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Full Length Research Paper

Effects of different concentrations of sodium chloride on plant growth and glucosinolate content and composition in pakchoi

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Pakchoi (*Brassica campestris* L. ssp. *chinensis* var. *communis*) is one of the most important *Brassica* vegetables in China, and the consumption of *Brassica* vegetables reduces the risk of cancer occurrence. The aim of this study was to investigate the contents and composition of glucosinolates in pakchoi shoots exposed to 0, 50 and 100 mM sodium chloride (NaCl) for two weeks. The results showed that salt stress significantly decreased the fresh weight of whole plant and the dry weight of shoots and roots of pakchoi, as compared to the control. Under 50 mM NaCl, the contents of total glucosinolates, aliphatic and indole glucosinolates were significantly increased, however, under 100 mM NaCl, the content of indole glucosinolates was significantly increased and aromatic glucosinolate (gluconasturtiin) content was significantly decreased. For individual glucosinolates, the contents of glucoalyssin, gluconapin, glucobrassicin and neglucobrassicin were significantly enhanced under 50 mM NaCl, however, only significant increases in the contents of gluconapin and glucobrassicin were observed under 100 mM NaCl. It could be concluded that NaCl stress considerably influenced the glucosinolate content and composition in pakchoi shoots.

Key words: Gluconapin, glucoalyssin, glucobrassicanapin, glucobrassicin, neoglucobrassicin, gluconasturtiin, 4-methoxyglucobrassicin.

INTRODUCTION

Glucosinolates are a category of secondary compounds mainly found in the cruciferous plants such as cauliflower, broccoli, cabbage, etc (Yan and Chen, 2007). All the glucosinolates share a chemical structure comprising a sulfonated oxime moiety, a β-D-thioglucose group and a variable side chain derived from methionine, tryptophan, phenylalanine or various branched chain amino acids. Based on their precursor amino acids, glucosinolates can be categorized into three classes: aliphatic, indole and aromatic glucosinolates (Halkier and Gershenzon, 2006).

Glucosinolates are hydrolyzed by myrosinases into different degradation products (isothiocynates, thiocynates, nitriles and epithionitriles) with a variety of biological activities. These degradation products are responsible for the characteristic flavor, pathogen defense system and insect attractants of *Brassica*. Moreover, isothiocyanates have been shown to possess anti-carcinogenic (Mithen et al., 2000). For example, the hydrolysis product of gluconasturtiin and 2-phenylethyl isothiocyanate can prevent cancer by inhibiting phase I enzymes and inducing phase II enzymes, resulting in carcinogen excretion (Hecht et al., 1999; Engelen-Eigles et al., 2006). 4-Methylsulfinylbutyl isothiocyanate (sulforaphane) may prevent tumor growth by blocking the cell cycle and promoting apoptosis, and exhibits potential for treating *Helicobacter pylori*-caused gastritis and stomach cancer (Fahey et al., 2002; Thornalley, 2002; Halkier and Gershenzon, 2006).

Because glucosinolates are sulfur and nitrogen containing compounds, most studies on the influence of mineral elements on glucosinolates concentration in plants focus

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on nitrogen and sulfur (Schonhof et al., 2007; Li et al., 2007). Little is known about glucosinolates accumulation in response to salt stress, although the previous studies indicate that environmental factors such as light (Engelen-Eigles et al., 2006), temperature (Velasco et al., 2007) and heavy metals (Coolong et al., 2004; Tolra et al., 2006) alter the glucosinolate content and composition.

Salt stress is a major abiotic stress reducing the productivity of crops in many areas of the world (Yamaguchi and Blumwald, 2005). Salinity affects the water balance and results in osmotic damage; however, osmotic adjustment is a plant adaptation mechanism used to maintain their water balance in plant (Sairam and Tyagi, 2004). It has been suggested that high concentrations of organic solutes in the cytoplasm, including proline, sucrose, glycinebetaine and secondary metabolites, such as glucosinolates, contributes to the osmotic balance (López-Berenguer et al., 2009).

Pakchoi (*Brassica campestris* L. ssp. *chinensis* var. *communis*) is an important cruciferous vegetable crop cultivated extensively in China. It contains high vitamins, mineral nutrients, glucosinolates and other phyto-chemicals. There is limited information about the effect of salinity on the content and composition of glucosinolates in pakchoi. The objectives of this study were to investigate the effects of different concentrations of NaCl on plant growth, contents of total and individual glucosinolates in pakchoi shoots.

MATERIALS AND METHODS

Plant materials, growth conditions and treatments

Seeds of pakchoi cv. Shang Hai Qing were purchased from Hangzhou Seed Company. Seeds were sown on growth medium containing a mixture of peat and vermiculite (7:3, v : v) in greenhouse. When the first true leaf was fully expanded, seedlings were grown in nutrient solutions with 2.5 mM $Ca(NO_3)_2$, 1.0 mM KH_2PO_4, 4.0 mM KNO_3, 1.0 mM $MgSO_4$, 0.5 mM NH_4NO_3, 10.0 µM H_3BO_3, 0.1 µM H_2MoO_4, 3.0 µM $MnSO_4$, 2.0 µM $ZnSO_4$, 0.8 µM $CuSO_4$ and 40.0 µM EDTA-Fe. There were 6 seedlings per 10 L pot. The nutrient solutions were aerated continuously with an air bubbler and changed every one week. The growth conditions were as follows: photoperiod of 14/10 h (day/night), temperature of 25/17°C (day/night), and the maximal photosynthetic photon flux density of 600 µmol m^{-2} s^{-1}. After three weeks of transfer to the hydroponic medium, the salt treatments were applied by adding 50 and 100 mM NaCl to the nutrient solution and maintained for two weeks. Each treatment had three replicates.

The plants were harvested after two weeks of treatments. The whole plant fresh weight was determined, and then the plants were separated into shoots and roots and immediately frozen with liquid nitrogen. The samples were freeze-dried, and the dry weight was determined and the shoots were ground to a fine powder and stored at -80°C for analysis.

Analysis of sodium

The analysis of sodium was determined by the method of Ruan et al. (2007) with minor modifications. 0.30 g of freeze-dried shoot powder was digested by concentrated acids mix (HNO_3 : $HClO_4$, 5:2 v/v) and analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES).

Extraction and determination of glucosinolates

Glucosinolates were analyzed according to the method of Krumbein et al. (2005) with minor modification. Duplicates of freeze-dried samples (0.25 g) were extracted in 4 ml of 70% methanol. Then the mixtures were kept at 75°C in a water bath for 10 min. For internal standardization, 100 µl of 5 mM sinigrin (Sigma-Aldrich Co., St. Louis, USA) were added to one of the duplicates before extraction. Then 1 ml of 0.4 M barium acetate were rapidly added and the vials vortexed for several seconds. The homogenates were centrifuged for 10 min at 2100 g. The supernatants were decanted and stored on ice and pellets were re-extracted twice with 3 ml of 70% methanol (75°C). The combined supernatants were applied to a 0.5 ml DEAE Sephadex™ A-25 column (Amersham Biosciences, Sweden) and washed with 5 ml of distilled water produced by Milli-Q system (Milli-pore Co., USA) and incubated overnight with aryl sulfatase (Sigma-Aldrich Co., St. Louis, USA) for desulfation. The resultant desulphoglucosinolates were eluted with 3 ml of ultra pure water produced by Milli-Q system and stored at -20°C until they were analyzed by high performance liquid chromatography (HPLC).

HPLC analysis was performed using an Agilent 1200 system (Santa Clara, USA) equipped with a C_{18} reverse-phase column (250 × 4 mm, 5 µm, Bischoff, Germany) using the following gradient: H_2O (2 min), a linear gradient of 0 - 20% acetonitrile (Tedia, USA) (32 min), 20% acetonitrile (6 min), followed by 100% acetonitrile and 0% acetonitrile prior to the injection of the next sample. The flow rate was 1.3 ml min^{-1}.

Statistical analysis

Measurements were analyzed using the SAS 8.0 (SAS Institute, Cary, NC) for the general linear models procedure (GLM). The least significant difference (LSD) was used to compare the means.

RESULTS AND DISCUSSION

Plant growth and Na accumulation

As shown in Table 1, salinity inhibited pakchoi growth. The fresh weight of the whole plant was significantly decreased with increasing salt concentration. Moreover, plant shoot dry weight was reduced by 26 and 39% at 50 and 100 mM NaCl, respectively. However, root dry weight was deceased by 24 and 33%, respectively. It suggested that shoot were more sensitive than root response to salt stress. It was also in accordance with previous finding of Viégas et al. (2001) who reported that salinity inhibits shoot growth more than root growth in young cashew. Many studies also reported that salinity results in a decline in metabolic activity of plant cells, which is inevitably reflected in inhibition of their growth (Cicek and Cakirlar, 2002).

NaCl application significantly increased Na absorption by pakchoi shoot compared with control (Table 1), which was in agreement with our previous study in tomato (He et al., 2009), indicating that the salt treatment in our

Table 1. Effects of salinity on the growth of pakchoi.

Concentration of NaCl (mM)	Fresh weight (g.plant^{-1})	Dry weight (g.plant^{-1})		Na (mg.g^{-1} DW)
		Shoots	Roots	
0	64.80±10.79 a	2.99±0.07 a	0.45±0.14 a	1.28±0.13 c
50	42.86±12.08 b	2.21±0.37 b	0.34±0.07 b	9.79±0.17 b
100	33.45±2.67 b	1.81±0.30 c	0.30±0.05 b	11.14±0.23 a

Data are the means ± standard deviation of three replicates. Means denoted by the same letter indicates no significant difference between treatments at P < 0.05 levels.

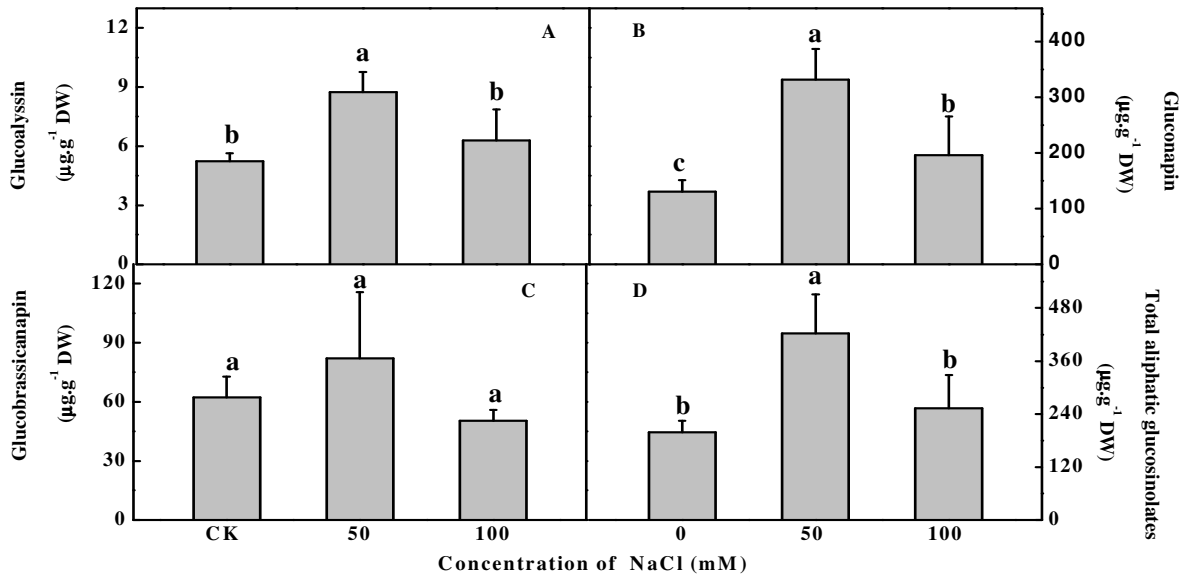

Figure 1. Effects of different concentrations of NaCl on the contents of aliphatic glucosinolates in pakchoi shoots. Data are the means ± standard deviation of three replicates and means denoted by the same letter indicates no significant difference between treatments at P < 0.05 levels.

experiment was effective.

Aliphatic glucosinolates

Three individual aliphatic glucosinolates including glucoalyssin (Figure 1A), gluconapin (Figure 1B) and glucobrassicanapin (Figure 1C) were identified in pakchoi shoots. 50 mM NaCl treatment significantly increased the contents of glucoalyssin (by 67%), gluconapin (by 154%), and total aliphatic glucosinolates (by 113%) in pakchoi shoots (Figure 1). However, under 100mM NaCl treatment, only a significant increase was observed in gluconapin content (by 50%) as compared to the control.

Aliphatic glucosinolates are derived from methionine (Halkier and Gershenzon, 2006). The increased content of aliphatic glucosinolates in the present experiment (Figure 1) could be attributed to the enhancement of precusor amino acid synthesis. Methionine synthase is significantly induced under salt stress at the levels of both mRNA and protein in barley leaves (Narita et al., 2004).

Indole glucosinolates

Three individual indole glucosinolates, neoglucobrassicin (Figure 2A), glucobrassicin (Figure 2B) and 4-methoxyglucobrassicin (Figure 2C), were detected in the present experiment. The content of neoglucobrassicin in pakchoi shoots were significantly increased by 43% in 50 mM NaCl treatment, however, the increases in 100 mM NaCl treatment were slight. Glucobrassicin content was significantly increased by 87 and 120%, respectively, in 50 and 100 mM NaCl treatments. The content of 4-methoxyglucobrassicin was not significantly affected by salt stress as compared to the control. The total indole

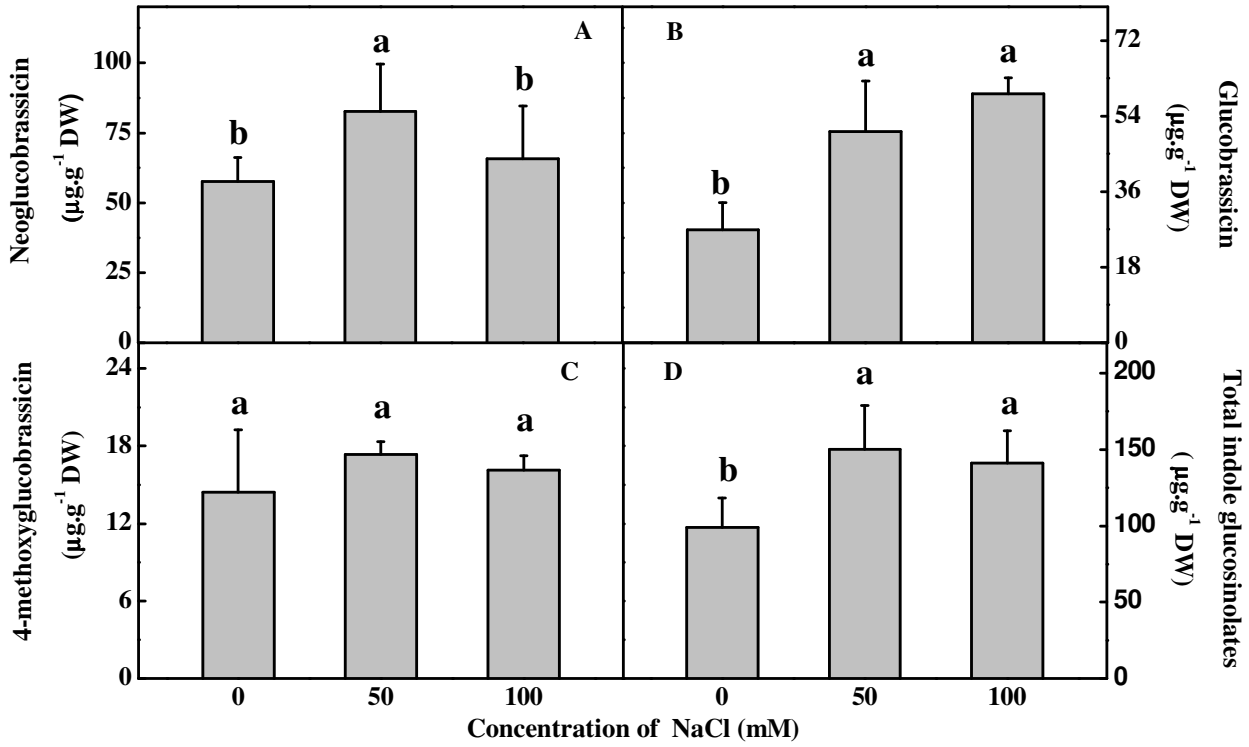

Figure 2. Effects of different concentrations of NaCl on the contents of indole glucosinolates in pakchoi shoots. Data are the means ± standard deviation of three replicates and means denoted by the same letter indicates no significant difference between treatments at P < 0.05 levels.

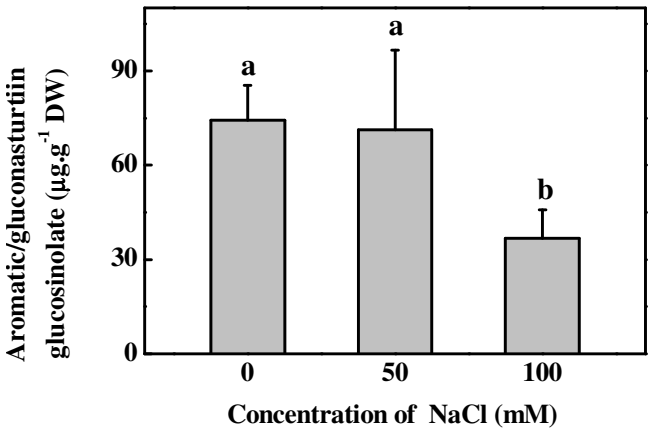

Figure 3. Effects of different concentrations of NaCl on aromatic/gluconasturtiin glucosinolate content in pakchoi shoots. Data are the means ± standard deviation of three replicates and means denoted by the same letter indicates no significant difference between treatments at P < 0.05 levels.

glucosinolate content was significantly enhanced by 52 and 43% at 50 and 100 mM NaCl treatments, respectively (Figure 2D).

The increase in the indole glucosinolate content under salt stress was predominantly due to the increase of neoglucobrassicin and glucobrassincin. Glucobrassicin is the parent compound of indole-3-carbinol, found in cruciferous vegetables (Montaut et al., 2009). A recent study has pointed out that glucobrassicin can decrease levels of urinary metabolites of tobaccospecific lung carcinogen in smokers (Hetcht et al., 2004). This study provided the possibility of enhancing glucobrassicin in pakchoi through artificial salt stress. Mechanical wounding and jasmonic acid also significantly increase the glucobrassicin content in woad (Galletti et al., 2006).

Aromatic glucosinolate

As for aromatic glucosinolate, only gluconasturtiin was identified in pakchoi shoots (Figure 3). In contrast to aliphatic and indole glucosinolates, a significant decrease (by 51%) in the aromatic glucosinolate content was observed under 100mM NaCl. Aromatic glucosinolates are derived from phenylalanine (Mithen et al., 2000). Wielanek and Urbanek (2006) have found that aromatic glucosinolate glucotropaeolin content is 2-fold enhanced after treatment with phenylalanine ammonia lyase inhibitor in nasturtium. Meanwhile, Dunn et al. (1998) have reported that NaCl stress exhibit a reduction in phenylalanine ammonia lyase activity in citrus. However, the mechanisms on the decrease of aromatic glucosinolate by salinity are

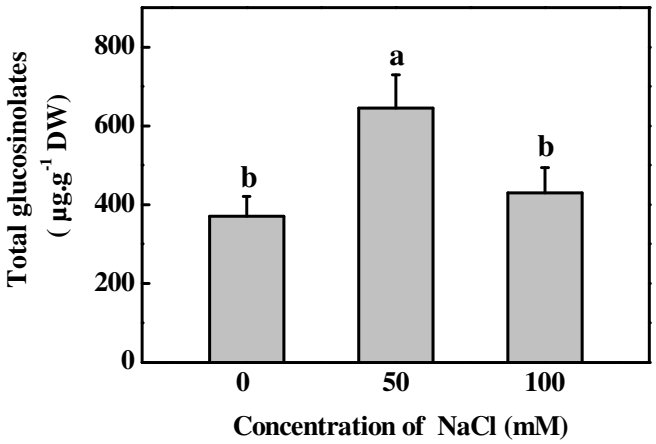

Figure 4. Effects of different concentrations of NaCl on total glucosinolate contents in pakchoi shoots. Data are the means ± standard deviation of three replicates and means denoted by the same letter indicates no significant difference between treatments at P < 0.05 levels.

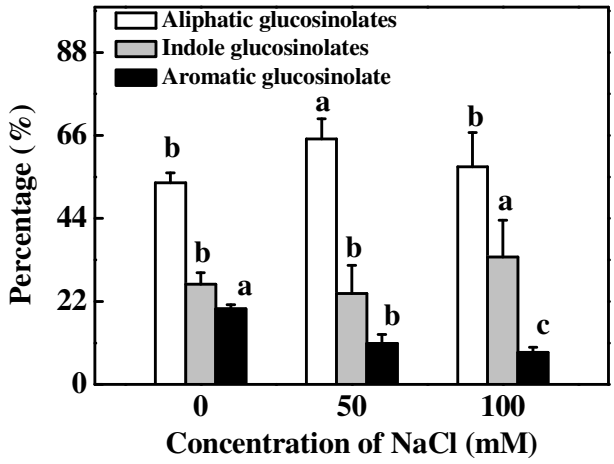

Figure 5. Effects of different concentrations of NaCl on the percentage of aliphatic, indole and aromatic glucosinolates to total glucosinolates. Data are the means ± standard deviation of three replicates and means denoted by the same letter indicates no significant difference between treatments at P < 0.05 levels.

at 100 mM NaCl. In the present study, total indole glucosinolate percentage increased by 27% at 100 mM still poorly understood.

Total glucosinolates

The content of total glucosinolates was expressed by the sum of seven identified individual glucosinolates. Total glucosinolate contents influenced by salinity were shown in Figure 4. Total glucosinolate content in pakchoi shoots was significantly enhanced under 50 mM NaCl, however, the increase under 100 mM NaCl was not significant. Qasim et al. (2003) have found that salinity increase total glucosinolate concentration in oilseed rape. Glucosinolates may have a potential role in osmotic adjustment and might be an adaptive component of salt tolerance (López-Berenguer et al., 2009). Other studies have reported that glucosinolate accumulation occurs due to biotic stress since glucosinolates are involved in the plant defense response against herbivores and pathogen attack (Mewis et al., 2005; Brader et al., 2006; Bennett and Wallsgrove, 1994). Moreover, mechanical impacts also increase glucosinolate concentration in *Brassica* vegetables (Bodnaryk, 1992). Some abiotic stress factors, such as UV-B (Schreiner et al., 2009) and water stress (Zhang et al., 2008), lead to increased glucosinolate concentration in nasturtium and turnip.

Relative contents

As shown in Figure 5, aliphatic glucosinolates were the most abundant in pakchoi shoots. The percentage of the total aliphatic glucosinolate increased by 22% at 50 mM NaCl in pakchoi shoots, but the impact was not significant NaCl treatment compared with the control. However, 50 mM NaCl stress had no distinct influence on total indole glucosinolate percentage composition. Aromatic glucosinolate percentage significantly decreased by 46 and 58% at 50 and 100 mM NaCl treatments, respectively. These results indicated that salt stress increased the percentage of aliphatic and indole glucosinolates in pakchoi shoots, but decreased aromatic glucosinolate percentage.

Conclusion

From these results, we concluded that NaCl stress affected the levels of individual glucosinolates content, and changed the composition of aliphatic glucosinolate, indole glucosinolate and aromatic glucosinolate in pakchoi shoots. This provided the opportunity to modulate the profile of glucosinolates, and to increase the levels of aliphatic and indole glucosinolates with health-promoting properties. Moreover, these results suggested that NaCl stress offered a strategy to produce glucosinolates especially for glucobrassicin as components for functional foods, nutraceuticals and pharmaceuticals. In addition, under salt stress, an increase in glucosinolate contents appears to be involved in the response of pakchoi to NaCl stress, but more biosynthetic and metabolic information about glucosinolates under salinity would deserved further attention.

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