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Leaf and root glucosinolate profiles of Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) as a systemic response to methyl jasmonate and salicylic acid elicitation*

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Abstract: Glucosinolates (GSs) are an important group of defensive phytochemicals mainly found in Brassicaceae. Plant hormones jasmonic acid (JA) and salicylic acid (SA) are major regulators of plant response to pathogen attack. However, there is little information about the interactive effect of both elicitors on inducing GS biosynthesis in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). In this study, we applied different concentrations of methyl jasmonate (MeJA) and/or SA onto the leaf and root of Chinese cabbage to investigate the time-course interactive profiles of GSs. Regardless of the site of the elicitation and the concentrations of the elicitors, the roots accumulated much more GSs and were more sensitive and more rapidly responsive to the elicitors than leaves. Irrespective of the elicitation site, MeJA had a greater inducing and longer lasting effect on GS accumulation than SA. All three components of indole GS (IGS) were detected along with aliphatic and aromatic GSs. However, IGS was a major component of total GSs that accumulated rapidly in both root and leaf tissues in response to MeJA and SA elicitation. Neoglucobrassicin (neoGBC) did not respond to SA but to MeJA in leaf tissue, while it responded to both SA and MeJA in root tissue. Conversion of glucobrassicin (GBC) to neoGBC occurred at a steady rate over 3 d of elicitation. Increased accumulation of 4-methoxy glucobrassicin (4-MGBC) occurred only in the root irrespective of the type of elicitors and the site of elicitation. Thus, accumulation of IGS is a major metabolic hallmark of SA- and MeJA-mediated systemic response systems. SA exerted an antagonistic effect on the MeJA-induced root GSs irrespective of the site of elicitation. However, SA showed synergistic and antagonistic effects on the MeJA-induced leaf GSs when roots and leaves are elicitated for 3 d, respectively.

Key words: Chinese cabbage, Methyl jasmonate, Salicylic acid, Glucosinolate, Interactive effect
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1 Introduction

Plants are frequently exposed to diverse biotic and abiotic stresses during growth and development. One of the plant strategies to combat against the different stresses is to accumulate defensive secondary metabolites and change the accumulation profiles targeted to either a specific or a wide spectrum of stress through elaborate signaling transduction

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pathways (Pieterse and Dicke, 2007; Akula and Ravishankar, 2011; Pieterse *et al.*, 2013). Glucosinolates (GSs) are one of the major defensive chemicals used by cruciferous plants to mediate plant innate immunity. Upon tissue damage occurring, GSs are hydrolyzed into bioactive isothiocyanates, thiocyanates, or nitriles by the action of myrosinase (Ratzka *et al.*, 2002; Borgen *et al.*, 2010). These GS hydrolysis products are toxic to fungi, bacterial pathogens, or herbivores (Agrawal and Kurashige, 2003; Wittstock *et al.*, 2003; Brader *et al.*, 2006; Burow *et al.*, 2006; Mumm *et al.*, 2008; Redovniković *et al.*, 2008).

Indole GS (IGS) breakdown products are known to be effective against both insect herbivores and pathogens (Agerbirk *et al.*, 2009; Stotz *et al.*, 2011). 4-Methoxy glucobrassicin (4-MGBC) hydrolysis products have a strong insect deterrent activity (Kim and Jander, 2007; Kim *et al.*, 2008), as well as antimicrobial activity (Osbourn, 1996; Bednarek *et al.*, 2009; Clay *et al.*, 2009). The breakdown products of neoglucobrassicin (neoGBC) are shown to exert mutagenic or genotoxic effects on mammalian and bacterial cells (Glatt *et al.*, 2011). Aliphatic GSs were also demonstrated to play an important role in plant-herbivore interactions and non-host resistance in *Arabidopsis* (Beekwilder *et al.*, 2008; Müller *et al.*, 2010; Fan *et al.*, 2011). The necrotrophic pathogen *Alternaria brassicicola* was strongly affected by aliphatic GSs and isothiocyanates (Buxdorf *et al.*, 2013). *Arabidopsis* ecotype lines accumulating methylsulfinyl GS were more resistant to the generalist caterpillar *Spodoptera exigua* (Hübner) and to the specialist caterpillar *Pieris brassicae* (L.) than the lines containing hydroxypropyl GS as main compounds (Rohr *et al.*, 2006). 4-Methylsulphinylbutyl isothiocyanate was found to inhibit a wide range of fungi and bacteria (Tierens *et al.*, 2001). Increased accumulation of aromatic GSs was shown to stimulate salicylic acid (SA)-mediated defenses, while suppressing jasmonic acid (JA)-dependent defenses (Brader *et al.*, 2006). On the other hand, the GS metabolism was tightly related to some physiological processes under abiotic stresses, such as salinity, drought, heat, light, and nutrient deprivation (del Carmen Martínez-Ballesta *et al.*, 2013).

As plant stress hormones, both methyl jasmonate (MeJA)/JA and SA can induce accumulation of GSs in cruciferous plants. Exogenous JA application dramatically increased the level of IGS, resulting in the enhanced resistance of *Arabidopsis* to both phloem-feeding and chewing insects (Mikkelsen *et al.*, 2003; Mewis *et al.*, 2005). Upon MeJA treatment, neoGBC significantly accumulated in the leaves of *Brassica* crops such as pak choi (Wiesner *et al.*, 2013), cabbage (Fritz *et al.*, 2010), oilseed rape (Brader Loivamäki *et al.*, 2004), broccoli (Pérez-Balibrea *et al.*, 2011), Chinese kale (Sun *et al.*, 2012), and turnip (Smetanska *et al.*, 2007). SA treatment on *Brassica* plants increased the accumulation of aromatic, indole, and aliphatic GSs, though accumulation profiles of the individual components vary greatly depending on the plants (Kiddle *et al.*, 1994; Mikkelsen *et al.*, 2003; Smetanska *et al.*, 2007; Pérez-Balibrea *et al.*, 2011; Sun *et al.*, 2012).

In general, JA induces resistance against necrotrophic pathogens, some phloem-feeding insects, and chewing herbivores, while SA induces resistance against biotrophic pathogens and some phloem-feeding insects (Thaler *et al.*, 2012). However, there are many exceptions to this basic framework, and recent works suggest that interactions between the JA and SA pathways may play important roles in fine-tuning defense responses (Takahashi *et al.*, 2004; Flors *et al.*, 2008; Smith *et al.*, 2009; Robert-Seilaniantz *et al.*, 2011). SA-applications did not disturb the JA-induced GS accumulation in *Brassica oleracea* and *Brassica nigra* (van Dam *et al.*, 2004), but attenuated in several *Arabidopsis* genotypes (Cipollini *et al.*, 2004). All these studies were based on the separate pre- and post-treatment of the tissues with either SA or JA. However, there has been no report on how the combined application of SA and JA onto leaves or roots affects GS accumulation profiles in Chinese cabbage. Due to the economical and nutritional importance in Asia, abundance in GSs, and the availability of whole genome information (Wang *et al.*, 2011), Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) has a good potential to improve disease resistance by engineering specific GS profiles. In this study we investigated the interplay of MeJA and SA in inducing the accumulation of GSs in Chinese cabbage. The results would be useful to further researches on the crosstalk between JA- and SA-mediated signaling pathways in the regulation of GS biosynthesis.

2 Materials and methods

2.1 Plant materials and cultivation

Seeds of Chinese cabbage (*B. rapa* ssp. *pekinensis*, cv. ZSN5) were germinated in vermiculite in the greenhouse. The average temperature was (22–28) °C/(15–20) °C (day/night) under natural light. Relative humidity fluctuated between 60% and 70%. Eight seedlings with one fully expanded true leaf were transferred into a trough with 20 L of half-strength Hoagland's nutrient solution, and were aerated continuously with an air bubbler. The nutrient solutions were renewed each week.

2.2 Plant elicitation and sampling

After growing in a greenhouse for 30 d, the Chinese cabbage plants with about 8–10 leaves were sprayed with 0.1 or 0.2 mmol/L MeJA, 0.2 or 2.0 mmol/L SA, 0.2 mmol/L MeJA+2.0 mmol/L SA (dissolved in 1% ethanol), or 1% ethanol in aqueous solution as a control, or added to the root nutrient solution with 0.2 mmol/L MeJA, 2.0 mmol/L SA, 0.2 mmol/L MeJA+2.0 mmol/L SA (dissolved in 1% ethanol), or 1% ethanol in aqueous solution as a control. They were harvested at 12, 24, 48, or 72 h after treatment. The samples were immediately frozen in liquid nitrogen, then lyophilized and pulverized for subsequent GS analysis.

2.3 Extraction of GSs

GS content was determined as previously described by Yang *et al.* (2009) with minor modifications. About 0.25 g of lyophilized leaf powder was boiled in 4 ml of 70% methanol solution (75 °C) for about 10 min, in which 0.1 ml of 5 mmol/L sinigrin (Sigma-Aldrich Co., MO, USA) was added as an internal standard for high performance liquid chromatography (HPLC) analysis. After centrifugation, the supernatant was transferred to a new tube, and the residue was re-extracted twice. The combined aqueous extracts were added to 1 ml of 0.4 mol/L barium acetate, and the mixture was centrifuged as above. They were then applied to an activated DEAE Sephadex™ A-25 column (Amersham Biosciences, Sweden). The column was left for desulfation with 500 μl of 2 mg/ml sulfatase (Sigma-Aldrich Co., MO, USA) at room temperature for 16 h. The resultant desulfoglucosinolates were eluted with 2.5 ml of ultra pure water and passed through a 0.45-μm syringe filter (Toyo Roshi Kaisha, Ltd., Japan) and stored at −20 °C for later HPLC analysis.

2.4 Determination of GS levels

The GS profile was analyzed in an Agilent 1200 HPLC system (Agilent Technologies, Inc., Shimadzu, Kyoto, Japan) with a prontosil ODS2 column (250 mm× 4 mm, 5 μm; Bischoff, Germany). Twenty microliters of eluents were monitored with a ultraviolet-visible (UV-VIS) detector (SPD 10-A, Shimadzu, Japan) set at 229 nm. Chromatography was performed at 35 °C in a linear gradient of 0%–20% acetonitrile (Tedia, USA) for 32 min, followed by a constant 20% acetonitrile for 6 min, a gradient of 20%–100% acetonitrile for 5 min, and 0% acetonitrile prior to the injection of the next sample. The flow rate was 1.3 ml/min. The GS content was expressed as μmol/g dry weight (DW).

2.5 Statistical analysis

The GSs in three replicates of each sample were analyzed by HPLC. Data were subjected to statistical analysis by analysis of variances (ANOVA) in the SAS (SAS, Inc., USA) package program 9.1. Means were separated by Duncan's multiple range tests. Differences at $P<0.05$ or $P<0.01$ were considered significant. Data are presented as mean±standard deviation.

3 Results

3.1 Effect of foliar MeJA and SA treatments on GS accumulation in leaves

In total, eight GSs were detected in the Chinese cabbage leaves, belonging to three classes: aliphatic GSs (progoitrin, glucoalyssin, gluconapin, and glucobrassicanapin), IGSs (glucobrassicin (GBC), 4-MGBC, and neoGBC), and aromatic GS (gluconasturtiin (GST)).

Tables 1 and 2 represent the leaf and root GS profiles of Chinese cabbage that responded to the foliar applications of different concentrations of MeJA, SA, or MeJA plus SA, respectively. The results showed that in response to MeJA or SA treatment leaf and root aliphatic GSs accumulated 48 and 12 h slower than those of IGSs, respectively. The levels of aromatic GS (in this case GST) and aliphatic

GSs from different leaf samples were significantly induced within 48 and 72 h, respectively. The concentration of total IGSs was much higher than those of the aliphatic and aromatic GSs. MeJA and/or SA induced accumulations of IGS occurred within 24 h, earlier than the other two types of GSs. The increased accumulation level of leaf total GSs in response to MeJA or SA treatment is similar to that of total IGS.

With MeJA treatment, GS levels continued to increase to about 11-fold over 72-h of treatment time. The MeJA-induced enhancement of GS also depended on its concentration. Unlike MeJA, SA treatments with different dosage and duration did not affect accumulation levels of GSs. High concentrations (2.0 mmol/L) of the SA application did not cause any symptom of localized cell death that may occur due to the high levels of reactive oxygen species accumulations. When compared with the MeJA treatment, the supplement of SA did not exert an antagonistic effect on the GS accumulation until 48 h later.

3.2 Effect of foliar MeJA and SA treatments on GS accumulation in roots

Unlike leaves, only one aliphatic GS (glucobrassicanapin) was detectable from roots of Chinese cabbage and was enhanced to the highest level after 24 h of foliar treatments (Table 2). The concentrations of GST in root samples were much higher than those in leaves. They reached to the top levels in roots and leaves after 24 and 48 h, respectively (Tables 1 and 2). Total IGS levels accumulated earlier than the other two types and reached to the top level with an approximately 9-fold increase after 24 h (Table 2). The

Table 1 Effects of foliar MeJA and SA treatments on glucosinolate (GS) levels in leaves of Chinese cabbage after 12, 24, 48, and 72 h

Time (h)	Treatment	GS concentration (μmol/g DW)			
		GST	Total AGS	Total IGS	Total GS
12	Control	0.87±0.29^{a}	1.31±0.43^{a}	6.33±1.15^{a}	8.50±1.87^{a}
	$MJ_{0.1}$	1.87±0.08^{a}	1.62±0.09^{a}	8.79±1.50^{ab}	12.28±1.50^{ab}
	$MJ_{0.2}$	1.86±0.69^{a}	1.86±0.26^{a}	10.71±1.61^{ab}	14.43±2.05^{b}
	$SA_{0.2}$	1.34±0.37^{a}	1.57±0.75^{a}	9.18±2.20^{ab}	12.09±1.08^{ab}
	SA_2	1.78±0.45^{a}	2.01±0.11^{a}	9.53±1.42^{ab}	13.31±1.08^{ab}
	$MJ_{0.2}$+SA_2	1.56±0.73^{a}	1.26±0.60^{a}	13.20±2.49^{b}	16.03±2.63^{b}
24	Control	1.05±0.19^{a}	1.40±0.26^{a}	6.36±1.52^{Aa}	8.75±1.97^{Aa}
	$MJ_{0.1}$	1.64±0.29^{a}	1.62±0.36^{a}	14.18±1.31^{ABbc}	17.43±1.24^{ABbc}
	$MJ_{0.2}$	1.90±0.50^{a}	2.43±0.79^{a}	17.74±2.93^{Bc}	22.08±4.22^{Bc}
	$SA_{0.2}$	1.48±0.75^{a}	1.71±0.26^{a}	11.55±1.95^{ABb}	14.74±1.46^{ABab}
	SA_2	1.58±0.15^{a}	1.91±0.46^{a}	11.56±1.35^{ABb}	15.05±1.66^{ABab}
	$MJ_{0.2}$+SA_2	1.53±0.28^{a}	1.89±0.45^{a}	16.27±2.01^{Bbc}	19.68±2.74^{Bbc}
48	Control	0.95±0.13^{Aa}	1.31±0.34^{a}	6.99±0.76^{Aa}	9.25±1.24^{Aa}
	$MJ_{0.1}$	2.09±0.26^{Bb}	1.88±0.23^{a}	46.83±4.35^{Bc}	50.80±4.32^{Bc}
	$MJ_{0.2}$	1.94±0.69^{ABb}	2.03±0.19^{a}	58.15±5.06^{Bd}	62.12±5.56^{Bd}
	$SA_{0.2}$	1.64±0.45^{ABb}	2.46±0.65^{a}	17.99±1.03^{Ab}	22.09±2.12^{Ab}
	SA_2	1.78±0.52^{ABb}	2.12±0.25^{a}	17.22±1.60^{Ab}	21.13±2.07^{Ab}
	$MJ_{0.2}$+SA_2	1.62±0.78^{ABb}	2.23±0.54^{a}	57.59±3.25^{Bd}	61.44±3.49^{Bd}
72	Control	1.03±0.03^{a}	1.40±0.16^{a}	6.59±1.47^{Aa}	9.03±1.66^{Aa}
	$MJ_{0.1}$	1.60±0.11^{ab}	1.68±0.48^{ab}	51.99±3.07^{Bc}	55.28±3.44^{Bc}
	$MJ_{0.2}$	1.95±0.27^{b}	2.24±0.40^{ab}	70.72±3.43^{Cd}	74.90±4.11^{Cd}
	$SA_{0.2}$	1.66±0.12^{ab}	2.66±0.54^{b}	16.72±0.95^{Ab}	21.03±1.61^{Ab}
	SA_2	1.60±0.63^{ab}	2.24±0.05^{ab}	17.36±1.28^{Ab}	21.21±1.87^{Ab}
	$MJ_{0.2}$+SA_2	1.81±0.38^{b}	2.70±0.37^{b}	52.33±3.26^{Bc}	56.84±2.51^{Bc}

Data represent GS levels of different leaf samples harvested at 12, 24, 48, and 72 h after foliar treatment, respectively. They were calculated as the mean of three replicates with standard deviation in μmol/g DW. Significant differences of GS levels among different samples (control, $MJ_{0.1}$, $MJ_{0.2}$, $SA_{0.2}$, SA_2, $MJ_{0.2}$+SA_2) are labeled with different capital letters (P<0.01) or lower-case letters (P<0.05). GST, gluconasturtiin; Total AGS, total aliphatic glucosinolates; Total IGS, total indole glucosinolates; Total GS, total glucosinolates. $MJ_{0.1}$, 0.1 mmol/L MeJA; $MJ_{0.2}$, 0.2 mmol/L MeJA; $SA_{0.2}$, 0.2 mmol/L SA; SA_2, 2.0 mmol/L SA; $MJ_{0.2}$+SA_2, 0.2 mmol/L MeJA+ 2.0 mmol/L SA

increased accumulation levels of total root GSs in response to MeJA or SA treatments were higher than those of total IGS (Table 2). Thus, in contrast to leaves, the accumulation of glucobrassicanapin (GBN) and GST significantly contributed to the increase in total GSs in roots. The effect of MeJA on root GS induction was stronger than that of SA. Unlike leaves, MeJA-induced enhancement of GS was not concentration-dependent. When compared with the MeJA treatment, the supplement of SA reduced the MeJA-induced levels of GS after 48 h (Table 2).

3.3 Effect of exogenous MeJA and SA in nutrient solution on GS accumulation in leaves

As shown in Table 3, the accumulation levels of GST and total aliphatic GSs were significantly increased after 12 h of root MeJA treatment, while the levels of total aliphatic GSs were not affected by root SA application. Root application of 0.2 mmol/L MeJA significantly increased the total leaf IGS levels after 12 h and reached the highest level after 48 h, though they were lower than those of the foliar MeJA application (Table 3). The levels of total IGS were also enhanced by SA treatment, but the effect of MeJA on IGS induction was about 4 times stronger than that of SA, especially after 48 h (Table 3). The increased accumulation levels of leaf total GSs in response to root MeJA or SA treatments are similar to those of total IGS (Table 3). Compared with root MeJA treatment, the supplement of SA dramatically enhanced the effect of MeJA on induction of total IGS after 24 h (Table 3).

Table 2 Effects of foliar MeJA and SA treatments on glucosinolate (GS) levels in roots of Chinese cabbage after 12, 24, 48, and 72 h

Time (h)	Treatment	GS concentration (μmol/g DW)			
		GST	GBN	Total IGS	Total GS
12	Control	11.75 ± 1.48^{a}	0.34 ± 0.11^{a}	13.10 ± 1.40^{Aa}	25.20 ± 2.77^{a}
	$MJ_{0.1}$	15.78 ± 1.14^{a}	0.69 ± 0.22^{ab}	21.81 ± 4.27^{ABcd}	38.28 ± 5.64^{bc}
	$MJ_{0.2}$	16.57 ± 0.88^{a}	0.78 ± 0.13^{b}	27.47 ± 3.82^{Bd}	44.82 ± 4.57^{c}
	$SA_{0.2}$	12.07 ± 3.32^{a}	0.47 ± 0.15^{ab}	14.75 ± 1.05^{Aab}	27.29 ± 4.51^{ab}
	SA_2	17.05 ± 1.68^{a}	0.46 ± 0.09^{ab}	20.77 ± 1.97^{ABbcd}	38.23 ± 3.56^{bc}
	$MJ_{0.2}$+SA_2	16.03 ± 1.66^{a}	0.56 ± 0.12^{ab}	18.16 ± 1.13^{ABabc}	34.75 ± 2.91^{abc}
24	Control	11.52 ± 1.24^{Aa}	0.39 ± 0.07^{Aa}	10.93 ± 0.40^{Aa}	22.85 ± 1.57^{Aa}
	$MJ_{0.1}$	41.56 ± 3.69^{Cc}	3.49 ± 0.66^{Cc}	91.09 ± 0.40^{Cd}	136.14 ± 4.75^{Cd}
	$MJ_{0.2}$	44.46 ± 3.07^{Cc}	2.82 ± 0.44^{BCc}	93.65 ± 4.23^{Cd}	140.93 ± 6.85^{Cd}
	$SA_{0.2}$	22.35 ± 4.05^{ABb}	1.18 ± 0.17^{Aab}	46.50 ± 1.49^{Bb}	69.69 ± 2.40^{Bb}
	SA_2	25.71 ± 1.62^{Bb}	1.66 ± 0.29^{ABb}	57.36 ± 4.01^{Bc}	84.72 ± 2.10^{Bc}
	$MJ_{0.2}$+SA_2	41.56 ± 0.52^{Dd}	3.63 ± 0.46^{Cc}	93.18 ± 3.20^{Cd}	138.38 ± 3.15^{De}
48	Control	13.29 ± 2.06^{Aa}	0.43 ± 0.17^{Aa}	13.61 ± 0.66^{Aa}	27.33 ± 2.56^{Aa}
	$MJ_{0.1}$	42.54 ± 2.16^{Cc}	2.57 ± 0.58^{Bb}	70.57 ± 3.24^{Cd}	114.15 ± 5.98^{Dd}
	$MJ_{0.2}$	34.21 ± 3.49^{BCb}	2.17 ± 0.75^{ABb}	77.67 ± 3.64^{Cd}	114.05 ± 6.38^{Dd}
	$SA_{0.2}$	15.89 ± 1.64^{Aa}	0.86 ± 0.19^{ABa}	25.37 ± 2.96^{Ab}	42.12 ± 4.79^{ABb}
	SA_2	18.76 ± 2.58^{Aa}	0.72 ± 0.32^{ABa}	28.73 ± 5.33^{Ab}	48.21 ± 3.07^{Bb}
	$MJ_{0.2}$+SA_2	30.56 ± 1.99^{Bb}	0.87 ± 0.16^{ABa}	48.29 ± 5.32^{Bc}	79.71 ± 3.17^{Cc}
72	Control	10.41 ± 0.74^{Aa}	0.58 ± 0.14^{Aa}	13.43 ± 1.73^{Aa}	24.41 ± 2.33^{Aa}
	$MJ_{0.1}$	30.51 ± 2.87^{Dd}	2.79 ± 0.26^{Bb}	55.57 ± 6.07^{Cc}	88.88 ± 3.46^{Cc}
	$MJ_{0.2}$	25.71 ± 2.88^{CDc}	1.42 ± 0.64^{ABa}	59.67 ± 2.02^{Cc}	86.80 ± 5.54^{Cc}
	$SA_{0.2}$	12.39 ± 0.93^{Aab}	0.74 ± 0.31^{Aa}	19.05 ± 1.99^{ABa}	32.19 ± 3.23^{Aa}
	SA_2	15.76 ± 1.66^{ABb}	0.72 ± 0.32^{Aa}	18.73 ± 0.32^{ABa}	35.21 ± 1.66^{Aa}
	$MJ_{0.2}$+SA_2	22.06 ± 1.55^{BCc}	0.71 ± 0.20^{Aa}	31.79 ± 6.70^{Bb}	54.56 ± 5.35^{Bb}

Data represent GS levels of different root samples harvested at 12, 24, 48, and 72 h after foliar treatment, respectively. They were calculated as the means of three replicates with standard deviation in μmol/g DW. Significant differences of GS levels among different samples (control, $MJ_{0.1}$, $MJ_{0.2}$, $SA_{0.2}$, SA_2, $MJ_{0.2}$+SA_2) are labeled with different capital letters ($P<0.01$) or lower-case letters ($P<0.05$). GST, gluconasturtiin; GBN, glucobrassicanapin; Total IGS, total indole glucosinolates; Total GS, total glucosinolates. $MJ_{0.1}$, 0.1 mmol/L MeJA; $MJ_{0.2}$, 0.2 mmol/L MeJA; $SA_{0.2}$, 0.2 mmol/L SA; SA_2, 2.0 mmol/L SA; $MJ_{0.2}$+SA_2, 0.2 mmol/L MeJA+2.0 mmol/L SA

Table 3 Effects of MeJA and SA irrigation treatments on glucosinolate (GS) levels in leaves of Chinese cabbage after 12, 24, 48, and 72 h

Time (h)	Treatment	GS concentration (μmol/g DW)			
		GST	Total AGS	Total IGS	Total GS
12	Control	0.91±0.11^{a}	1.21±0.13^{a}	7.16±0.33^{a}	9.29±0.30^{a}
	$MJ_{0.2}$	1.68±0.19^{b}	2.36±0.46^{b}	10.19±0.26^{b}	14.23±0.27^{b}
	SA_2	1.62±0.04^{ab}	1.65±0.15^{ab}	7.58±0.66^{a}	10.85±0.78^{a}
	$MJ_{0.2}$+SA_2	2.26±0.36^{b}	2.24±0.05^{b}	10.11±1.78^{b}	14.62±2.20^{b}
24	Control	1.12±0.37^{a}	1.30±0.11^{a}	6.82±0.54^{Aa}	9.24±0.80^{Aa}
	$MJ_{0.2}$	1.56±0.08^{a}	2.15±0.64^{a}	14.37±2.05^{Ab}	18.08±2.62^{ABb}
	SA_2	2.26±0.26^{a}	2.19±0.22^{a}	12.68±1.12^{Ab}	17.13±1.23^{ABb}
	$MJ_{0.2}$+SA_2	1.30±0.48^{a}	2.04±0.40^{a}	25.37±2.18^{Bc}	28.71±2.27^{Bc}
48	Control	0.96±0.33^{a}	1.55±0.35^{a}	6.98±0.57^{Aa}	9.49±0.12^{Aa}
	$MJ_{0.2}$	1.67±0.11^{a}	2.10±0.24^{a}	41.52±2.09^{Bb}	45.29±2.32^{Bc}
	SA_2	2.07±0.57^{a}	1.67±0.69^{a}	12.35±1.04^{Aa}	16.09±1.15^{Ab}
	$MJ_{0.2}$+SA_2	2.07±0.28^{a}	2.26±0.11^{a}	55.27±1.81^{Cc}	59.60±1.99^{Cd}
72	Control	0.94±0.12^{a}	1.36±0.05^{a}	7.81±0.47^{Aa}	10.11±0.64^{Aa}
	$MJ_{0.2}$	2.19±0.58^{a}	2.55±0.73^{a}	35.06±2.97^{Bb}	39.80±4.28^{Bb}
	SA_2	2.05±0.37^{a}	2.37±0.56^{a}	10.73±0.38^{Aa}	15.10±0.55^{Aa}
	$MJ_{0.2}$+SA_2	2.23±0.26^{a}	2.33±0.12^{a}	87.52±5.01^{Cc}	92.08±5.26^{Cc}

Data represent GS levels of different leaf samples harvested at 12, 24, 48, and 72 h after irrigation treatment, respectively. They were calculated as the means of three replicates with standard deviation in μmol/g DW. Significant differences of GS levels among different samples (control, $MJ_{0.2}$, SA_2, $MJ_{0.2}$+SA_2) are labeled with different capital letters (P<0.01) or lower-case letters (P<0.05). GST, gluconasturtiin; Total AGS, total aliphatic glucosinolates; Total IGS, total indole glucosinolates; Total GS, total glucosinolates. $MJ_{0.2}$, 0.2 mmol/L MeJA; SA_2, 2.0 mmol/L SA; $MJ_{0.2}$+SA_2, 0.2 mmol/L MeJA+2.0 mmol/L SA

3.4 Effect of exogenous MeJA and SA in nutrient solution on GS accumulation in roots

Table 4 displays the fact that MeJA or SA treatment on roots dramatically increased the levels of three types of root GSs in 12 h, after which they decreased rapidly, and then increased gradually until 72 h. The increased accumulation levels of total root GSs in response to MeJA or SA treatments are much higher than total root IGS (Table 4). Thus, in contrast to leaves, accumulation of GBN and GST significantly contributed to the increase in total GSs in roots. Compared with root MeJA treatment, the supplement of SA dramatically reduced the effect of MeJA on induction of GSs (Table 4).

3.5 Accumulation profiles of individual IGSs in leaves and roots

Foliar SA treatment induced only GBC among three types of IGS, whereas MeJA treatment solely or MeJA combined with SA significantly increased the GBC and neoGBC levels of leaves (Figs. 1a, 1c, and 1e). All three types of IGS were remarkably induced in roots by foliar MeJA or SA treatment (Figs. 1b, 1d, and 1f). Among three types of IGS, root SA treatment significantly induced only leaf GBC, while root MeJA treatment alone or MeJA combined with SA significantly increased the levels of GBC and neoGBC, but not 4-MGBC in leaves (Figs. 2a, 2c, and 2e). All three types of root IGS were dramatically induced by root MeJA or SA treatment (Figs. 2b, 2d, and 2f).

We also examined the time-dependent accumulation levels of individual IGS. It appears that the 4-MGBC level remained constant in leaves but increased in the first 12 h and then decreased in roots (Figs. 1c, 1d, 2c, and 2d). GBC and neoGBC levels increased steadily in leaves with the duration time. The accumulation levels of leaf GBC at 72 h were about 12- or 20-times higher than the initial level of leaf GBC in foliar or root MeJA+SA treatment (Figs. 1a and 2a). The accumulation levels of leaf neoGBC at 72 h were about 37- or 24-times higher than the initial level of neoGBC in foliar or root MeJA treatment (Figs. 1e and 2e). On the other hand, the root GBC and neoGBC levels reached the top in 24 or 12 h with foliar or root treatment, respectively (Figs. 1b, 1f, 2b, and 2f).

Table 4 Effects of MeJA and SA irrigation treatments on glucosinolate (GS) levels in roots of Chinese cabbage after 12, 24, 48, and 72 h

Time (h)	Treatment	GS concentration (μmol/g DW)			
		GST	GBN	Total IGS	Total GS
12	Control	8.89 ± 2.62^{Aa}	0.50 ± 0.25^{Aa}	10.95 ± 0.84^{Aa}	20.34 ± 2.02^{Aa}
	$MJ_{0.2}$	96.85 ± 5.28^{Cd}	5.24 ± 0.23^{Cc}	139.26 ± 8.37^{Cc}	241.35 ± 2.86^{Dd}
	SA_2	88.06 ± 3.15^{Cc}	3.37 ± 0.44^{Bb}	100.15 ± 0.13^{Bb}	191.42 ± 3.47^{Cc}
	$MJ_{0.2}+SA_2$	61.22 ± 1.39^{Bb}	3.47 ± 0.77^{Bb}	89.42 ± 6.77^{Bb}	154.11 ± 8.93^{Bb}
24	Control	9.52 ± 0.51^{a}	0.45 ± 0.09^{a}	9.93 ± 1.44^{Aa}	19.90 ± 0.84^{Aa}
	$MJ_{0.2}$	16.46 ± 1.48^{b}	0.92 ± 0.26^{a}	27.37 ± 0.65^{Cb}	44.76 ± 1.87^{Bc}
	SA_2	8.44 ± 1.97^{a}	0.35 ± 0.16^{a}	11.32 ± 1.35^{ABa}	19.78 ± 3.17^{Aa}
	$MJ_{0.2}+SA_2$	11.24 ± 1.43^{ab}	0.74 ± 0.12^{a}	21.37 ± 3.22^{BCb}	33.35 ± 4.78^{ABb}
48	Control	9.01 ± 1.01^{ABab}	0.35 ± 0.12^{Aa}	8.17 ± 1.44^{Aa}	17.53 ± 0.55^{Aa}
	$MJ_{0.2}$	19.96 ± 2.18^{Bc}	1.02 ± 0.07^{Bc}	33.49 ± 0.82^{Bc}	54.48 ± 2.94^{Cd}
	SA_2	14.92 ± 2.12^{ABbc}	0.49 ± 0.10^{ABab}	18.65 ± 3.75^{Ab}	34.05 ± 1.73^{Bc}
	$MJ_{0.2}+SA_2$	8.20 ± 1.59^{Aa}	0.72 ± 0.07^{ABb}	20.15 ± 0.75^{Ab}	29.08 ± 2.27^{Bb}
72	Control	8.68 ± 0.53^{Aa}	0.48 ± 0.09^{a}	12.97 ± 1.70^{Aa}	22.14 ± 1.08^{Aa}
	$MJ_{0.2}$	24.39 ± 2.93^{Ab}	2.15 ± 0.24^{b}	60.21 ± 1.47^{Cc}	86.76 ± 1.70^{Cc}
	SA_2	45.13 ± 3.30^{Bc}	1.17 ± 0.20^{a}	31.58 ± 1.43^{Bb}	77.88 ± 4.53^{Cc}
	$MJ_{0.2}+SA_2$	13.70 ± 2.30^{Aa}	1.02 ± 0.35^{a}	26.65 ± 1.37^{Bb}	41.37 ± 1.28^{Bb}

Data represent GS levels of different root samples harvested at 12, 24, 48, and 72 h after irrigation treatment, respectively. They were calculated as the means of three replicates with standard deviation in μmol/g DW. Significant differences of GS levels among different samples (control, $MJ_{0.2}$, SA_2, $MJ_{0.2}+SA_2$) are labeled with different capital letters ($P<0.01$) or lower-case letters ($P<0.05$). GST, gluconasturtiin; GBN, glucobrassicanapin; Total IGS, total indole glucosinolates; Total GS, total glucosinolates. $MJ_{0.2}$, 0.2 mmol/L MeJA; SA_2, 2.0 mmol/L SA; $MJ_{0.2}+SA_2$, 0.2 mmol/L MeJA+2.0 mmol/L SA

4 Discussion

Among several elicitors, MeJA was found to be most effective for inducing the biosynthesis of IGS, particularly the neoGBC (Smetanska *et al.*, 2007; Wiesner *et al.*, 2013). These findings were confirmed in our study. Regardless of the type of elicitors and induction site, IGS was a major portion of total leaf GSs (Tables 1 and 3), whereas indole, aliphatic, and aromatic GSs all significantly contributed to total root GSs (Tables 2 and 4). Unlike leaves, roots accumulated only one type of aliphatic GS, GBN, in Chinese cabbage. The biomass was not affected by MeJA or SA treatment levels (data not shown).

Foliar application of MeJA led to about a 9- to 11-fold increase of total GSs in leaf and root at the highest accumulation level. Foliar application of SA increased total leaf IGS 2.6 times and total root IGS 5.2 times at the top level. The root application of MeJA resulted in about a 5- to 6-fold increase of total leaf and root GSs at the highest accumulation level (Tables 3 and 4). The root application of SA increased total leaf IGS 2 times and total root IGS 9 times at the top level (Tables 3 and 4). Besides IGS, GST and GBN accumulation in roots increased 9.9 and 6.7 times by SA root treatment, respectively (Table 4). Thus, both leaf and root application of MeJA and SA led to the induced systemic GS response. This is in contrast to the *B. oleracea* in which only root JA application yielded a systemic response (van Dam *et al.*, 2004).

Accumulation profiles of individual components of IGS differed depending on the elicitors. Irrespective of the site of elicitation and the type of elicitors, the level of 4-MGBC remained relatively constant in leaf but increased in roots. GBC and neoGBC significantly increased in both leaves and roots in response to foliar MeJA treatment, whereas SA treatment increased only the GBC level in the leaves and roots regardless of the induction site (Figs. 1 and 2). GBC was reported as an important insect resistant chemical for green peach aphid resistance (Kim *et al.*,

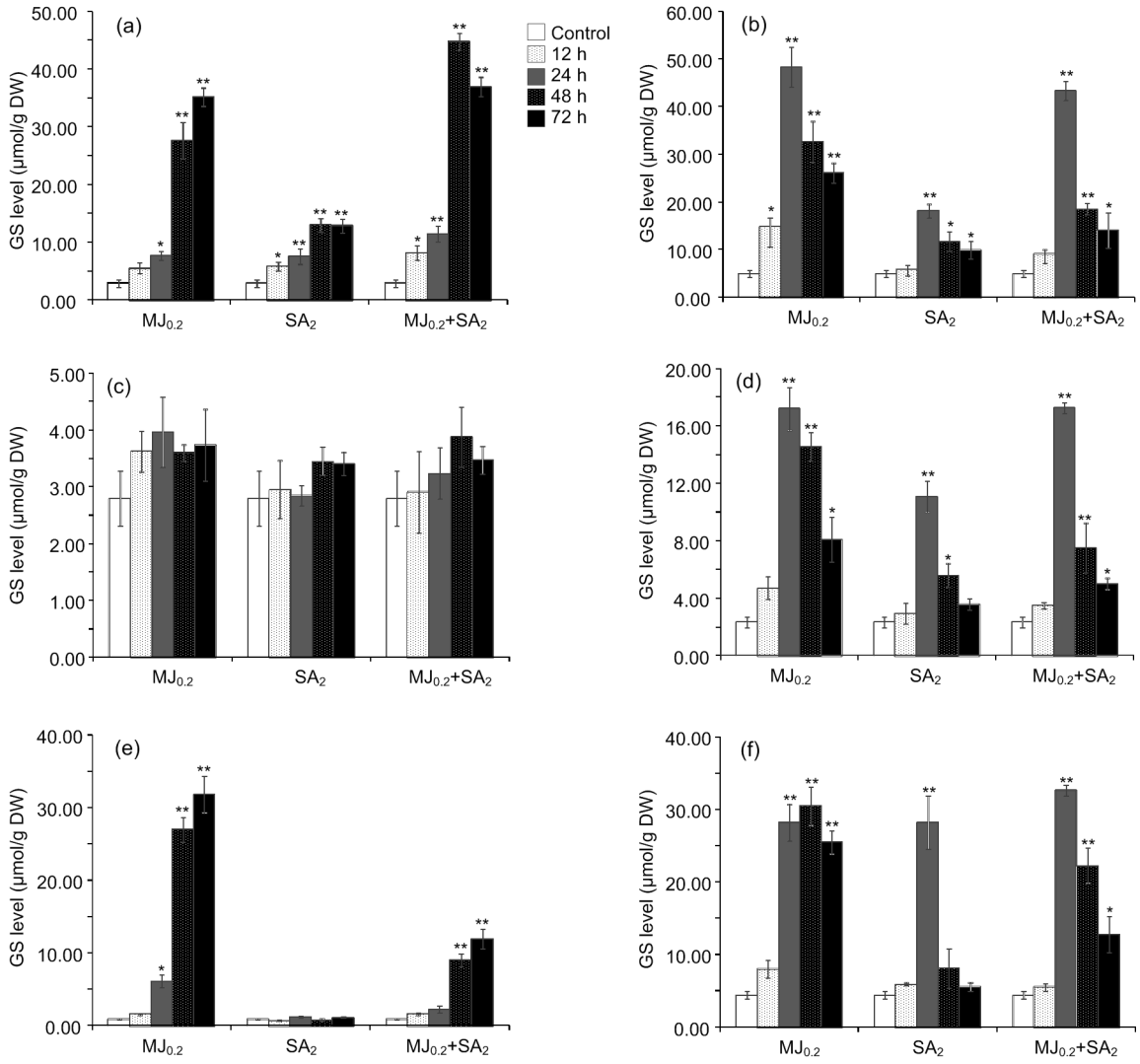

Fig. 1 Accumulation kinetics of GBC, 4-MGBC, and neoGBC in leaves (a, c, e) and roots (b, d, f) of ZSN5 with foliar treatments

$MJ_{0.2}$, SA_2, and $MJ_{0.2}$+SA_2 denote 0.2 mmol/L MeJA, 2.0 mmol/L SA, and 0.2 mmol/L MeJA+2.0 mmol/L SA treatments in foliar application, respectively. The data were calculated as the mean of three replicates with standard deviation in μmol/g DW. Significant differences of glucosinolate (GS) levels compared to control are labeled with ** (P<0.01) or * (P<0.05). 12, 24, 48, and 72 h represent the sampling time of 12, 24, 48, and 72 h after different treatments on ZSN5 (Zao Shu Wu Hao). GBC, glucobrassicin; 4-MGBC, 4-methoxy glucobrassicin; neoGBC, neoglucobrassicin

2008). So, MeJA- and SA-induced accumulation of GBC could enhance the insect resistance of Chinese cabbage plant. The SA application increased the neoGBC level only in roots. As a result, 4-MGBC is a root metabolite that responds to both elicitors, whereas neoGBC is a leaf metabolite that does not respond to SA but to the MeJA elicitor. The inducing effect of SA on leaf and root GS accumulation was always much lower than that of MeJA despite the use of a 10-fold higher molar concentration. However, the specific GS profiles of Chinese cabbage in response to leaf and root MeJA application were not altered by an SA application. All these results indicate that the signal transduction pathways triggered by MeJA and SA converge downstream and then diverge to activate the subset of specific genes at different magnitudes. Along this line, signaling pathways of systemic acquired resistance (SAR) mediated by SA and induced systemic resistance (ISR) mediated by JA are known to share the components controlled by the same

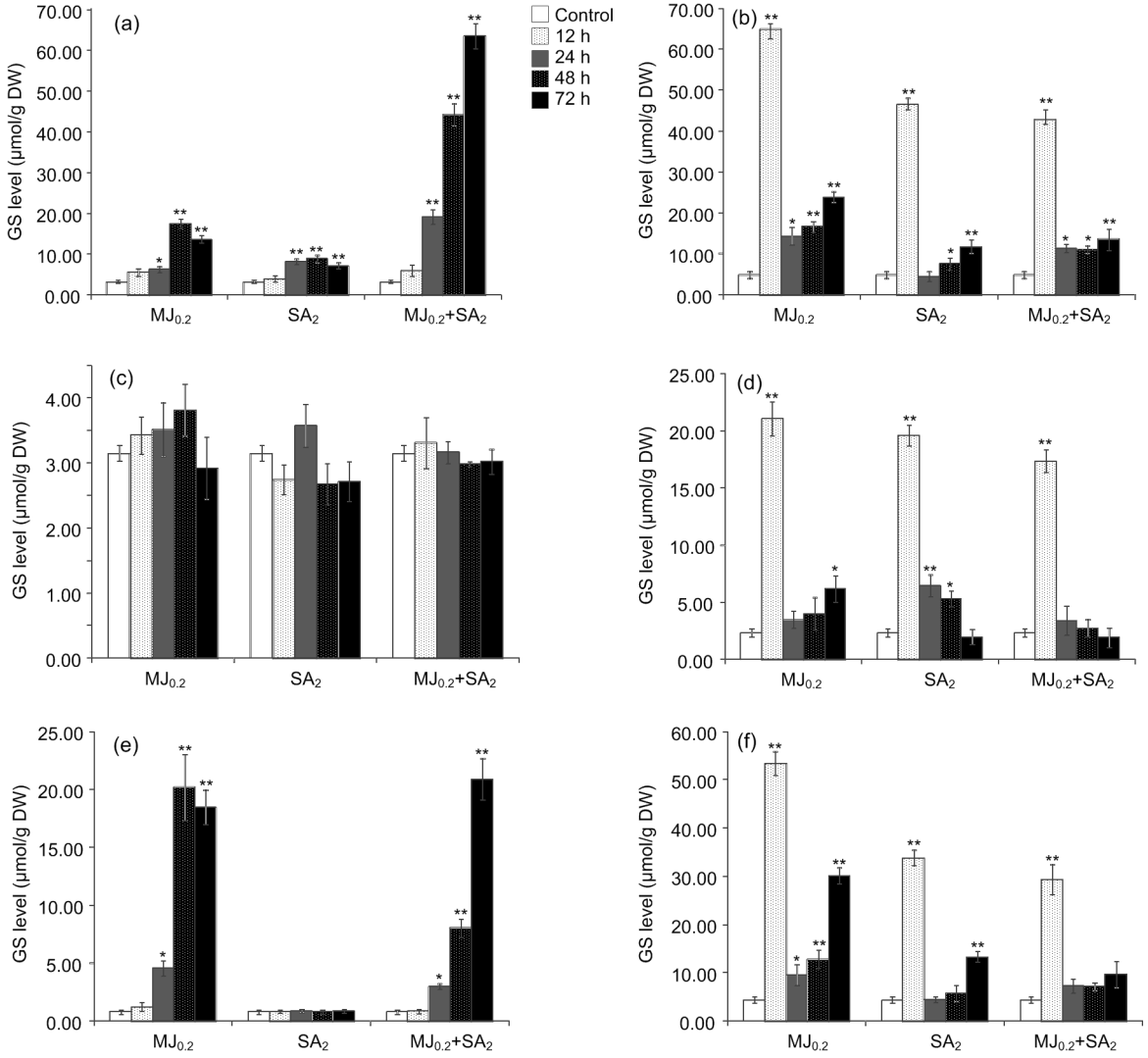

Fig. 2 Accumulation kinetics of GBC, 4-MGBC, and neoGBC in leaves (a, c, e) and roots (b, d, f) of ZSN5 with root treatments

$MJ_{0.2}$, SA_2, and $MJ_{0.2}$+SA_2 denote 0.2 mmol/L MeJA, 2.0 mmol/L SA, and 0.2 mmol/L MeJA+2.0 mmol/L SA treatments in root application, respectively. The data were calculated as the mean of three replicates with standard deviation in μmol/g DW. Significant differences of glucosinolate levels compared to control are labeled with ** (P<0.01) or * (P<0.05). 12, 24, 48, and 72 h represent the sampling time of 12, 24, 48, and 72 h after different treatments on ZSN5 (Zao Shu Wu Hao). GBC, glucobrassicin; 4-MGBC, 4-methoxy glucobrassicin; neoGBC, neoglucobrassicin

transcriptional regulator NPR1, along with NPR1-independent pathways for immediate early genes (Uquillas *et al.*, 2004; Kachroo and Kachroo, 2013). Competition for NPR1 as a part of crosstalk control by SA-mediated transduction systems could inhibit the signaling of JA.

GSs were also recognized for their distinctive benefits to human nutrition (Verkerk *et al.*, 2009). GS breakdown products, particularly isothiocyanates and nitriles, have been shown to induce the phase II detoxification enzymes, resulting in cell cycle arrest or apoptosis (Lund, 2003). Indole-3-carbinol produced by the breakdown of GBC is known to exert an anti-carcinogenic effect. An inverse association between the GS intake, especially aliphatic GS, and the risk of prostate cancer was recently found (Steinbrecher *et al.*, 2009). Thus, induced accumulation of GSs in Chinese cabbage will be beneficial for human health. However, high accumulation of neoGBC may pose a risk to human consumption since

it has a high mutagenic activity (Wiesner *et al*., 2014), and thus this must be taken into consideration for treatment conditions and/or metabolic engineering of Chinese cabbage in order to minimize the accumulation of neoGBC. In this regard, an SA application is preferred to an MeJA application for enhanced leaf GSs because our study showed that an SA application increased the neoGBC level only in roots.

Several differences in GS accumulation patterns between the leaf and root were observed in response to the foliar treatment of MeJA or SA. Firstly, overall accumulation levels and rates of total GSs were much higher and faster in roots than in leaves. Secondly, the rate of IGS accumulation was much faster than that of aliphatic and aromatic GSs in both leaves and roots. Thirdly, the levels of total GSs were continuously increased over 72 h in leaves, but reached the highest level after 24 or 12 h followed by continuously decreased levels in roots with foliar or root treatment, respectively (Figs. 1 and 2). Fourthly, GS accumulation levels were not dependent on elicitor concentration in roots but in leaves, indicating that roots are saturated with 0.1 mmol/L MeJA or 0.2 mmol/L SA. Lastly, the antagonistic effect of foliar SA treatment on the MeJA-induced GS accumulation occurred after 72 h in leaves and 48 h in roots. All these findings indicate that roots are more sensitive and more rapidly responsive to elicitors than leaves. A previous study showed that MeJA and SA treatments triggered high rhizo-secretion of IGS with maximal elicitation occurring on the 10th day of treatment in *B. rapa* ssp. *rapa* plants (Schreiner *et al.*, 2011). Our treatment study was performed over three days. Most of the treatments steadily enhanced the GS levels. Taking the results together, repeated MeJA and SA treatments with a suitable concentration in a regular time interval will bring about a sustained level of GS accumulation that leads to enhanced disease resistance in Chinese cabbage.

The maximum levels of total leaf GS were similar between the elicitor treatment to the leaves and to the roots. When leaves and roots are treated with both stress hormones, SA exerts an antagonistic effect on MeJA-induced root GSs (Tables 2 and 4). However, SA showed a synergistic effect on the MeJA-induced leaf GSs accumulation when roots are treated with both elicitors for 72 h (Table 3). In contrast, SA had an antagonistic effect on the MeJA-induced leaf GS accumulation when leaves are treated with both elicitors for 72 h. Similar observations of the attenuation effect of SA on JA-induced GS accumulation were made in *Arabidopsis* and *B. oleracea* (Cipollini *et al.*, 2004; van Dam *et al.*, 2004). It is intriguing how a plant distinguishes the site of elicitation to reprogram the metabolic profiles. Further researches by comparative RNA-Seq analyses of the leaf and root RNAs from the two different elicitation sites may provide important information on the molecular mode of the specificity of plant responses and the dissection of the interactive signaling networks of MeJA and SA.

5 Conclusions

The present work demonstrated that roots accumulate much more GSs and are more sensitive and more rapidly responsive to elicitors than leaves in Chinese cabbage. However, unlike leaf GSs, accumulation levels of root GSs continue to decrease after 24-h elicitation time. Irrespective of the induction site, MeJA had a greater inducing and longer lasting effect on GSs accumulation than SA. SA exerted an antagonistic effect on the MeJA-induced root GSs irrespective of the site of elicitation. However, SA showed synergistic and antagonistic effects on the MeJA-induced leaf GSs when roots and leaves are elicitated for 3 d, respectively. Plant will respond specifically depending on the organ that is elicitated. Accumulation of IGS is a major metabolic hallmark of SA- and MeJA-mediated systemic response systems. The rate of conversion of GBC to neoGBC appears to occur at a steady rate with the duration of the elicitation. NeoGBC is an MeJA-responsive leaf metabolite that does not respond to SA elicitor, thus, temporal and spatial expression studies of the genes responsible for conversion of GBC to neoGBC may provide an important insight into the molecular mechanism of the elicitor and tissue specificity, by which the gene responds to only MeJA in the leaf tissue but to both SA and MeJA in the root tissue. This study may serve as a practical method to induce high levels of GSs for enhancement of disease resistance as well as for an anti-cancer diet of Chinese cabbage.

Compliance with ethics guidelines

Yun-xiang ZANG, Jia-li GE, Ling-hui HUANG, Fei GAO, Xi-shan LV, Wei-wei ZHENG, Seung-beom HONG, and Zhu-jun ZHU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：茉莉酸甲酯与水杨酸诱导的大白菜叶片与根系硫苷含量系统性变化研究

目　的：通过单独或混合施用茉莉酸甲酯与水杨酸，研究两者在诱导大白菜硫苷合成方面的差异及其相互作用。

创新点：试验中，首次通过混合喷施或灌施茉莉酸甲酯与水杨酸，研究两者在诱导大白菜硫苷合成过程中的相互作用。

方　法：试验过程中采用高效液相色谱法分析各硫苷组分的具体含量，为分析茉莉酸甲酯与水杨酸单独或混合施用在诱导大白菜硫苷合成过程中的作用奠定了基础。

结　论：茉莉酸甲酯与水杨酸处理后，大白菜根系比叶片积累更多的硫苷，吲哚族硫苷比其他种类的硫苷积累更快；茉莉酸甲酯诱导硫苷合成的效果好于水杨酸，而且诱导时间更长；茉莉酸甲酯与水杨酸在诱导大白菜根系硫苷合成过程中具有反协同效应。

关键词：大白菜；硫苷；茉莉酸甲酯；水杨酸