Mechanisms of salt stress tolerance in two rose rootstocks: *Rosa chinensis* ‘Major’ and *R. rubiginosa*

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Abstract

The responses of two rose rootstocks *Rosa chinensis* ‘Major’ and *R. rubiginosa* were investigated under salt stress. The distribution of chloride and sodium ions in all plant parts was determined. The salt treatments were applied through irrigation water containing 0, 5, 10, 20 and 30 mM NaCl. Necrosis on the leaves as a result of the NaCl treatments was observed with in rootstocks after two months. Leaf injury was more pronounced in *R. chinensis* ‘Major’ than *R. rubiginosa*. The rootstock *R. rubiginosa* showed a higher tolerance to the NaCl stress than *R. chinensis* ‘Major’. The survival of the plants under increased NaCl stress as well as the extent of leaf injury could be used in the determination of tolerance of the rose genotypes. The lower older leaves contained higher concentrations of Cl\(^-\) than the young upper leaves. Leaf samples had higher concentrations of Cl\(^-\) than stem samples taken from the same positions. The roots contained higher amounts of Cl\(^-\) than the stem samples. The plants accumulated higher amounts of Cl\(^-\) in comparison with Na\(^+\). The lower leaves of *R. chinensis* ‘Major’ had higher amounts of Na\(^+\) than in all other parts whereas *R. rubiginosa* had higher concentrations of Na\(^+\) in the roots than in all other parts. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Rosa species; Salt stress; Ion distribution; Sodium; Chloride

1. Introduction

Understanding the physiology of salt tolerance in plants is important for the effective solution of the problem of salinity in agricultural and horticultural soils (Larcher, 1994). The general characteristic of saline soils is the occurrence of...
high concentrations of soluble salts. Because of its increased osmotic potential, the water in saline soils is less readily available to plants leading to a physiological water stress. On the other hand the excess concentration of ions, mainly Cl\(^{-}\) and Na\(^{+}\), leads not only to ion imbalances in the plant but also to ion toxicity. The restriction of the uptake of chlorine and sodium in the roots and the accumulation of these ions in stems and leaves are important mechanisms in the resistance of salt stress in glycophytes. This depends on the permeability and selection of the roots to these ions during uptake which differs among genotypes (Marschner, 1993). Yeo (1983) defined salt stress resistance as the ability of a plant to maintain growth and metabolism under saline conditions. The transport of chloride ions in plants takes place mainly in the transpiration stream (Marschner, 1993). This explains the occurrence of high concentrations of chlorine in the leaves which leads eventually to leaf injury. Reduction in growth in plants under salt stress can be attributed to necrosis of the leaves which reduces the photosynthetically active area (El-Siddig and Lüdders, 1994). Chloride ions are normally rapidly taken up by the roots and are then easily transported to the shoot system. This explains the occurrence of high concentrations of chloride ions in the plants in comparison with sodium ions and chloride is therefore usually the major cause of injuries arising from salt stress in plants (Greenway and Munns, 1980). The uptake of sodium ions in plants occurs actively through ion pumps in the roots but passive uptake takes place to a lesser degree. The passive uptake of sodium increases under saline conditions and leads to a reduction in the uptake of potassium (Brunold, 1996). Due to the sensitivity of enzymes to high levels of sodium, plants maintain low sodium levels in the cytoplasm through exclusion mechanisms. The exclusion of ions from cytoplasm is normally limited and the ability to restrict the transport of sodium from the roots to shoots is very important for the survival of plants under salt stress conditions (Cheeseman, 1988). The aim of this investigation was to determine the tolerance of different rose rootstocks to salt stress. The mechanisms by which roses adapt to salt stress conditions and the relationship between the distribution of chloride and sodium ions in different plant parts and salt stress tolerance was determined.

2. Materials and methods

2.1. Plant materials

Plants of two rootstocks *R. chinensis* ‘Major’ and *R. rubiginosa* were cultivated in a mixture of peat and perlite (1:1) in 12 l Mitscherlich pots. Planting date was June 1996. The rooted cuttings were grown for six weeks before the onset of NaCl treatments. The plants were irrigated daily with 200 ml water containing 0,
5, 10, 20 and 30 mM NaCl. Ten plants of each genotype were used in every treatment. The experiments were carried out at the experimental station, Koepenick at the Humboldt University in Berlin, Germany.

2.2. Leaf injury

The occurrence of injury on the leaves in the form of chlorosis and necrosis was checked at two-week intervals. A detailed investigation of leaf injury was undertaken in both rootstocks after three months of treatment. The numbers of healthy shoots and those with leaves showing necrosis per plant were determined.

2.3. Chloride ions analysis

In order to determine the applicability of the distribution of Cl\(^-\) in different plant parts as an indicator of salt stress tolerance, its content in lower and upper leaves, lower and upper stem parts, base of the stem (original cutting) and in roots was analysed. Three mixed samples of each plant part obtained from the 10 plants in each treatment were used in the analysis. The lower leaf samples were usually necrotic as a result of the salt injury. The upper leaves were obtained from the top of the plants and were healthy. The lower stem consisted of 20 cm of the base of the shoots obtained after pruning while 10 cm long tips of the shoots formed the upper stem samples. Only the long shoots were utilised for analysis. The samples were dried in an oven at 121°C for 24 h and then ground. Samples of 1 g of the ground tissue were used for the Cl\(^-\) ions analysis. Chloride ions are normally extracted from plant tissue by the use of distilled water and shaking in boiling water bath for 1 h and then the content of chloride ions is determined from the filtrate. However, the samples from rose tissues in this investigation formed a slime after shaking in water bath and no filtrate could be obtained. The Cl\(^-\) ions were therefore extracted by shaking the samples in open air and then centrifuging the contents instead of filtering. Investigations revealed that the contents of Cl\(^-\) ions obtained from samples shaken in open air and in boiling water bath were very similar. The chloride ions were extracted in 25 ml double distilled water. Another 25 ml of double distilled water was added after shaking to make the final volume 50 ml which was then centrifuged for 10 min at 4500 rpm. Samples of 1 ml were taken from the clear region after centrifuging and used for the determination of Cl\(^-\) ions content using a chloride meter (Model 6610, Eppendorf, Hamburg, Germany).

2.4. Sodium ions analysis

The content of Na\(^+\) was determined from the same samples used for the analysis of Cl\(^-\) ions. Samples of 0.5 g were weighed in porcelain pots and burnt
to ash in a muffle oven at 490°C for 3 h. The ash was dissolved in 5 ml 10% HCl and then heated on a water bath until almost dry. Another 5 ml of 20% HCl was added after cooling and the content of the porcelain emptied into 50 ml volumetric flasks. The pots were washed with boiled demineralised water and the contents again emptied into the volumetric flasks. The volume was adjusted after cooling, filtered and the content of Na\(^+\) ions determined directly from the filtrate or after dilution using a flame photometer (Model M8D, Dr. Bruno Lange GmbH, Berlin, Germany).

3. Results

3.1. Effect of NaCl on leaf injury

The response of the two rose rootstocks to NaCl stress was determined by recording the occurrence of symptoms of leaf injury. Necrosis on the leaves was observed after two months of treatment in both genotypes. However, this symptom of leaf injury was more pronounced in *R. chinensis* ‘Major’ than *R. rubiginosa*. The necrosis began at the leaf tips and then progressed inward towards the petiole. Injury began on the lower leaves and thereafter progressed to the upper leaves. However, the new leaves at the top of the shoots were not affected. Leaf injury increased with rising level of NaCl concentration in irrigation water as well as duration of the treatment.

The plants were pruned in December 1996 after five months of treatment. New shoots developed towards the end of February 1997. Leaf injury occurred on the new shoots earlier than on the original plants. The new shoots in the control and 5 mM NaCl treatments showed no symptoms of leaf injury two weeks after their formation. However, a few plants in the 10 and 20 mM treatments and a large number of plants in the 30 mM NaCl treatment showed necrosis on the leaves of the new shoots. Leaf injury on the plants, the 5 mM NaCl treatment occurred after six weeks. The new shoots in the 20 and 30 mM NaCl treatments were partly or wholly dead eight weeks after their formation in *R. chinensis* ‘Major’. A few plants dried up completely in the 30 mM NaCl treatment. Death of the new shoots or complete drying up of the plants was not observed in *R. rubiginosa*. The occurrence of injured leaves in *R. rubiginosa* was less in comparison with *R. chinensis* ‘Major’ and was more pronounced only in the 20 and 30 mM NaCl treatments. Chlorosis which normally precedes necrosis under salt stress conditions was not observed in this case. The rootstock *R. chinensis* ‘Major’ was found to be more sensitive to the NaCl treatment than that of *R. rubiginosa*.

The NaCl treatment had a significant influence on the proportion of shoots with injured leaves determined three months after the beginning of the treatments in all the genotypes tested (Table 1). *R. chinensis* ‘Major’ had a higher proportion of
shoots with injured leaves than *R. rubiginosa* in all NaCl treatments. The 10 mM NaCl treatment induced a significant increase in the proportion of shoots with injured leaves in *R. rubiginosa*, but further increase in the concentration of NaCl had no additional significant effect.

### 3.2. Distribution of chloride ions in plant parts

The data shown in Tables 2 and 3 were obtained towards the end of the experiments in July 1998. Plants of *R. rubiginosa* received the NaCl treatment for 25 months but those of *R. chinensis* ‘Major’ for 13 months because new plants were established in June 1997 as a result of the extensive injury on leaves and shoots after the pruning of the original plants planted in June 1996. The lower leaves of *R. chinensis* ‘Major’ had more than twice the amount of Cl$^-$ in comparison with the upper leaves in all treatments (Table 2). Addition of only 5 mM NaCl to the irrigation water resulted in more than double the amount of Cl$^-$ in both leaf samples in comparison with the control. The roots, lower and upper stem samples had similar amounts of Cl$^-$ (Table 2). A reduced

### Table 1

Effect of NaCl on the proportion of shoots with injured leaves in *R. chinensis* ‘Major’ and *R. rubiginosa* after three months of treatment, *N*=10

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment/proportion of shoots with injured leaves (%)$^a$</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 5 mM NaCl 10 mM NaCl 20 mM NaCl 30 mM NaCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>R. chinensis</em> ‘Major’</td>
<td>0.00 d 8.90 c 40.00 b 93.30 a 90.80 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>R. rubiginosa</em></td>
<td>2.30 b 0.00 b 17.30 a 34.00 a 40.00 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Mean separation for each genotype within rows by chi-square test, *P*=0.05.

### Table 2

Effect of NaCl on the distribution of Cl$^-$ ions in plant parts in *R. chinensis* ‘Major’ after 13 months of treatment

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment/Cl$^-$ content (% DM)$^a$</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 5 mM NaCl 10 mM NaCl 20 mM NaCl 30 mM NaCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper leaves</td>
<td>0.21 e 0.58 d 0.77 c 1.17 b 1.34 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower leaves</td>
<td>0.70 e 1.81 d 2.12 c 2.51 b 2.63 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper stem</td>
<td>0.18 c 0.39 b 0.41 b 0.56 a 0.62 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower stem</td>
<td>0.17 c 0.22 c 0.36 b 0.55 a 0.60 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stem basis</td>
<td>0.06 d 0.13 c 0.20 b 0.28 a 0.29 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>0.28 c 0.48 b 0.50 b 0.60 a 0.64 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Mean separation for each parameter within rows by Student–Newman–Keuls test, *P*=0.05.
concentration of Cl$^-$ in the basis of the stem in comparison with the other plant parts was determined. *R. chinensis* ‘Major’ was observed to accumulate the highest amounts of Cl$^-$ in the lower leaves. Both leaf samples had higher amounts of Cl$^-$ in comparison with the stem samples taken at the same positions.

*R. rubiginosa* also showed an increased amount of Cl$^-$ in the lower leaves than in the upper leaves as with *R. chinensis* ‘Major’ (Table 3). However, the lower stem parts contained a higher amount of Cl$^-$ than the upper stem in contrast to the findings in *R. chinensis* ‘Major’. Apart from the 30 mM NaCl treatment, the lower stem and the bases of the stems had similar amounts of Cl$^-$. The roots accumulated higher amount of Cl$^-$ than all the stem parts and both leaf samples apart from 20 mM NaCl treatment. The sensitive rootstock *R. chinensis* ‘Major’ accumulated higher amounts of Cl$^-$ in all plant parts apart from the roots as compared to *R. rubiginosa*. The decreased Cl$^-$ content in *R. rubiginosa* could be responsible for the increased tolerance to NaCl observed. The rootstock, *R. rubiginosa* also accumulated higher amounts of Cl$^-$ in the roots than in any other part of the plant and it accumulated a higher concentration of Cl$^-$ ions in the roots than *R. chinensis* ‘Major’. The two rootstocks showed a similar tendency to accumulate Cl$^-$ in the lower leaves. The lower leaves were usually necrotic and were shed in time. This leaf fall could have been utilised by the plants to rid themselves of the toxic levels of Cl$^-$. 

3.3. Distribution of sodium ions in plant parts

The lower leaves of *R. chinensis* ‘Major’ contained higher amounts of Na$^+$ in the 20 and 30 mM NaCl treatments than the upper leaves (Table 4). A similar tendency was determined regarding the lower and upper stem parts especially from 10 mM NaCl treatment. The leaf and stem samples accumulated
almost similar amounts of Na$^+$. The bases of the stems and the root samples contained similar amounts of Na$^+$. However, the lower stem samples had higher Na$^+$ contents than the stem bases and the roots. Thus, the distribution of Na$^+$ in the plant parts in *R. chinensis* ‘Major’ approximated that of Cl$^-$ (Tables 2 and 4) although the plants accumulated higher amounts of Cl$^-$ than Na$^+$.

Application of NaCl in the irrigation water showed no corresponding increase in the accumulation of Na$^+$ in the lower and upper leaves of *R. rubiginosa* (Table 5). The Na$^+$ contents in both leaf samples were also very similar. Similar observations were obtained in the upper and lower stem parts. Increasing the concentration of NaCl resulted in a higher accumulation of Na$^+$ in the stem bases and in the root system. The root system contained the highest amount of Na$^+$ in comparison with all other plant parts just as was determined in the distribution of Cl$^-$ in *R. rubiginosa*. This genotype retained Na$^+$ ions in the roots just like the Cl$^-$ ions.

Table 4  
Effect of NaCl on the distribution of Na$^+$ ions in plant parts in *R. chinensis* ‘Major’ after 13 months of treatment

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment/Na$^+$ content (% DM)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control  5 mM NaCl 10 mM NaCl 20 mM NaCl 30 mM NaCl</td>
</tr>
<tr>
<td>Upper leaves</td>
<td>0.39 bc 0.29 c 0.25 c 0.46 b 0.71 a</td>
</tr>
<tr>
<td>Lower leaves</td>
<td>0.21 c 0.20 c 0.25 c 0.62 b 1.10 a</td>
</tr>
<tr>
<td>Upper stem</td>
<td>0.12 c 0.13 c 0.17 c 0.44 b 0.67 a</td>
</tr>
<tr>
<td>Lower stem</td>
<td>0.06 c 0.15 bc 0.35 b 0.78 a 0.97 a</td>
</tr>
<tr>
<td>Stem basis</td>
<td>0.10 b 0.18 b 0.40 a 0.44 a 0.46 a</td>
</tr>
<tr>
<td>Roots</td>
<td>0.12 c 0.29 b 0.38 ab 0.48 a 0.52 a</td>
</tr>
</tbody>
</table>

$^a$ Mean separation for each parameter within rows by Student–Newman–Keuls test, $P=0.05$.

Table 5  
Effect of NaCl on the distribution of Na$^+$ ions in plant parts in *R. rubiginosa* after 25 months of treatment

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment/Na$^+$ content (% DM)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control  5 mM NaCl 10 mM NaCl 20 mM NaCl 30 mM NaCl</td>
</tr>
<tr>
<td>Upper leaves</td>
<td>0.13 a 0.11 a 0.14 a 0.13 a 0.11 a</td>
</tr>
<tr>
<td>Lower leaves</td>
<td>0.10 a 0.10 a 0.11 a 0.11 a 0.08 a</td>
</tr>
<tr>
<td>Upper stem</td>
<td>0.14 a 0.08 a 0.16 a 0.16 a 0.15 a</td>
</tr>
<tr>
<td>Lower stem</td>
<td>0.07 a 0.15 a 0.17 a 0.22 a 0.23 a</td>
</tr>
<tr>
<td>Stem basis</td>
<td>0.14 b 0.16 b 0.31 a 0.38 a 0.39 a</td>
</tr>
<tr>
<td>Roots</td>
<td>0.22 b 0.33 b 0.45 b 0.57 b 1.12 a</td>
</tr>
</tbody>
</table>

$^a$ Mean separation for each parameter within rows by Student–Newman–Keuls test, $P=0.05$. 

4. Discussion

Leaf injury occurred in both rootstocks after two months of treatment. Necrosis on the leaf tips which spread inward towards the petiole during the course of treatment was the characteristic response of the plants to the NaCl treatment. The necrosis appeared first on the lower older leaves and then spread upwards. The affected leaves eventually abscised. Similar observations have been reported in *Rosa multiflora* (Weber and Reimann-Phillip, 1989), orange (Banuls and Primo-Millo, 1995) and sugarcane (Greenway and Munns, 1980). Hughes and Hanan (1978) observed the occurrence of necrosis and chlorosis on the leaves of *Rosa hybrida* ‘Bacarra’ after 45 days of treatment.

New shoots formed after pruning of the plants in *R. chinensis* ‘Major’ died partly or in whole after eight weeks in the 20 and 30 mM NaCl treatments. A few plants also dried up especially in the 30 mM NaCl treatment. Such extreme injuries were not observed in *R. rubiginosa*. The survival and formation of new shoots in roses after pruning was used to characterise the tolerance of plants under salt stress conditions (Cid et al., 1989). Ebert (1998) reported that plants responded to increased salinity by the occurrence of injury on the leaves which was normally followed by leaf fall. Part of the shoot, the whole shoot or even the whole plant may die in extreme cases. Banuls and Primo-Millo (1995) showed a close relationship between the Cl\(^–\) content of the leaves and leaf fall associated with salt stress. Leaf injury in *Citrus* spp. was attributed to toxic levels of Cl\(^–\) and Na\(^+\) in leaves (Storey and Walker, 1999).

A higher Cl\(^–\) than Na\(^+\) content was determined especially in the upper and lower leaves. Accumulation of toxic levels of Cl\(^–\) could therefore have been responsible for the leaf necrosis. Chloride ions are normally taken up by plants faster than Na\(^+\) ions and hence their concentration in plants is always higher (Greenway and Munns, 1980). Chartzoulaskis (1994) also observed a higher Cl\(^–\) content in leaf tissues of cucumbers under saline conditions in comparison with Na\(^+\) content. The reduction in growth and yield in avocado (Wiesman, 1995) and in citrus (Bar et al., 1998) has been attributed to the accumulation of toxic levels of Cl\(^–\).

The lower older leaves contained a higher Cl\(^–\) content than the upper leaves. The lower and upper stem parts had a lower Cl\(^–\) content in comparison to leaf samples obtained from same positions. The sensitive genotype *R. chinensis* ‘Major’ accumulated a higher amount of Cl\(^–\) than *R. rubiginosa*, while *R. rubiginosa* accumulated higher amounts of Cl\(^–\) in roots as compared to *R. chinensis* ‘Major’ but it retained the Cl\(^–\) ions in the roots. Similar observations have been reported in apples (Döring and Lüdders, 1987), citrus (Banuls and Primo-Millo, 1995), sunflower (Ballesteros et al., 1997) and in potatoes (Heuer and Nadler, 1998). Accumulation of Cl\(^–\) in older leaves and the subsequent leaf fall has been described as a mechanism of ridding the plants of excess salts.
thereby protecting the younger leaves (Ebert, 1998). Accumulation of Cl⁻ and Na⁺ ions in plant tissues under salinity has been associated with osmotic adaptations (Döring and Lüdders, 1987; Heuer and Nadler, 1998; Pardossi et al., 1999). Accumulation of Cl⁻ and Na⁺ in roots improved the water status in *Vaccinium ashei* (Wright et al., 1995) and *Pistacia* spp. (Picchioni and Miyamoto, 1990). Perez-Alfocea et al. (1996) attributed salt stress tolerance in tomato to the ability of the plant to exclude Na⁺ from the shoot system combined with good selectivity during ion uptake and good distribution of both mineral salts and assimilates.

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**References**


