Native Mediterranean *Pyrus* Rootstock, *P. amygdaliformis* and *P. elaeagrifolia*, Present Higher Tolerance to Salinity Stress Compared with Asian Natives

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The salt tolerance of native Asian pear species, *Pyrus betulaefolia* Bunge., *P. pyrifolia* Nakai, and *P. xerophila* Yu, and native Mediterranean ones, *P. amygdaliformis* Vill. and *P. elaeagrifolia* Pall., were examined by irrigating them with 75 mM and 150 mM NaCl solutions for 30 days. Native Mediterranean species did not develop leaf injury during the 30-day NaCl treatments, but native Asian species developed leaf injury. Concerning the shank, fine roots, and whole plant, bioregional differences in Na and Cl contents were small. However, the Na and Cl contents of the leaves of native Mediterranean species were markedly less than those of native Asian species in both NaCl concentrations. Therefore, the native Mediterranean *P. amygdaliformis* and *P. elaeagrifolia* may have some salt exclusion mechanisms in the shank which restrict Na and Cl transport to the leaves. The stem water potential and Ci/Ca ratios were decreased by NaCl treatments in native Mediterranean and native Asian species. Thus, the photosynthetic decline by NaCl treatment observed in these species might have been caused by stomatal closure, which is likely to be induced by decreased water potential in the plant body. However, the photosynthetic rate of native Mediterranean species under NaCl stress was higher than native Asian species. Therefore, low concentrations of Na and Cl in the leaves of native Mediterranean species might prevent crucial decreases of photosynthetic rates under NaCl stress. The present results imply that native Mediterranean *P. amygdaliformis* and *P. elaeagrifolia* have a higher salt tolerance than native Asian *P. betulaefolia*, *P. pyrifolia* and *P. xerophila*. These native Mediterranean species would be useful in providing genetic resources as salt-tolerant rootstock for the Japanese pear.

Key Words: ion uptake, photosynthesis, *Pyrus betulaefolia*, salt tolerance.

Introduction

Salt tolerance is one of the major factors influencing crop productivity in arid or semi-arid environments. Cultivated pear species are not tolerant to salinity, although *Pyrus communis* L. has been cultivated in arid or semi-arid areas (Bernstein, 1965). The Asian pear, including *P. pyrifolia* Nakai, *P. ussuriensis* Max., and *P. breitschneider* Rehd., has recently been cultivated in arid or semi-arid areas and its cultivation is increasing due to the unique, attractive features of the fruit, such as its juicy crisp flesh (Kajiura, 2002; Lombard and Westwood, 1987; Rathore, 1991).

The salt tolerance of fruit trees can be enhanced through the use of tolerant rootstocks (Ruiz et al., 1997). Soil salinity directly affects roots; therefore, the selection of suitable rootstocks is essential for sustainable fruit production in salt-affected areas (Bernstein and Kafkafi, 2002). In *Citrus* and *Persea*, studies on salt-tolerant rootstocks have been conducted, and some tolerant rootstocks, especially Cl- and Na-excluding rootstocks, were selected for use in cultivation (Bernstein et al., 2001; Storey and Walker, 1999). The *Pyrus* genus has 22 primary species; however, systematic investigations on salt tolerance in these species are limited (Bell, 1991).

In a previous study, we compared the salt tolerance of five Asian native rootstocks and revealed that *P. betulaefolia* Bunge. had the highest salt tolerance (Matsumoto et al., 2006). Moreover, this study also suggested that *P. betulaefolia* restricts the translocation of Na and Cl ions from roots to shoots and prevents overaccumulations of these ions in leaves. Lombard and Westwood (1987) reported that *P. amygdaliformis* and *P. elaeagrifolia* present a higher tolerance to lime-induced chlorosis. However, adaptation of these two species to soil salinity has not been investigated and no study has directly compared the salt tolerance of native Mediterranean and native Asian *Pyrus* rootstocks.

In general, native Asian wild pear species survive under wet climate conditions, whereas native Mediterranean pear species survive under dry, salty soil environments (Lombard and Westwood, 1987). Therefore, there is a strong possibility that these two groups have different genetic backgrounds and different patterns
of adaptation to soil salinity. To develop salt-tolerant rootstocks and reveal the mechanisms of salt tolerance, comparisons of growth characteristics and the intrinsic physiology of these two groups is critical.

In this study, we compared the shoot growth, leaf injury, and ion content of each plant organ, and examined the relationship between ion uptake and salt tolerance. Additionally, photosynthetic characteristics were assessed under salt treatments.

**Materials and Methods**

*Plant materials and treatments*

Three-year-old seedlings of *Pyrus betulaefolia* Bunge., *P. pyrifolia* Nakai, *P. xerophila* Yu, *P. amygdaliformis* Vill., and *P. elaeagrifolia* Pall. were used. *P. betulaefolia*, *P. pyrifolia* and *P. xerophila* originated in East Asia, whereas *P. amygdaliformis* and *P. elaeagrifolia* in Mediterranean coastal areas. Seeds were collected from the pear germplasm collection orchard in Tottori University, Tottori, Japan. The seedlings were grown at an orchard in Tottori University for three years, and then transplanted into 20 L terracotta pots filled with decomposed granite soil and cultivated in a greenhouse under natural conditions. The seedlings were irrigated with 500 mL of 75 mM or 150 mM NaCl solutions once for 72 h, the dry weight was measured and samples were lightly ground into powder. To determine the Na, K, Ca, and Mg contents, powdered samples were digested using the H$_2$SO$_4$–H$_2$O$_2$ Kjeldahl digestion method (Ohyama et al., 1991). One milliliter of H$_2$SO$_4$ was added to 50 mg of each powdered sample in a test tube, left for 12 h, and then heated at 180°C for 3 h. During heating, 0.9 mL of H$_2$O$_2$ was added to completely digest samples. Samples were diluted with 50 mL distilled water, and this solution was used for Na, K, Ca, and Mg analyses. These mineral concentrations were determined using an atomic absorption spectrophotometer (170–30, Hitachi Ltd., Tokyo, Japan). The Cl content was determined using the spectrophotometric mercury thiocyanate-iron method of Iwasaki et al. (1956). Fifty milligrams of powdered sample and 10 mL of 0.1 N acetic acid were put into a plastic tube, shaken for 1 h, and then left stationary for 12 h. Supernatants were then centrifuged for 5 min at 12000 rpm and 0.1 mL of each sample solution was mixed with 0.2 mL 13.2 mM mercuric thiocyanate solution, 0.1 mL 0.5 M ferric nitrate solution, and distilled water. Absorbance of ferric thiocyanate was measured using a spectrophotometer at 460 nm (U-2000, Hitachi Ltd.).

**Results**

*Growth increment of shoots*

Shoot growth in all pear rootstocks was reduced by each NaCl treatment (Table 1). The increment of shoot length with 150 mM NaCl did not differ among species. However, the relative shoot growth (% of control) of *P. xerophila* with 150 mM NaCl was less than other species and the rate was 46.4% of the control. The rate of *P. amygdaliformis* and *P. elaeagrifolia* was 57.0 and 57.1% of the control, respectively. The NaCl effect on *P. pyrifolia* and *P. betulaefolia* was less than other species and the rates were 64.0% and 67.0% of the control, respectively.

*Leaf injury*

*P. amygdaliformis* and *P. elaeagrifolia* showed no visible symptoms of leaf injury during the experimental
period in any NaCl concentrations (Fig. 1). In contrast, *P. betulaefolia*, *P. pyrifolia*, and *P. xerophila* presented leaf injury. The most sensitive species was *P. xerophila*; injury was observed even when irrigated with 75 mM NaCl for 20 days. *P. betulaefolia* exhibited leaf injury only when irrigated with 150 mM NaCl (Fig. 1).

Mineral contents

The total Na and Cl contents per plant did not differ among species with 75 mM NaCl (Fig. 2). Total Na and Cl contents of *P. pyrifolia* with 150 mM NaCl were higher than the native Mediterranean *P. amygdaIiformis* and *P. elaeagrifolia*. However, there was no difference among other species (Fig. 2).

Leaf Na and Cl contents of native Mediterranean *P. amygdaIiformis* and *P. elaeagrifolia* were remarkably lower than the native Asian species, even with 150 mM NaCl (Fig. 3). In the native Asian species, leaf Na and Cl contents of *P. betulaefolia* were lower than *P. pyrifolia* and *P. xerophila*. Leaf Na and Cl contents of *P. xerophila* with 75 mM NaCl were the highest among species.

The Na and Cl contents of the shank increased with

![Table 1](https://example.com/table1.png)

**Table 1.** Effect of NaCl treatments on shoot growth for 30 days in *Pyrus* rootstock species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Shoot length increment (cm)</th>
<th>Relative shoot growth (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mM</td>
<td>75 mM</td>
</tr>
<tr>
<td><em>P. amygdaIiformis</em></td>
<td>116.2 a</td>
<td>82.5 ab</td>
</tr>
<tr>
<td><em>P. elaeagrifolia</em></td>
<td>130.7 a</td>
<td>70.7 b</td>
</tr>
<tr>
<td><em>P. xerophila</em></td>
<td>147.5 a</td>
<td>86.3 ab</td>
</tr>
<tr>
<td><em>P. pyrifolia</em></td>
<td>146.0 a</td>
<td>114.5 a</td>
</tr>
<tr>
<td><em>P. betulaefolia</em></td>
<td>118.4 a</td>
<td>90.3 ab</td>
</tr>
</tbody>
</table>

**Analysis of variance**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species (A)</td>
<td>4</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>NaCl (B)</td>
<td>2</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>A × B</td>
<td>8</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are the means of five replications.

* Different letters within the same column show a significant difference by Tukey-Kramer’s HSD tests at the 5% level.

NS, **, *** indicate non-significant and significant differences at P<0.01 and 0.001, respectively.

![Fig. 1](https://example.com/fig1.png)

**Fig. 1.** Occurrence of leaf injury in *Pyrus* species treated with different concentrations of NaCl for 20 or 30 days. Values are the means of five replications. Leaf injury index: 0 = no visual symptoms to 5 = all leaves burned or abscised. Vertical bars indicate the standard error of the mean. P.a.: *P. amygdaIiformis*; P.e.: *P. elaeagrifolia*; P.x.: *P. xerophila*; P.p.: *P. pyrifolia*; and P.b.: *P. betulaefolia*. nd indicates non-developed.

![Fig. 2](https://example.com/fig2.png)

**Fig. 2.** Effect of 30 days NaCl treatment on Na (left side) and Cl (right side) contents in whole plants among five *Pyrus* species. Vertical bars indicate the standard error of the mean for five plants. P.a.: *P. amygdaIiformis*; P.e.: *P. elaeagrifolia*; P.x.: *P. xerophila*; P.p.: *P. pyrifolia*; and P.b.: *P. betulaefolia*. 
NaCl applications, but there were no marked differences among species (Fig. 3). The Na and Cl contents in fine roots also increased with NaCl applications in all species. Moreover, there was little difference between NaCl treatments (Fig. 3).

There were no significant differences in K, Ca, and Mg contents among all species, with or without NaCl treatment (data not shown).

**Stem water potential**

Stem water potential decreased with NaCl application except in *P. amygdaliformis* at 75 mM NaCl (Table 2). Stem water potential with 150 mM NaCl did not differ among species (Table 2).

**Photosynthetic rate**

The decrease in the light-saturated net photosynthesis of native Asian *P. betulaeifolia*, *P. pyrifolia*, and *P. xerophila* was greater than native Mediterranean *P. amygdaliformis* and *P. elaeagrifolia* under saline conditions (Fig. 4). The values of native Asian species

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**Table 2.** Effect of 4 days NaCl treatment on stem water potential of five *Pyrus* species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Stem water potential (MPa)</th>
<th>Control</th>
<th>75 mM</th>
<th>150 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. amygdaliformis</em></td>
<td>−1.30 ab</td>
<td>−1.24 a</td>
<td>−1.45  a</td>
<td></td>
</tr>
<tr>
<td><em>P. elaeagrifolia</em></td>
<td>−1.07 bc</td>
<td>−1.29 a</td>
<td>−1.34  a</td>
<td></td>
</tr>
<tr>
<td><em>P. xerophila</em></td>
<td>−0.94 ab</td>
<td>−1.52 b</td>
<td>−1.63  a</td>
<td></td>
</tr>
<tr>
<td><em>P. pyrifolia</em></td>
<td>−1.04 abc</td>
<td>−1.26 a</td>
<td>−1.45  a</td>
<td></td>
</tr>
<tr>
<td><em>P. betulaeifolia</em></td>
<td>−0.77 a</td>
<td>−1.13 a</td>
<td>−1.45  a</td>
<td></td>
</tr>
</tbody>
</table>

Analysis of variance

Rootstock (A) **
NaCl (B) ***
A × B **

Values are the means of five replications.

* Different letters within the same column show a significant difference by Tukey-Kramer’s HSD tests at the 5% level.

**, *** indicate significant difference at P<0.01 and 0.001, respectively.

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**Fig. 3.** Effect of 30 days NaCl treatment on Na (left side) and Cl (right side) contents of the leaves, shank, and fine roots among five *Pyrus* species. Vertical bars indicate the standard error of the mean for five plants. The horizontal lines within the panels of Na and Cl contents of leaves indicate of 87 and 141 μmol·g⁻¹DW, respectively. *P.a.*: *P. amygdaliformis*; *P.e.*: *P. elaeagrifolia*; *P.x.*: *P. xerophila*; *P.p.*: *P. pyrifolia*; and *P.b.*: *P. betulaeifolia*.

**Fig. 4.** Effect of 17 days NaCl treatment on the photosynthetic light-response curve of five *Pyrus* species.
were less than 20% of the control, but those of native Mediterranean species were 64% and 56% of the control, respectively, even with 150 mM NaCl (Fig. 4). The photosynthetic rate of NaCl-treated native Asian species decreased when the photosynthetic photon flux density (PPFD) exceeded 300 μmol·m⁻²·s⁻¹. In contrast, native Mediterranean species did not show a decreased photosynthetic rate, even when the PPFD was as high as 700 μmol·m⁻²·s⁻¹, by NaCl treatment. The Ci/Ca rates were decreased by NaCl treatment in all species (Fig. 5).

**Discussion**

Soil adaptation of pear rootstock varies within the *Pyrus* genus (Bell, 1991; Lombard and Westwood, 1987). However, no study has directly compared the salt tolerance of native Asian *Pyrus* rootstock species with native Mediterranean *Pyrus* rootstock species. In the present study, we found that native Mediterranean *P. amygdaliformis* and *P. elaeagrifolia* have a higher salt tolerance than native Asian *P. betulaefolia*, *P. pyrifolia*, and *P. xerophila*.

Native Mediterranean *P. amygdaliformis* and *P. elaeagrifolia* did not show any leaf injury symptoms during the 30-day experimental period, even with 150 mM NaCl (Fig. 1). On the other hand, native Asian *P. betulaefolia*, *P. pyrifolia*, and *P. xerophila* developed some leaf injuries. In general, leaf injury is caused by an overaccumulation of Na and Cl in leaves (Munns, 2002). Bernstein (1965) suggested that leaf injury in fruit trees develops when the leaf ion content exceeds 87 μmol·g⁻¹ DW for Na and/or 141 μmol·g⁻¹ DW for Cl. In this study, leaf injury was observed only when the leaf Na and Cl contents exceeded these threshold values. The Na and Cl contents in leaves of *P. amygdaliformis* and *P. elaeagrifolia* were lower than these threshold values, even at the highest salinity level (Fig. 3).

Moreover, the Na and Cl contents in leaves of *P. betulaefolia* which presented the highest salt tolerance among native Asian species in a previous study (Matsumoto et al., 2006) with 75 mM NaCl were also lower than these threshold values. These results suggested that the higher tolerance of native Mediterranean *P. amygdaliformis* and *P. elaeagrifolia* to salt stress was related to the low concentration of Na and Cl in leaves, and that the contents of Na and Cl in leaves were an important factor to decide salt tolerance.

In Avocado rootstock species, salt tolerance is different among their bioregions, and salt tolerance species exclude Na and Cl from the leaves (Mickelbart and Arpaia, 2002). In the present study, the differences of the total Na and Cl contents in whole plants among the species were small; the contents with 75 mM NaCl particularly did not differ among species (Fig. 2). Moreover, the Na and Cl contents of the shank and fine roots with NaCl treatments did not differ among species (Fig. 3). However, Na and Cl contents in leaves of native Mediterranean *P. amygdaliformis* and *P. elaeagrifolia* were extremely lower than native Asian *P. betulaefolia*, *P. pyrifolia*, and *P. xerophila* (Fig. 3). These results suggests that both native Mediterranean and native Asian species might absorb almost the same amounts of Na and Cl in the plant body, but, native Mediterranean species did not transport this Na and Cl from the shank to the leaves.

Numerous studies have been conducted to elucidate the mechanisms of Na and Cl transport to leaves in various plant species. Several studies suggested that non-vigorous growth restricted Na and Cl transport to the leaves (Massai et al., 2004). However, in the present study, the difference in shoot length increments was small, and there was no relationship between shoot growth and salt tolerance (Table 1). Thus, vigorous
growth did not affect the contents of Na and Cl in leaves. Greenway and Munns (1980) and Munns (2002) suggested that the salt exclusion function in glycophytes is linked with the ability to limit uptake and/or transport of Na and Cl from the root to above-ground parts. Fisarakis et al. (2001) reported consistently higher contents of Na and Cl in roots as compared to the leaves of Sultana vines and suggested that the capability to store Na in roots is a tolerance characteristic of rootstocks. Boland et al. (1997) also suggested that the trunk wood of Pear (P. communis) acted as a storage organ for Na and restricted the accumulation of Na in leaves. The details of exclusion mechanisms were not clear solely from the present experiment. Moreover, further experiments must be performed using scion cultivars grafted on these rootstocks. However, it is suggested that native Mediterranean P. amygdaliformis and P. elaeagrifolia have some salt exclusion mechanisms in the shank and/or roots and restrict the transport of Na and Cl to the leaves. Further research may reveal the mechanisms and some effective usages of native Mediterranean species for rootstock and/or genetic resources will be clarified.

In this study, photosynthetic activity was also affected differently between the native Mediterranean the native Asian species. On the 17th day, the effect of NaCl treatment on the light saturated photosynthetic activity of the native Mediterranean species was weaker than on native Asian ones (Fig. 4). Some reports suggested that the accumulation of Na and Cl in leaves induces the collapse of many physiological processes and reduces the photosynthetic rate (Garcia-Legaz et al., 1993; Ziska et al., 1990). In the present study, the photosynthetic rates under low light were not changed by NaCl treatment in native Mediterranean species; however, the rates of native Asian species were decreased (Fig. 4). Thus, there is a possibility that the photosynthetic apparatus of native Asian species is injured by NaCl treatment. However, James et al. (2002) reported in wheat that the photosynthetic apparatus was not affected directly when the leaf Na and Cl contents were over 200 and 300 mM in the tissue water base, respectively. And these values were higher than the contents of the present experiment. Because, the reduction of the photosynthetic rate under saline conditions may be induced by many factors, ionic and osmotic, it is difficult to determine the relative effect of these factors. Therefore, further investigations are necessary to elucidate the cause of the difference between native Asian and Mediterranean species. But, the Ci/Ca rate was decreased by NaCl treatment in all species in the present experiment (Fig. 5). Moreover, the stem water potential was also decreased by NaCl treatment in all species (Table 2). Ouerghi et al. (2000) reported that the decline of the Ci/Ca rate implied a decline in the photosynthetic rate caused by stomatal limitation. Therefore, the following phenomenon may be induced commonly by NaCl treatment among the examined species: NaCl treatment decreases water potential in the plant body, and this leads to a reduced photosynthetic rate via stomatal closure (Holbrook et al., 2002; Tardieu et al., 1996).

In conclusion, the present results imply the existence of a restriction mechanism that blocks the transport of saline substrates from the shank to leaves. The salt tolerance mechanisms of the native Mediterranean species P. amygdaliformis and P. elaeagrifolia are superior to native Asian species, and may reflect environmental adaptations. Therefore, native Mediterranean Pyrus species present a higher salt tolerance. Until now, the use of P. amygdaliformis and P. elaeagrifolia for pear rootstocks has been limited and used only in Mediterranean coastal areas (Lombard and Westwood, 1987; Procopiou and Wallace, 2000). Moreover, it is not clear whether these rootstocks can be adapted to the Japanese pear or not. As the Japanese pear and its rootstocks are adapted to wet climates in East Asia, their genetic resources for salt tolerance might be limited. The present results indicate that native Mediterranean species would be useful in providing genetic resources as salt tolerant rootstock for the Japanese pear.

**Literature Cited**


地中海沿岸原産のナシ台木種 P. amygdaiformis および P. elaeagrifolia の耐塩性は
アジア原産の台木種に比べ強い

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1鳥取大学農学部 680–8553 鳥取市湖山町南
2忠南大学校農業生命科学大学院芸学科 305–764 大田広域市 大韓民国

アジア原産ナシ台木種: Pyrus betulaefolia, P. pyrifolia および P. xerophila と地中海沿岸原産ナシ台木種: P. amygdaiformis および P. elaeagrifolia の耐塩性の差異を、75 mM および 150 mM の NaCl 溶液を 30 日間処理し、調査した。地中海沿岸原産台木種は 30 日間の NaCl 処理期間中、いずれの処理区においても葉に障害を発生しなかった。一方、アジア原産台木種は NaCl 処理により葉に障害が発生した。根幹、細根および 1 個体当たりの Na および Cl 含量に原産地による大きな差異はみられなかった。しかし、葉の Na および Cl 含量は、いずれの NaCl 濃度においても地中海沿岸原産台木種の方がアジア原産台木種に比べ著しく少なかった。したがって、地中海沿岸原産台木種 P. amygdaiformis および P. elaeagrifolia は、根幹に Na および Cl の葉への移動を抑制する何らかの機構を備えているものと考えられた。

Stem water potential 値および Ci/Ca 値は NaCl 処理に伴い原産地にかかわらず低下したため、光合成速度の低下要因のひとつは体内の水ポテンシャルの低下による気孔の閉鎖であることが示唆された。しかしながら、地中海沿岸原産台木種における NaCl ストレス下の光合成速度はアジア原産台木種に比べ高かったことから、地中海沿岸原産台木種の葉の Na および Cl 含量が低いことが光合成速度の低下抑制に寄与した可能性が示唆された。本実験結果は地中海沿岸原産ナシ台木種: P. amygdaiformis および P. elaeagrifolia はアジア原産ナシ台木種: P. betulaefolia, P. pyrifolia および P. xerophila に比べ強い耐塩性を持つことを示すものである。地中海沿岸原産台木種はニホンナシの耐塩性台木を育成する上で重要な遺伝資源となるものと考えられた。