Difference in Sodium Spatial Distribution in the Shoot of Two Canola Cultivars Under Saline Stress

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Among different mechanisms of salt resistance, regulation of ion distribution among various tissues and intracellular compartmentation are of great importance. In this study, we investigated the effects of salt stress on growth, photosynthesis, and Na⁺ accumulation and distribution in leaf apoplast and symplast of two canola (Brassica napus L.) cultivars (NYY 1 and BZY 1). The results showed that the declines in shoot dry mass, leaf water potential and net photosynthetic rate of BZY 1 (salt sensitive) were higher than those of NYY 1 (salt resistant) in response to salt stress. Stomatal limitation to photosynthesis was mainly affected under moderate salinity, whereas the reduction in assimilation rate under severe salt stress was due to both stomatal and non-stomatal limitations. We also found that more Na⁺ was distributed to leaf veins in NYY 1 than in BZY 1; simultaneously, less Na⁺ accumulated in the leaf blade in NYY 1 than in BZY 1. The percentage of Na⁺ in the leaf symplast in NYY 1 was markedly lower than that in BZY 1. Also, Na⁺ diffusion in leaves through apoplastic and symplastic pathways of BZY 1 was stronger than that in NYY 1, and the transpiration rate in BZY 1, especially at the leaf edges, decreased more than in NYY 1. Our results showed that NYY 1 accumulated less Na⁺ in the shoot, especially in leaf blades, and confined Na⁺ to the apoplast to avoid leaf salt toxicity, which could be one reason for the higher resistance of NYY 1 than BZY 1 plants to salt stress.

Keywords: Apoplast • Canola seedlings • NaCl stress • Na⁺ transport.

Abbreviations: AWF, apoplastic washing fluid; Ce, intercellular CO₂ concentration; gs, stomatal conductance; Lm, stomatal limitation; Pn, net photosynthetic rate; PPFD, photosynthetic photon flux density; Tr, transpiration rate.

Introduction

Soil salinization not only is a serious environmental problem but also poses a severe threat to plant growth and agricultural productivity (Allakhverdiev et al. 2000). Approximately 20% of the world’s cultivated land and 50% of cropland has already been lost to salinization (Lakhdar et al. 2009). More seriously, it is estimated that >50% of all arable land will suffer from salinization by the year 2050 (Wang et al. 2003). The detrimental effects of high salinity on plants can be observed at the whole-plant level as death of plants and/or decreases in productivity (Munns 2002, Zheng et al. 2010b). The development of salt-resistant crops is believed to be the most effective way to improve productivity of plants grown in saline soils (Zheng et al. 2009).

The impairment process under saline conditions is very complex and depends on the specific cultivars, the salt concentration and the type of ion in the growth medium (Zheng et al. 2009). Generally, plants grown on salt-affected soils suffer from osmotic stress, ion toxicity and nutrient deficiency (Cheeseman 1988, Silberbush and Ben-Asher 2001, Zhu 2001). During the first of these phases, it becomes difficult for plant roots to take up water from the soil (Munns 2009). Dehydration and reduced growth rates are typical in salt-sensitive plants. In the subsequent phase, excess salt in leaves, imported through the xylem, can inhibit enzyme activity and disturb metabolism. For example, leaf photosynthesis in crop plants is reduced by salinity (Parida and Das 2005), leading to suppressed growth and development of the plants. Once the stress exceeds a certain level, the plants die (Munns 2002). To reduce these detrimental effects, it is vital to restrict the entry of Na⁺ efficiently and to exclude Na⁺ rapidly from plants to reduce toxic levels in the cytoplasm (Lacan and Durand 1996, Nass et al. 1997). There are two different modes of Na⁺ exclusion by which Na⁺ is transferred from the cytoplasm to the vacuole and apoplast, mainly mediated by an Na⁺/H⁺ antiporter in the tonoplast and plasma membrane (Niu et al. 1995). Compared with cytoplasm, the apoplast contains fewer enzymes and, therefore, is likely to be a more appropriate place to store Na⁺. Several contradictory studies have reported on the relevance of plant salt resistance and Na⁺ distribution in the leaf apoplast (Speer and Kaiser 1991, Mühling and Läuchli 2002, Ottow et al. 2005), and
whether \(\text{Na}^+\) accumulated in the apoplast is beneficial to salt resistance is not yet clear. Plant growth is the result of integrated and regulated physiological processes. The dominant physiological process is photosynthesis (Munns 2002). Plant growth as biomass production is a measure of net photosynthesis, and, therefore, environmental stresses affecting growth also affect photosynthesis (Parida and Das 2005). There are many reports of suppression of photosynthesis by salt stress (Steduto et al. 2000, Parida and Das 2005, Li et al. 2008, Praxedes et al. 2010).

Canola (\textit{Brassica napus} L.) is a moderately salt-tolerant crop, grown mainly for its edible oil (Akkari et al. 2011). However, its production and quality are greatly reduced by soil salinity (Ruiz and Blumwald 2001, Lomonte et al. 2010). Improvement of salt resistance in this crop would therefore be of considerable economic value. In this study, we examined the effect of gradually developing salinity stress on plant growth in two canola cultivars differing in salt resistance. A vacuum infiltration technique was used to investigate whether the concentration of apoplastic \(\text{Na}^+\) was associated with salt resistance. Leaf water potential, photosynthesis, transpiration rate and \(\text{Na}^+\) accumulation in different parts of a plant were also investigated. We addressed two specific questions: (i) whether there are differences between different salt-resistant canola varieties with respect to \(\text{Na}^+\) distribution in the leaf apoplast and (ii) how \(\text{Na}^+\) distribution in the leaf affects leaf gas-exchange parameters and stomatal and non-stomatal limitations to carbon assimilation.

### Results

#### Effect of salt stress on plant growth and leaf water potential

As shown in Fig. 1, under mild salt stress conditions (3 g kg\(^{-1}\) NaCl), there was no significant decrease in shoot dry mass of NYY 1 compared with the control; however, an obvious decline was found in BZY 1. When exposed to moderate and severe salt stress levels (6 and 9 g kg\(^{-1}\) NaCl), the shoot dry mass of both canola cultivars was reduced significantly, with reductions of 37 and 63% in NYY 1, and 59 and 73% in BZY 1, respectively. Outwardly, some leaf edges of BZY 1 developed local necrosis, but NYY 1 leaves were apparently unaffected. Similarly, in the treatment with 3 g kg\(^{-1}\) NaCl, the leaf water potential (\(\Psi_w\)) of NYY 1 did not change, whereas that of BZY 1 declined significantly under mild salt stress compared with the control. Treatments with 6 and 9 g kg\(^{-1}\) NaCl decreased the \(\Psi_w\) of both canola cultivars: 37 and 42% in NYY 1, and 44 and 68% in BZY 1, respectively (Fig. 2).

#### Effect of salt stress on gas-exchange parameters

In NYY 1, net photosynthetic rate (\(P_n\)), stomatal conductance (\(g_s\)), intercellular CO\(_2\) concentration (\(C_i\)), transpiration rate (\(T_r\)) and stomatal limitation (\(L_s\)) in leaves grown under mild salt stress (3 g kg\(^{-1}\) NaCl) were unaffected; however, 3 g kg\(^{-1}\) NaCl treatment significantly decreased \(P_n\), \(g_s\) and \(C_i\), and increased \(L_s\) of BZY 1 (Table 1). After moderate and severe salt stress (6 and 9 g kg\(^{-1}\) NaCl treatments), there was a remarkable reduction in \(P_o\), \(g_o\), \(C_o\) and \(T_o\), and an increase in \(L_s\) in both canola cultivars. \(P_n\) decreased by 43 and 67% in NYY 1 and by 46 and 78% in BZY 1 under 6 and 9 g kg\(^{-1}\) NaCl stress, respectively (Table 1). We also calculated the ratios of the \(T_r\) between the leaflet edge and center: \((T_r\text{ of LE})/(T_r\text{ of LC})\) (Fig. 3). In NYY 1, the \((T_r\text{ of LE})/(T_r\text{ of LC})\) of seedlings remained unaffected under 3 g kg\(^{-1}\) NaCl, whereas the ratio significantly declined with 6 and 9 g kg\(^{-1}\) NaCl treatment. In contrast, the ratio in BZY 1 seedlings grown under all NaCl treatments was significantly decreased. The ratio decreased by 5 and 14% in NYY 1 and by 28 and 33% in BZY 1 under 6 and 9 g kg\(^{-1}\) NaCl stress, respectively (Fig. 3).

#### Effect of salt stress on Na\(^+\) concentration in the petiole, leaf main vein and blade

Treatment with NaCl significantly increased the Na\(^+\) concentration in the petiole, leaf main vein and blade of both canola cultivars compared with controls and increased it to a greater extent in BZY 1 than in NYY 1 (Table 2). Especially in blade cells, the Na\(^+\) concentration increased by 167, 360 and 670% in NYY 1 after mild, moderate and severe salt stress, respectively, compared with 296, 592 and 1,036% in BZY 1 under the same conditions.
NaCl treatments. We also calculated the $\frac{[\text{Na}^+]_{\text{main vein}}}{[\text{Na}^+]_{\text{blade}}}$ which were 1.17, 1.38, 1.47 and 1.97 in NYY 1, and 0.96, 0.83, 0.87 and 1.21 in BZY 1 after 0, 3, 6 and 9 g kg$^{-1}$ NaCl treatment, respectively. This suggests that there was more Na$^+$ in the main vein of leaves in NYY 1 than in BZY 1.

**Effect of salt stress on Na$^+$ concentrations in the leaf apoplast and symplast**

All treatments with increasing NaCl led to a significant increase in Na$^+$ in the leaf apoplast and symplast of both canola cultivars (Fig. 4A, B). We calculated the $\frac{[\text{Na}^+]_{\text{sym}}}{[\text{Na}^+]_{\text{apo}}}$ (Table 3) using the data of Fig. 4A and B. In the center of the leaf, the ratios in BZY 1 were 2.35, 1.74 and 1.50 times those of the ratios in NYY 1 after mild, moderate and severe salt stress, respectively. At the leaf edge, the ratios in BZY 1 were 2.11, 1.96 and 1.46 times those of NYY 1, respectively (Table 3). To better understand Na$^+$ distribution in different leaf spaces, we prepared a schematic diagram showing the Na$^+$ concentration in the cell symplast and apoplast of the two canola varieties under 9 g kg$^{-1}$ NaCl (Fig. 5). From Fig. 4 and Table 3 we found that there was relatively more Na$^+$ in the cell symplast, and this phenomenon was more significant in BZY 1. Thus, BZY 1 clearly showed a steeper Na$^+$ gradient across the leaf cell plasma membrane than did NYY 1 under salt stress (Fig. 4, Table 3). We also calculated the increased rate of $[\text{Na}^+]_{\text{apo}}$ and $[\text{Na}^+]_{\text{sym}}$ (Table 3) from Fig. 4A and B. Under mild, moderate and severe salt stress, the increased rate of $[\text{Na}^+]_{\text{apo}}$ in the leaf center of NYY 1 was 2.23, 1.93 and 1.48 times that of BZY 1, respectively. The value of the increased rate of $[\text{Na}^+]_{\text{apo}}$ in the leaf edge of NYY 1 was also 1.3 times that of BZY 1 exposed to severe salt stress (9 g kg$^{-1}$ NaCl). However, there were no obvious changes in the increased rate of $[\text{Na}^+]_{\text{apo}}$ at the leaf edge between the two canola cultivars under mild and moderate stresses (Table 3). However, the values of the increased rate of $[\text{Na}^+]_{\text{sym}}$ were higher in the leaf center and edge of BZY 1 compared with those of NYY 1 for all NaCl treatments. The values of the increased rate of $[\text{Na}^+]_{\text{sym}}$ in the leaf center of BZY 1 were 1.47, 1.29 and 1.46 times higher than those in NYY 1 under mild, moderate and severe salt stresses, respectively (Table 3). Table 3 shows that values of $\frac{[\text{Na}^+]_{\text{apo}}}{\text{le}}$ and $\frac{[\text{Na}^+]_{\text{apo}}}{\text{le}}$ in BZY 1 were much higher than those in NYY 1.

**Discussion**

**Canola NYY 1 is more resistant to salinity stress than BZY 1**

Plant growth can be largely suppressed by excess salt in soil (Flowers 2004, Parida and Das 2005). When canola plants were...
Fig. 3 Effects of four NaCl levels (0, 3, 6 and 9 g kg\(^{-1}\)) on the (T, of LE)/
(T, of LC) ratios of canola seedlings. Canola seedlings of NYY 1 and
BZY 1 were irrigated with one-quarter strength Hoagland nutrient
solution. When the fourth true leaf emerged from the plants, they
were transplanted to soil placed in pots with or without additional
NaCl (3, 6 and 9 g kg\(^{-1}\)) for 20 d (for details, see the Materials and
Methods). The values of the transpiration rate (T, mmol H\(_2\)O m\(^{-2}\)
\(\cdot\) s\(^{-1}\)) in the leaflet center (LC) and edge (LE) were monitored from
three plants in each treatment, and we calculated the (T, of LE)/(T, of
LC) ratios in the figure representing the means ± SE of three exper-
iments. Different letters in the same cluster indicate statistical differ-
ence according to Duncan’s multiple range test (\(P < 0.05\)).

Table 2 Effects of four NaCl levels (0, 3, 6 and 9 g kg\(^{-1}\)) on Na\(^+\)
concentration in shoot of canola seedlings

<table>
<thead>
<tr>
<th>Canola cultivars</th>
<th>NaCl treatments (g kg(^{-1}))</th>
<th>Na(^+) concentration, mg (g DW(^{-1}))</th>
<th>Petiole</th>
<th>Leaf main vein</th>
<th>Leaf blade</th>
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<tbody>
<tr>
<td>NYY 1</td>
<td>0</td>
<td>12.32 ± 0.53d</td>
<td>3.77 ± 0.07c</td>
<td>3.23 ± 0.40c</td>
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<td>3</td>
<td>31.20 ± 2.26c</td>
<td>11.85 ± 0.43c</td>
<td>8.61 ± 0.95c</td>
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<td></td>
<td>6</td>
<td>48.31 ± 3.57b</td>
<td>21.89 ± 2.77b</td>
<td>14.86 ± 2.02b</td>
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<td></td>
<td>9</td>
<td>66.72 ± 4.75a</td>
<td>48.89 ± 2.87a</td>
<td>24.88 ± 1.19a</td>
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<tr>
<td>BZY 1</td>
<td>0</td>
<td>13.04 ± 0.46d</td>
<td>4.12 ± 0.24d</td>
<td>4.27 ± 0.60d</td>
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<td>3</td>
<td>43.35 ± 1.56c</td>
<td>13.98 ± 1.98c</td>
<td>16.92 ± 1.07c</td>
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<tr>
<td></td>
<td>6</td>
<td>64.01 ± 1.65b</td>
<td>25.76 ± 1.19b</td>
<td>29.55 ± 2.02b</td>
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<td></td>
<td>9</td>
<td>72.75 ± 0.60a</td>
<td>58.51 ± 1.53a</td>
<td>48.52 ± 1.31a</td>
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Canola seedlings of NYY 1 and BZY 1 were irrigated with one-quarter strength
Hoagland nutrient solution. When the fourth true leaf emerged from the plants,
they were transplanted to soil placed in pots with or without additional NaCl
(3, 6 and 9 g kg\(^{-1}\)) for 20 d (for details, see the Materials and Methods).
Leaves were harvested from three plant in each treatment for Na\(^+\) concen-
tration determination in petiole, leaf main vein and blade, and the data represent
the means ± SE of three experiments. Different letters in the same column in-
dicate statistical difference according to Duncan’s multiple range test (\(P < 0.05\)).

Fig. 4 Effects of four NaCl levels (0, 3, 6 and 9 g kg\(^{-1}\)) on Na\(^+\)
concentration in the leaf apoplast (A) and leaf symplast (B) in canola
seedlings. Canola seedlings of NYY 1 and BZY 1 were irrigated with
one-quarter strength Hoagland nutrient solution. When the fourth true leaf emerged from the plants, they were transplanted to soil placed in pots with or without additional NaCl (3, 6 and 9 g kg\(^{-1}\))
for 20 d (for details, see the Materials and Methods). The discs were
obtained by a punch from the leaflet center (LC) and edge (LE) of
three plants in each treatment for Na\(^+\) concentration determination
in the leaf apoplast (A) and leaf symplast (B), respectively (for details,
see the Materials and Methods). The data in the figure represent the
means ± SE of three experiments. Different letters in the same cluster indicate
statistical difference according to Duncan’s multiple range test (\(P < 0.05\)).

leaves of canola seedlings gradually decreased with exposure to
increasing NaCl concentrations (0, 100, 150, 200, 250 and
300 mM NaCl) for 24 h (Dai et al. 2009), showing that canola
seedling leaves lose more water with the increase of NaCl
concentration. In order to screen suitable canola varieties

grown in the presence of a salt concentration of 50, 100 and
200 mM NaCl, both the fresh and dry weight of the roots and
shoots of all plants decreased as the salt concentration
increased (Sergeeva et al. 2006). The relative water content in
Increased rate of \([\text{Na}^+]_{\text{apo}}\) obtained by a punch from the leaflet center (LC) and edge (LE) of three plants in each treatment (for details, see the Materials and Methods). The values were calculated from the ratio of \([\text{Na}^+]_{\text{sym}}/[\text{Na}^+]_{\text{apo}}\) of LE and LC in canola seedlings.

### Table 3: Effects of four NaCl levels (0, 3, 6 and 9 g kg\(^{-1}\)) on \([\text{Na}^+]_{\text{sym}}/[\text{Na}^+]_{\text{apo}}\)

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<tr>
<td>NYY 1</td>
<td>LC 13.27 ± 0.70a</td>
<td>LE 9.80 ± 0.77b</td>
<td>LC 4.41 ± 0.32d</td>
<td>LE 2.03 ± 0.43f</td>
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<td>(4.42 ± 0.35f)</td>
<td>(7.77 ± 0.40f)</td>
<td>(11.48 ± 1.26b)</td>
<td>(5.25 ± 0.58d)</td>
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<td>(3.69 ± 0.11g)</td>
<td>(4.35 ± 0.18f)</td>
<td>(16.58 ± 1.67a)</td>
<td>(10.97 ± 0.20b)</td>
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<td>(9.33 ± 0.87bc)</td>
<td>(6.22 ± 0.27def)</td>
<td>(1.98 ± 0.27fg)</td>
<td>(1.43 ± 0.23g)</td>
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<td>(5.95 ± 0.71d)</td>
<td>(3.49 ± 0.52ef)</td>
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<td>(11.24 ± 0.85b)</td>
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<td>(1.26b)</td>
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<td>(13.27)</td>
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<td>NYY 1</td>
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<td>(1.26)</td>
<td>(1.02)</td>
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<td>BZY 1</td>
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### Fig. 5: Schematic diagram of Na\(^+\) distribution

The figure illustrates the Na\(^+\) distribution in 9 g kg\(^{-1}\) NaCl-treated leaves of canola seedlings. Canola seedlings of NYY 1 and BZY 1 were irrigated with one-quarter strength Hoagland nutrient solution. When the fourth true leaf emerged from the plants, they were transplanted to soil placed in pots with or without additional NaCl. The data represent the means ± SE of three experiments. Different letters in the same row indicate statistical difference according to Duncan’s multiple range test (P < 0.05).

**Difference of sodium distribution between the leaves of the two canola plants**

In this study, the major and particular observation made on canola was the differences in the ratio of [Na\(^+\)] in symplast to that in apoplast. Subcellular Na\(^+\) distribution is a vital feature of the physiological response of a plant to salinity (Munns 2002, Parida and Das 2005). Salt-tolerant plants differ from salt-sensitive plants in having a low rate of Na\(^+\) and Cl\(^-\) transport into leaves and the ability to compartmentalize these ions in vacuoles to prevent a build-up in the cytoplasm or cell walls, and thus avoid salt toxicity (Munns 2002). The capacity of the cells to compartmentalize Na\(^+\) in the vacuole was positively related to plant salt tolerance (Parida and Das 2005). However, different opinions on the relationship between the apoplastic Na\(^+\) level and salt resistance have emerged based on different species and measuring techniques. Pea plant is more sensitive to salt stress than spinach, due to an inability to compartmentalize Na\(^+\) in the apoplast.
insights into the role of the leaf apoplastic space in salt resistance. Salt-sensitive BZY 1 accumulated more Na\(^+\) in the shoot than did the salt-resistant NYY 1 (Table 2). Speer and Kaiser (1991) similarly found that salt-sensitive pea accumulated more salt in the shoot than did salt-resistant spinach. However, the salt-sensitive BZY 1 showed steeper Na\(^+\) gradients across the leaf plasma membrane than were seen in salt-resistant NYY 1 under salt stress (Figs. 4, 5). This result is opposite that found in the study by Speer and Kaiser (1991), who suggested that salt-sensitive pea could accumulate several times more Na\(^+\) in the apoplast compared with salt-resistant spinach; nevertheless, the Na\(^+\) concentration in the leaf symplast of pea was slightly higher than that in spinach. Speer and Kaiser (1991) concluded that the salt-resistant spinach was able to accumulate less Na\(^+\) in the apoplast and maintain steeper Na\(^+\) gradients across the leaf cell plasmalemma. Mühling and Läuchli (2002) found that the Na\(^+\) concentrations in the leaf apoplast of salt-sensitive corn and salt-tolerant cotton plants significantly increased with higher Na\(^+\) supply, and higher Na\(^+\) concentrations were found in the leaf apoplast of salt-tolerant cotton compared with those in salt-sensitive corn, particularly in fully expanded leaves. However, higher Na\(^+\) concentrations were found in the leaf apoplast of salt-sensitive canola compared with those of salt-resistant canola (Figs. 4, 5). Our results showed increasing [Na\(^+\)]\(_{apo}\) and [Na\(^+\)]\(_{sym}\) in both types of canola leaves subjected to increasing NaCl stress, and that the increase in [Na\(^+\)]\(_{apo}\) was much higher than the increase in [Na\(^+\)]\(_{sym}\), especially for the increasing [Na\(^+\)]\(_{apo}\) in NYY 1 leaves (Table 3). We consider that the difference in salt adaptation among cultivars was related to the increased apoplastic capacity of NYY 1 for Na\(^+\). Simultaneously, Na\(^+\) in the leaf symplast increased a great deal more in BZY 1 than in NYY 1 with increasing NaCl stress (Table 3). Together, these results demonstrate an inability of BZY 1 to control Na\(^+\) in the leaf symplast, and cause salt ion accumulation to excessive levels in its transpiring leaves, exceeding the ability of the cells to compartmentalize these ions in the vacuole. Ions then build up rapidly in the cytoplasm and inhibit enzyme activity (Munns 2002). It is reasonable to suppose that the plasma membrane of NYY 1 could control and/or exclude Na\(^+\) entry from the cytoplasm better than could BZY 1, which is consistent with the results of Ottow et al. (2005), who considered that apoplastic accumulation was a vital strategy to protect the cytosol against Na\(^+\) toxicity in *Populus euphratica*. Our previous study suggested that the higher salt resistance of NYY 11 than that of BZY 1 mainly resulted from higher K\(^+\)/Na\(^+\) in the shoot and, higher ratios of K\(^+\)/Na\(^+\) and Ca\(^{2+}\)/Na\(^+\) in the shoot and root (Zheng et al. 2010a). Also, the present study suggested that, compared with salt-sensitive BZY 1, the leaf cells of NYY 1 had a stronger ability to maintain ion homeostasis by excluding Na\(^+\) from the cytosol and storing it primarily in cell walls. Na\(^+\) export from the cytosol into the apoplast is most likely carried out by a homolog of the plasma membrane-bound Na\(^+\)/H\(^+\) antiporter (SOS1) (Shi et al. 2000). SOS1 also plays a crucial role in salt resistance of Arabidopsis (Shi et al. 2000). Thus, SOS1, HKT1, plasma membrane-bound proton ATPase, etc. should be identified in further research.

In fact, a steep Na\(^+\) gradient is more likely to exist at the tonoplast than at the plasma membrane. Intracellular compartmentalization, intended for keeping toxic ions away from the cytoplasm, involves an energy-dependent transport of ions into the vacuole (Glenn et al. 1999). The ability to compartmentalize Na\(^+\) and Cl\(^-\) is considered to be an underlying determinant of the resistance not only of halophytes but also of many crop species (Parida and Das 2005). This is supported by the evidence that the overexpression of tonoplast-bound Na\(^+\)/H\(^+\) antiporter in Arabidopsis greatly enhanced its salt resistance, by transporting and accumulating salts in the vacuole (Apse et al. 1999). Thus, in order to determine the salt-resistant mechanism of canola, this aspect should be studied in further research and the roles of different strategies in the canola adaptation response to salt stress should be clarified.

In our study, we analyzed the Na\(^+\) concentration of the leaf edge and leaflet center (Figs. 4, 5, Table 3). The [Na\(^+\)]\(_{le}\)/[Na\(^+\)]\(_{lc}\) ratio represents the Na\(^+\) diffusion strength from the inside to the outside of leaves (Table 3). We found that the values of [Na\(^+\)]\(_{apo}\) of LE/[Na\(^+\)]\(_{apo}\) of LC and [Na\(^+\)]\(_{sym}\) of LE/[Na\(^+\)]\(_{sym}\) of LC in BZY 1 were much higher than those in NYY 1, which suggests that the increased Na\(^+\) accumulation in leaves of BZY 1 may accelerate Na\(^+\) diffusion into leaves. It is likely that Na\(^+\) diffusion in leaves through the apoplast and symplast pathway in BZY 1 was probably stronger than that in NYY 1. As a result, Na\(^+\) in the leaf symplast of BZY 1 increases much more, which was consequently responsible for a larger Tc decrease and greater growth inhibition.

As documented, mechanisms adopted by some halophytes to survive very high salt concentrations largely involve absorption and storage of Na\(^+\) in shoot vacuoles as an energetically cheap osmoticum (Glenn et al. 1999). Unlike halophytes, glycophytes usually cannot tolerate high salinity, and reduce Na\(^+\) transport to shoots, in particular reducing the rate at which salt accumulates in transpiring organs (Munns 2002). Numerous studies have demonstrated a significant inverse correlation between Na\(^+\) concentration and dry mass accumulation of crop plants (Schachtman and Munns 1992, Asch et al. 2000, Rahnama et al. 2011). The selective absorption, translocation and distribution of Na\(^+\) into different organs or specific tissues, especially the leaf blade, where most biochemical processes take place, is crucial for salt tolerance in plants (Tester and Davenport 2003, Shabala and Cuin 2008). However, under saline stress, our measurement of Na\(^+\) concentrations in canola plant showed significantly more accumulation of Na\(^+\) and Cl\(^-\) in shoot than in root, and salt ion contents in shoot and root in BZY 1 were increased more than in NYY 1 (data not shown). Previous studies reported that Na\(^+\) transporters, such as AtHKT1, are involved in xylem loading by controlling the retrieval of Na\(^+\) from the xylem to alter the root/shoot Na\(^+\) distribution (Mäser et al. 2002, Davenport et al. 2007). In shoots, Na\(^+\) is preferentially accumulated in the leaf sheath to protect the leaf blade from damage, especially in young expanding
tissues (Wei et al. 2003, Rahnama et al. 2011). In the present study, the sharp increase in Na\(^+\) in the petiole, leaf main vein and leaf blade in both canola cultivars was responsible for an obvious decline in shoot dry weight. In shoots, much more Na\(^+\) was found, especially in the leaf blade in BZY 1 (Table 2), which is consistent with the larger reduction of shoot dry weight in BZY 1 compared with NYY 1 (Fig. 1). Additionally, under salt stress, a higher [Na\(^+\)]\(_{\text{main vein}}/[\text{Na}\(^+\)]\(_{\text{blade}}\) ratio was found in NYY 1 than in BZY 1, suggesting that the leaf vein of NYY 1 played a greater role in retention of Na\(^+\) than did that in BZY 1. A relatively lower Na\(^+\) content in the leaf blade of NYY 1 implies better ionic homeostasis, which is important for plant growth under salinity (Munns 2002).

**Difference of gas-exchange parameter characteristics between the leaves of the two canola plants**

Important processes that are closely associated with plant growth, such as photosynthesis, were seriously disturbed by Na\(^+\) stress in the two canola cultivars, especially in BZY 1 (Table 1). Previous studies demonstrated that NaCl stress greatly suppresses photosynthesis by altering both the leaf area and the photosynthetic rate (Steduto et al. 2000, Li et al. 2008, Praxedes et al. 2010). Additionally, the low CO\(_2\) concentration in the chloroplast induced by reduced stomatal and mesophyll conductance and decreased activity of Rubisco contribute to the decline in photosynthesis (Loreto et al. 2003, Parida et al. 2004, Chaves et al. 2009). Most studies dealing with stomatal and non-stomatal limitation to photosynthesis are on crops. In bean, a salt-sensitive species, the reduction in photosynthesis was found to be mostly due to stomatal limitation (Brugnoli and Lauteri 1991) or to both stomatal and non-stomatal limitations (Seemann and Critchley 1985). Several studies on stomatal and non-stomatal components in sunflower have referred to water stress conditions but reported conflicting results (Steduto et al. 2000). However, there are few reports on photosynthetic inhibition in canola. In our experiment, the reduction in \(P_n\) and \(g_s\) under increasing NaCl conditions was more drastic in BZY 1 than in NYY 1 (Table 1). Additionally, the significant decrease in \(C_i\) was accompanied by increasing \(L_i\) under increasing salt stress to 6 g kg\(^{-1}\) NaCl. These results indicated that stomatal limitation primarily accounted for the decline in photosynthetic rate under moderate salt stress in our experiment. However, \(g_s\), \(C_i\), and \(L_i\) did not differ markedly under 6 and 9 g kg\(^{-1}\) NaCl stress in the two canola varieties, and \(P_n\) of both canola varieties decreased further and significantly under 9 g kg\(^{-1}\) NaCl stress, suggesting that the reduction in photosynthesis was due to both stomatal and non-stomatal limitation under severe salt stress. There were no obvious differences in stomatal limitation between NYY 1 and BZY 1 at all salt stress levels. This suggests that the difference in \(P_n\) between the two canola varieties was probably due to a non-stomatal component. That is to say, photosynthetic activity of leaf mesophyll cells in BZY 1 probably decreased more than in NYY 1.

Transpiration rates of crop plants are strongly influenced by salinity (Munns 2002), and the decrease of transpiration in the salt-sensitive BZY 1 was more than that in the salt-tolerant NYY 1 (Table 1). Transpiration is usually strongly correlated with stomatal conductance; therefore, it is assumed that salinity reduced transpiration rates mainly through effects on stomatal opening (Munns 2002, Parida and Das 2004). In this study, the large decrease in stomatal conductance in BZY 1 caused a large decrease in transpiration rate (Table 1). We found that more Na\(^+\) was transported to the leaflet edge in BZY 1 than in NYY 1 (Figs. 4, 5, Table 3); therefore, it is assumed that transpiration of the leaf edge of the salt-sensitive BZY 1 was more affected by salt stress than that of NYY 1 and that the \(T_r\) of the leaf edge in BZY 1 decreased more than that of NYY 1. Thus, the value of the \((T_r\text{ of LE})/(T_r\text{ of LC})\) ratio in BZY 1 decreased more obviously than that in NYY 1 at all levels of NaCl stress.

In conclusion, the results of this study support the observation that under salinity conditions, there was a lower increase of Na\(^+\) in shoots of NYY 1 compared with BZY 1 when exposed to increasing NaCl concentrations and that this was responsible for the higher salt resistance of NYY 1. The leaf vein of the salt-resistant NYY 1 played a greater role in retention of Na\(^+\) compared with that of BZY 1. Compared with the Na\(^+\) concentration in the leaf symplast, the leaf apoplastic accumulation of NYY 1 accumulated more Na\(^+\) than did that of BZY 1 under salt stress. Additionally, Na\(^+\) diffusion into leaves though the apoplastic and symplastic channels in BZY 1 was stronger than in NYY 1. A large increase in Na\(^+\) accumulation in the apoplast and symplast of leaf cells greatly inhibited photosynthesis and transpiration, especially in BZY 1. Stomatal limitation to photosynthesis in canola was mainly found under moderate salinity stress, whereas the reduction in photosynthesis under severe salt stress was due to both stomatal and non-stomatal limitations. Differences in photosynthetic activity in the leaf mesophyll cells led to differences in salt resistance between the two canola varieties examined here.

In conclusion, this study on ion distribution at the apoplast and symplast levels provides an insight into how two canola plants respond to salinity stress. Compared with salt-sensitive BZY 1, salt-tolerant NYY 1 accumulates less Na\(^+\) in the shoot, especially in leaf blades, and excludes a higher proportion of Na\(^+\) to the leaf apoplast. Also, BZY 1 has much more Na\(^+\) in the leaf symplast and maintains a steeper Na\(^+\) gradient across the leaf cell plasma membrane than NYY 1. Hence, NYY 1 plants had more resistance to salt than BZY 1 plants. These physiological processes might be further included in the estimation of salt-tolerant cultivars.

**Materials and Methods**

**Plant material and growth conditions**

The seeds of canola (B. napus L.) cv. NYY 1 (Nanyanyou 1) and BZY 1 (Baozyou 1), from China, were surface-sterilized...
for 20 min with 1% sodium hypochlorite, vigorously rinsed in distilled water, and germinated on moist filter paper in the dark at 25°C. Three days later, the germinated seedlings were transferred to pots filled with sand. The pots were placed in one-quarter strength Hoagland nutrient solution containing: 5 mM KNO₃, 4 mM Ca(NO₃)₂, 2 mM MgSO₄, 1 mM NH₄H₂PO₄, 10 μM H₂BO₃, 1.6 μM MnSO₄, 10 μM ZnSO₄, 0.5 μM CuSO₄, 50 μM (NH₄)₆Mo₇O₂⁴ and 20 μM Fe-EDTA. When the fourth true leaf emerged, plants were transplanted to soil in plastic cylindrical pots 28 cm in diameter. Each pot contained 14.45 g kg⁻¹ oven-dried soil to a height of 19 cm. The main soil properties were determined after fixation at 105°C and drying to constant weight at 70°C. The field moisture capacity was 53(6)%; available P 1.58 g kg⁻¹; available K 116 mg kg⁻¹; total N 1.58 g kg⁻¹; available P 16.37 mg kg⁻¹; available K 116 mg kg⁻¹. Five days after plants were transplanted, the salt treatments were started. The experimental design consisted of four NaCl levels of 0, 3, 6 and 10 mM, which were combined with two day/night temperature and 420±10³ ppm atmospheric CO₂ concentration. Data were recorded after equilibration to a steady state (approximately 10 min). The measured leaves were labeled, and leaf areas were calculated (Li et al. 2009). Values of stomatal limitation were calculated using the following formula: \( L_{r} = 1 - C_{i}/C_{a} \) (where \( C_{i} \) is the CO₂ concentration in the air) (Farquhar and Sharkey 1982). The \((T_{r} of LE)/(T_{r} of LC)\) ratios were calculated using the following formula:

\[
T_{r} of LE/T_{r} of LC = \frac{\text{transpiration rate of leaf edge}}{\text{transpiration rate of leaf center}}.
\]

### Determination of Na⁺ concentration in the petiole, leaf main vein and leaf blade

After the shoot was dried to constant weight at 70°C, we divided the shoot into three parts, petiole, leaf vein and blade. The dried samples of these three parts were ground and ashed in a furnace at 6 h at 500°C. Then, the ash was dissolved in 20% sulfuric acid, diluted in distilled water and filtered through Whatman filter paper. Na⁺ concentrations were determined by flame-emission photometry (FP6410) (Ghoulam et al. 2002).

### Determination of Na⁺ concentration in the leaf apoplast and symplast

Twenty leaf discs were obtained from cut leaves using a punch (1.27 cm diameter) from the leaflet center and edge without the midrib. Then, the discs were carefully individually washed in distilled water, blotted dry and stacked carefully in a 50 ml glass tube whose intercellular space was filled with 100 mmol l⁻¹ sorbitol. The glass tube was put into a sealed container which was connected to a vacuum pump (MPC301-z). The infiltration procedure was carried out by the vacuum pump, which produced a reduced pressure of about 0.1 MPa for 3 min. Infiltrated leaves quickly became dark and sank. They were stacked in a 20 ml syringe which was positioned over a 50 ml centrifugation vial (Mußling and Lüchli 2002) and centrifuged at 2,000 g for 3 min. The infiltrated samples were rinsed with 1% sodium hypochlorite three times. Then, the ash was dissolved in 20% sulfuric acid, diluted in distilled water and filtered through Whatman filter paper. Na⁺ concentrations were determined by flame-emission photometry (FP6410). The Na⁺ concentration in the apoplasmic fluid was calculated by multiplying the Na⁺ concentration in the AWF by a dilution factor (\( F_{dil} = V_{water} + V_{air}/V_{water} \)) (Lohaus et al. 2001), where \( V_{water} \) is the apoplastic air space, and \( V_{water} \) is the apoplastic water space, as determined using the silicone oil method and a modified indigo carmine method, respectively (Husted and Schjoerring 1995). The Na⁺ concentration in the apoplastic fluid was regarded as the Na⁺ concentration in the residual leaf tissue that had been separated from the apoplastic fluid, which was filtrated through a 0.8 μm filter. The Na⁺ concentration in the leaf symplast and was determined using the method of Mußling and Lüchli (2002).

\[
\text{values of } [\text{Na}^+]_{sym} = [\text{Na}^+]_{apo} \times \frac{[\text{Na}^+]_{apo} \text{ of LC}}{[\text{Na}^+]_{apo} \text{ of LE}}
\]

### Gas-exchange measurements

Twenty days after treatments started, the rate of light-saturated photosynthesis of newly expanded leaves was measured using a Li-Cor 6400 portable photosynthesis open system. \( P_{n} \), \( g_{s} \), \( C_{i} \) and \( T_{r} \) were measured at a PPFD of 1,500 μmol photons m⁻² s⁻¹, 38.96 ± 5.58% relative humidity, 28.0 ± 0.2°C leaf temperature and 420 ± 1.5 μmol mol⁻¹ atmospheric CO₂ concentration. Data were recorded after equilibration to a steady state (approximately 10 min). The measured leaves were labeled, and leaf areas were calculated (Li et al. 2009). Values of stomatal limitation were calculated using the following formula: \( L_{r} = 1 - C_{i}/C_{a} \) (where \( C_{i} \) is the CO₂ concentration in the air) (Farquhar and Sharkey 1982). The \((T_{r} of LE)/(T_{r} of LC)\) ratios were calculated using the following formula:

\[
T_{r} of LE/T_{r} of LC = \frac{\text{transpiration rate of leaf edge}}{\text{transpiration rate of leaf center}}.
\]

### Determination of shoot dry weight

The shoots of canola plants were harvested and washed in distilled water. Then, the dry weight of the above-ground organs was determined after fixation at 105°C for 5 min and drying to constant weight at 70°C.

### Determination of leaf water potential

The fresh leaves without the midrib were cut into small pieces and placed in the sample cup, completely covering the bottom of the cup (Gao et al. 2010). The prepared cup was placed in a drawer, and leaf water potential was monitored using a dewpoint potentiometer (WP4).
and $[\text{Na}^+]_{\text{sym}}/L/C_{[\text{Na}^+]_{\text{sym}}}$ of LC were calculated using the following formulae:

$$[\text{Na}^+]_{\text{sym}}/L/C_{[\text{Na}^+]_{\text{sym}}}_{\text{apo}} = \frac{\text{Na}^+ \text{ concentration in the leaf symplast}}{\text{Na}^+ \text{ concentration in the leaf apoplast}}$$

Increased rate of $[\text{Na}^+]_{\text{apo}} = \frac{\text{Na}^+ \text{ concentration in the leaf apoplast under salinity stress}}{\text{Na}^+ \text{ concentration in the leaf apoplast with no salt stress}}$

$[\text{Na}^+]_{\text{apo}}_{\text{of LC}}/[\text{Na}^+]_{\text{apo}}_{\text{LC}} = \frac{\text{Na}^+ \text{ concentration in the apoplast of the leaf edge}}{\text{Na}^+ \text{ concentration in the apoplast of the leaf centre}}$

$[\text{Na}^+]_{\text{sym}}_{\text{of LC}}/[\text{Na}^+]_{\text{sym}}_{\text{LC}} = \frac{\text{Na}^+ \text{ concentration in the symplast of the leaf edge}}{\text{Na}^+ \text{ concentration in the symplast of the leaf centre}}$

Statistical analysis

One-way analysis of variance (ANOVA) was applied to assess differences for each parameter among treatments using the SAS 9.0 statistical software package. The means and calculated standard errors are reported. Significance was tested at the 5% level.

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