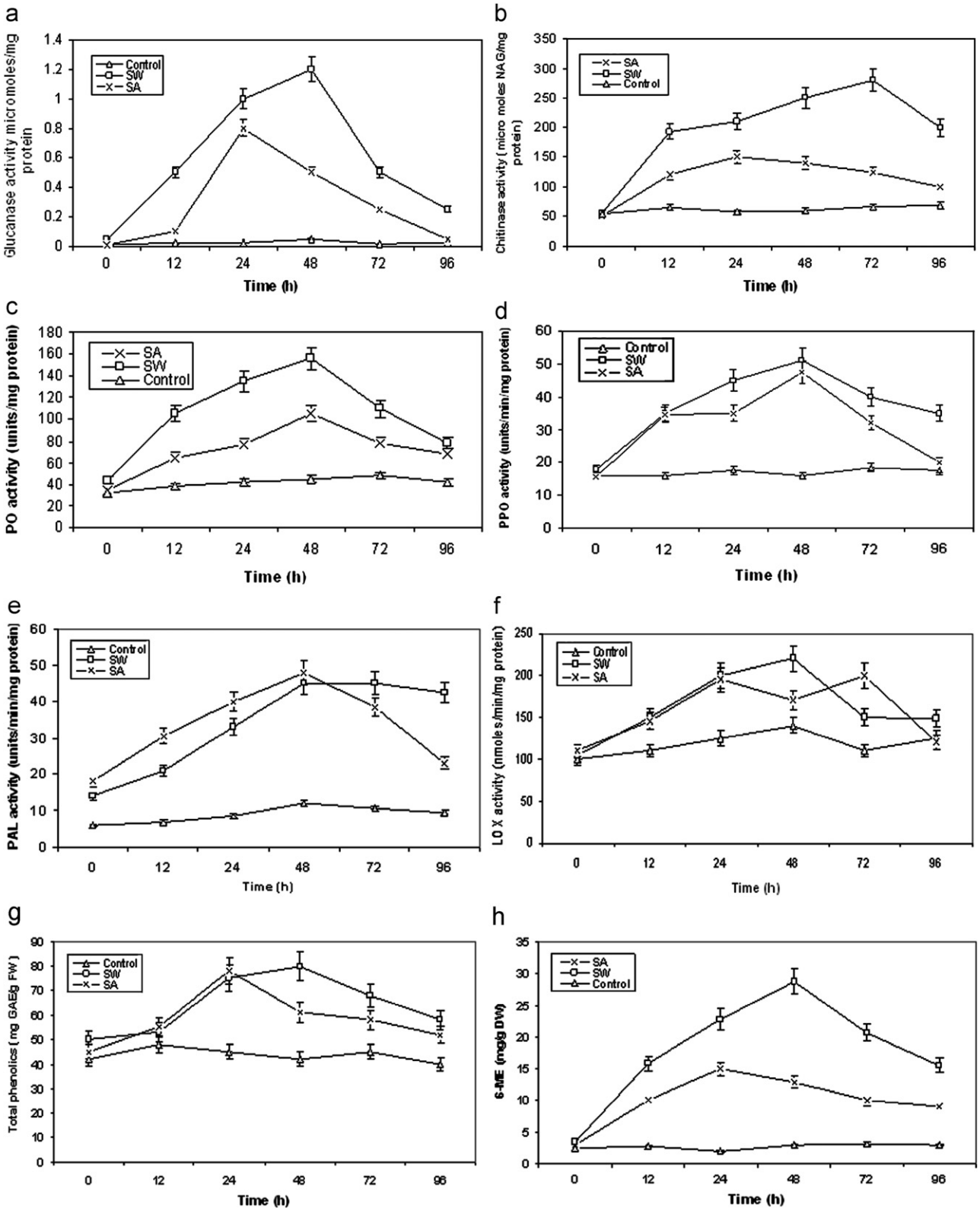

Fig. 3. Enzyme activities in carrot plants treated with seaweed extract and salicylic acid compounds compared to a water control. (a) Glucanase; (b) chitinase; (c) peroxidase; (d) polyphenol oxidase; (e) phenylalanine ammonia lyase; (f) lipoxigenase; (g) total phenolic content; (h) 6-methoxymellin (6-ME) content. Vertical bars indicate mean ± SE. Data are means of three replicates.

analysis of SW-treated plants showed the accumulation of defence gene transcripts at higher levels, with enhanced defence enzyme activities and accumulation of phenolics and phytoalexins.

Seaweed extract is reported to contain several carbohydrate molecules mostly in the form of oligosaccharides, including oligogalacturonides, and some polysaccharides (Dr. Jeffrey Norrie, Acadian SeaPlant, unpublished report). Oligosaccharides are

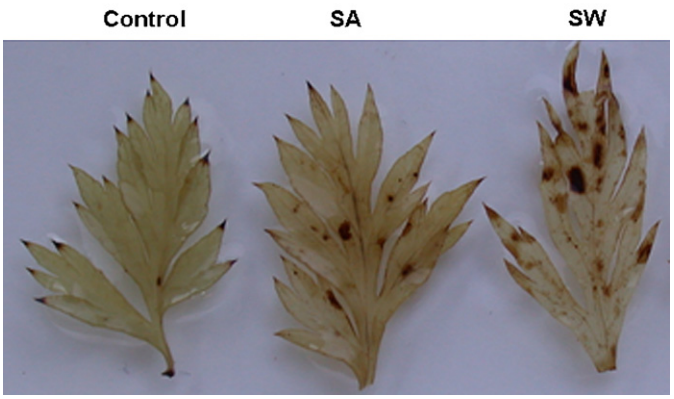

Fig. 4. Production of hydrogen peroxide following treatment with seaweed extract and salicylic acid. Carrot leaves were stained with 3′,3′-diaminobenzidine and decolorized in ethanol. Brown patches reflect H_2O_2 accumulation. Leaves were collected 12 h after treatment.

known to act as elicitor and signal transduction molecules in plants (Vidhyasekaran, 1997; Walters et al., 2005). The two algal polysaccharides, laminarin and carrageenans, were shown to induce efficient signalling and defence gene expression in tobacco leaves (Mercier et al., 2001). Defence genes encoding sesquiterpene cyclase, chitinases and proteinase inhibitor were induced locally, and the signalling pathways mediated by ethylene, jasmonic acid and SA were triggered (Mercier et al., 2001). In the present study, we observed an upregulation of various PR protein genes as well as enhanced activities of different defence enzymes following SW application. The disease control observed in SW-treated carrot plants could be attributed to the elicitor activity of carbohydrate (oligosaccharides) fractions present in the seaweed extract. Oligogalacturonide molecules can be mobile systemically, depending on their molecular weight, and can induce expression of various defence-related proteins and proteinase inhibitors *in vivo* (Ryan and Farmer, 1991). Cluzet et al. (2004) conducted gene expression profiling studies in *Medicago truncatula* following treatment with an elicitor from the green algae, *Ulva* sp. When the algal extract was infiltrated or sprayed onto plants, it induced the expression of defence-related marker gene *PR-10* without inducing necrosis. Spraying at a concentration of 500 $\mu g\,ml^{-1}$ was sufficient to obtain maximum induction of *PR-10* after 2 d. By using a cDNA array, Cluzet et al. (2004) observed increased expression of a broad range of defence-related transcripts, notably genes involved in phytoalexin, PR protein and cell wall protein production. Further treatment of plants with an extract of *Ulva* sp. reduced subsequent infection by *Colletotrichum trifolii*.

In the present study, we observed a co-upregulation of *NPR-1* and *PR-1* gene induction in both SW- and SA-treated plants. *NPR-1* is an essential modulator of the SAR signalling pathway at downstream levels and interacts with members of the TGA family transcription factors (Zhou et al., 2000). In our experiments, the SW- and SA-treated plants also had elevated levels of transcripts of *Chs* and *PAL* genes. Chalcone synthase is the key regulatory enzyme of flavonoids and isoflavonoid synthesis and it has been reported that its expression is regulated by chemical and biological stimuli which include elicitor agents (Ichinose et al., 1992). An elicitor mixture containing oligogalacturonides, endopolygalacturonase and a polygalacturonase-inhibiting protein induced the expression of chalcone synthase gene in citrus leaves (Nalumpang et al., 2002).

Seaweed extract enhanced the activities of chitinase, glucanase, PO, PPO, PAL and lipoxygenase enzymes in treated carrot plants comparable to that of SA treatment. The increased activities were stable and prolonged for up to 72 h. Several previous reports describe the induction of defence enzymes by other elicitors (Vallard and Goodman, 2004). Chitosan sprays on grapevine leaves caused a marked induction of lipoxygenase and PAL activities (Trotel-Aziz et al., 2006) as well as chitinase, β-1,3-glucanase and lipoxygenase in potato and tomato (Vasuikova et al., 2001). All of these enzymes have well-described antimicrobial activities (Jayaraj et al., 2004), and furthermore their hydrolytic activities might play an important role in the amplification of defence reactions through release of chitin or glucan from the pathogen cell walls (Jayaraj et al., 2004).

Elicitor applications may also increase the constitutive phenolic levels in plants. BTH treatment of strawberry plants caused an increased accumulation of soluble and cell wall bound phenolics in leaves (Hukkanen et al., 2007). Application of chitosan oligosaccharides to tomato induced several defence responses, including an accumulation of phenolics, which led to resistance to fungal infection (Benhamou et al., 1994). The enhanced accumulation of phenolics in elicitor-treated carrot plants might be related to the increased activity of PAL, PO and other phenol-oxidizing enzymes, which would have in turn caused an increase in the available free phenolic pool. The free phenolic pool may be utilized for both enzyme activities and polymerization, leading to lignin synthesis, thereby contributing to disease resistance (Vidhyasekaran, 1997).

The visible brown patches observed in carrot leaves treated with SW, following DAB staining, was due to the formation of an insoluble pigment as a consequence of reaction of H_2O_2 with DAB. The intensity of browning is correlated to the level of ROS or H_2O_2 (Ganesan and Thomas, 2001). There are earlier studies reporting the generation of ROS and H_2O_2 by elicitor treatment (Rakwal et al., 2002). Lipoxigenase activity generates ROS and superoxide anion radicals and singlet oxygen (Ohta et al., 1990) and a significant increase in LOX activity was observed in the present study, which might possibly be the result of upregulated LOX activity due to SW/SA treatment. The active oxygen may be involved in the oxidation of membrane lipids that results in the production of several antifungal compounds, including phytoalexins, initiation of cell wall lignification and signal transduction leading to resistance responses (Sutherland, 1991).

Previous reports have shown that seaweed extracts can reduce disease and promote plant growth. Pepper plants treated with an extract of the marine algae *Ascophyllum* had enhanced foliar resistance to *Phytophthora capsici* (Lizzi et al., 1998). Acquired resistance was found to be proportional to the concentration of the extract and the number of applications. Sprays of *Ascophyllum* extract induced PO activity and accumulation of phytoalexin. One application of extract at 0.8 or 1.6 $l\,ha^{-1}$ stimulated PO activity and two applications caused an eight-fold increase in PO activity. Treated leaves accumulated the highest capsidiol (a phytoalexin in peppers) concentrations when compared to the control (Lizzi et al., 1998). This compound is synthesized by the host plant in response to infection and has a fungistatic action on the development of *P. capsici* (Turelli et al., 1984). Incorporation of *A. nodosum* extract into the planting medium caused delayed and reduced incidence of Verticillium wilt of pepper plants. These plants also contained higher levels and early accumulation of phenolics (Garcia-Mina et al., 2004). The enhanced plant growth effects in seaweed extract-treated plants are also correlated with auxins, gibberellins, cytokinins, precursors of ethylene and betaine and cytokinins which are present and potentially involved in enhancing plant growth responses (Crouch and Staden, 1993). In the present study, enhancement of foliage plant growth could be the result of the hormonal activity of the seaweed extract.

In light of concerns over the use of chemical fungicides on horticultural crops, including carrot, alternative strategies that

utilize non-fungicidal products need to be evaluated. Induction of disease resistance in crop plants is an attractive strategy because it can activate various defences throughout the plant, providing protection to multiple pathogens (Anderson et al., 2006). In this context, the SW evaluated in carrot plants reduced leaf blights caused by *Alternaria* and *Botrytis* as effectively as the fungicide chlorothalonil. The plant protective role of seaweed extract and impact on disease and quality require further evaluation under different field cropping systems.

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