Effects of Salt Stress on Growth and Inorganic Ion Distribution in Narcissus tazetta L. var. chinensis Roem. Seedlings

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Abstract. Narcissus tazetta L. var. chinensis Roem. seedlings were subjected to substrate salinity and salt spray during greenhouse cultivation. The results demonstrated that N. tazetta L. seedlings treated with substrate salinity and salt spray exhibited slower growth rates than the control group. The sedimentation of Na+ and Cl− was primarily observed in the leaf apex, which was consistent with the location of lesions induced by salt stress. Under the two methods of salt stress, the mass fraction of ash in the leaf was significantly higher than that in the control group (P < 0.05). The sedimentation of Na+ and Cl− was mostly distributed in young leaves in the salt spray treatment, whereas the sedimentation was mostly distributed in old leaves under substrate salinity. There was a significant positive correlation between contents of Na+ and Cl− under the two methods of salt stress (P < 0.01). Not only the mass concentrations of Ca2+, Mg2+, Na+, K+, and Cl− in the seedlings exhibited variation, but also the distribution of mineral elements in the seedlings changed after both salt stress treatments. Moreover, the ratio of K+/Na+ under salt spray was greater than that under substrate salinity at the 300 mM NaCl treatment level. These results show that ion toxicity in N. tazetta L. seedlings was more serious under substrate salinity than under salt spray.

The ornamental and economic value of N. tazetta L. var. chinensis Roem. is high. This variety is widely cultivated in the coastal area of Zhangzhou City, Fujian Province. In this area, there is a large amount of precipitation and uneven distribution that varies greatly with seasonal change. This pattern of precipitation aggravates soil salinization. Strong wind and waves in coastal areas lead to the deposition of numerous water droplets with wind and waves in coastal areas lead to the deposition of numerous water droplets with wind and waves in coastal areas lead to the deposition of numerous water droplets with

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Materials and Methods

Plant materials and culture
Narcissus tazetta L. var. chinensis Roem. seedlings were selected as the research material. They were planted in plastic pots (25 × 18 cm) and maintained in the greenhouse of Minnan Normal University. The temperature of the greenhouse was maintained at 20 ± 2 °C/15 ± 2 °C, and the photoperiod was 14 h/10 h (day/night). When the leaves of N. tazetta L. seedlings grew to 10 cm length, 100 seedlings with consistent growth were transferred to plastic pots filled with 1/2 Hoagland hydroponic solution.

Narcissus tazetta L. seedlings were subjected to substrate salinity and salt spray treatments in a greenhouse environment. The growth rate, the allocation of five measures and provide a basis for screening salt tolerant species.
those that appear within seven growth days, old leaves are defined as those that have existed for at least 28 d, and mature leaves are those that have existed in the range of 14–21 d. After collecting young leaves, mature leaves, and old leaves, the salt remaining on the leaves was washed with distilled water. The leaves can be divided into three parts: leaf apex (1/3 crosscut near the leaf tip), leaf margin (1/4 longitudinal part on both ends of the leaf after removing the leaf tip), and leaf middle (leaf remainder after removing the leaf tip and leaf margin) (Wang and Lin, 1999).

**Morphological.** Narcissus tazetta L. seedlings were selected randomly and measured to determine plant height between root base and stem top, leaf number, and the growth situation, and symptoms of plant stress.

**The mass fraction of ash.** All plants were randomized to treatment groups, and 10 plants were measured for each treatment (Yasu et al., 1986). Narcissus tazetta L. seedlings were then rinsed in distilled water, subjected to heat at 100 °C for 10 min and dried to a constant weight at 80 °C for measurement of plant dry matter weight. The mass fraction of ash was then examined via the dry ashing method (525 °C ± 25 °C, for 4 h).

**The mass concentrations of Ca²⁺, Mg²⁺, Na⁺, K⁺, and Cl⁻.** Extraction and measurement of Ca²⁺, Mg²⁺, Na⁺, K⁺, and Cl⁻ in N. tazetta L. seedlings were performed according to Yue et al. (2012) with minor modifications. Narcissus tazetta L. plants were fully rinsed in distilled water, subjected to heat at 100 °C for 10 min, and dried to a constant weight at 80 °C. Dry matter was ground and screened with a 60-mesh sieve. Next, 100 mg of each sample was placed into a test tube (25 mL) and 20 mL of deionized H₂O was added. This mixture was boiled for 2 h, and then filtered and deionized H₂O was added to create a final volume of 50 mL. Concentrations and absolute contents of mineral elements including potassium, sodium, calcium, and magnesium were determined via atomic absorption spectrophotometry (AA800 type atomic absorption spectrometer). The content of chloride was determined via a AgNO₃ titration method (Adachi and Kobayashi, 2008). Measurement of K⁺/Na⁺ in N. tazetta L. seedlings was performed according to Wang et al. (2007).

### Statistical analysis

All data were analyzed using SPSS software (ver. 17.0) and presented as means ± SE for each treatment (n = 3, except in measurement of plant height, leaf number, and the mass fraction of ash, where n = 10). Differences among means were determined by Tukey’s multiple range test. P values less than 0.05 were considered statistically significant.

### Result

**Plant growth under salt stress.** Injury of N. tazetta L. seedlings was observed more often in the leaf apex under substrate salinity and salt spray. The growth in the salinity group was generally worse than in the control group. In the 200 mM NaCl substrate salinity treatment, the growth of new leaves in N. tazetta L. seedling was severely limited and disease spot distributed in the leaf apex of old leaves. In the 300 mM NaCl substrate salinity treatment, leaves turned yellow, whereas in the 300 mM NaCl salt spray treatment, the growth of new leaves in N. tazetta L. seedling was severely limited and disease spot distributed in the leaf apex of young leaf. Injury of N. tazetta L. under salt spray was lower than under substrate salinity at the 300 mM NaCl treatment level (Table 1).

**The mass fraction of ash under salt stress.** With increasing salinity, the mass fraction of ash in N. tazetta L. seedling leaves under substrate salinity was higher than those in the control group (P < 0.05; Figs. 1 and 2). Under the same salinity, the mass fraction of ash in the distinct parts of leaves was as follows: leaf apex > leaf middle > leaf margin. The mass fraction of ash in the different ages of leaves under substrate salinity was as follows: old leaf > mature leaf > young leaf. The mass fractions of ash in the different ages of leaves under salt spray were as follows: young leaf > mature leaf > old leaf. The ash content under substrate salinity was greater than that under salt spray when plants were treated with 300 mM NaCl (P < 0.05). SPSS covariance analysis results showed that there was significant effect for ash in the factors of salinity, leaf partition, and leaf age, but there was no interaction effect between them.

**The mass concentrations of Ca²⁺, Mg²⁺, Na⁺, K⁺, and Cl⁻ under salt stress.** Increasing salinity increased the mass concentrations of Na⁺, K⁺, Mg²⁺, and Cl⁻ in N. tazetta L. seeding leaves (P < 0.05). Different leaf parts (leaf margin, leaf apex, and leaf middle) exhibited variation in the distribution of each element, which was statistically significant (P < 0.05; Fig. 3). Under substrate salinity, Na⁺, K⁺, Cl⁻, and Mg²⁺ are distributed in the leaf apex, whereas Ca²⁺ is mainly distributed in the leaf middle and leaf margin. Under salt spray, Mg²⁺ and Ca²⁺ are mainly distributed in the leaf middle and leaf margin, whereas Na⁺, K⁺, and Cl⁻ are mostly distributed in the leaf apex. Under substrate salinity and salt spray, the mass concentrations of Na⁺ and Cl⁻ rapidly increased in all leaf parts with the increase in salinity. However, the mass concentration of Ca²⁺ exhibited little alteration. The mass concentrations of Na⁺ and Cl⁻ in the leaf parts of N. tazetta L. seedling leaves were significantly higher than the levels in the control group. The mass concentrations of K⁺, Mg²⁺, and Ca²⁺ were lower than those in the control group. The concentrations of Na⁺ and Cl⁻ under salt spray were greater than those under substrate salinity at the 300 mM NaCl treatment level.

Increasing salinity increased the mass concentrations of Na⁺, K⁺, Mg²⁺, and Cl⁻ in N. tazetta L. seeding leaves (P < 0.05). Age of leaf (old leaf, mature leaf, and young leaf) also affected the distribution of each element in the seedling leaves (P < 0.05; Fig. 4). In the control group, Na⁺, K⁺, and Cl⁻ were mostly distributed in young leaves. Mg²⁺ was mainly distributed in mature leaves, and Ca²⁺ was mainly distributed in old leaves. Under 100 and 200 mM NaCl substrate salinity treatments, Na⁺ and Cl⁻ were mostly distributed in the young leaves. However, Na⁺ and Cl⁻ were mostly distributed in the old leaves in the 300 and 400 mM NaCl substrate salinity treatment groups. Under substrate salinity, K⁺ was mostly distributed in the young leaves. Mg²⁺ was mostly distributed in the mature leaves, and Ca²⁺ was mostly distributed in the old leaves. The mass concentrations of Na⁺ and Cl⁻ in the various age groups of N. tazetta L. seedling leaves were higher than those in the control group, the mass concentrations of K⁺ and Mg²⁺ were lower than those in the control group, and the mass concentration of Ca²⁺ exhibited no significant difference. Under the salt spray treatment, Na⁺, K⁺, and Cl⁻ were mostly distributed in the young leaves, Mg²⁺ was mostly distributed in the mature leaves, and Ca²⁺ was mostly distributed in the old leaves. The mass concentrations of Na⁺ and Cl⁻ in the various age groups of N. tazetta L. seedling leaves subjected to salt spray were higher than those in the control group, whereas the mass concentrations of Mg²⁺, K⁺, and Ca²⁺ were lower than those in the control group. The concentrations of Na⁺, K⁺, and Cl⁻ under salt spray were lower than those under substrate salinity at the 300 mM NaCl treatment level.

One-Way analysis of variance analysis was completed using SPSS V10.0, the results show that under the two methods of salt stress there is the extremely significant strong

<table>
<thead>
<tr>
<th>NaCl concn/(mM)</th>
<th>Plant ht/cm</th>
<th>Number of leaves</th>
<th>Growth situation</th>
<th>Symptoms of plant stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>52.347 ± 0.387 a</td>
<td>11.323 ± 0.421 a</td>
<td>Well</td>
<td>No disease spot</td>
</tr>
<tr>
<td>100</td>
<td>51.325 ± 0.346 a</td>
<td>9.422 ± 0.341 b</td>
<td>Slow</td>
<td>Disease spot distribute in leaf apex of old leaf</td>
</tr>
<tr>
<td>300</td>
<td>49.769 ± 0.231 b</td>
<td>7.853 ± 0.315 c</td>
<td>Slow</td>
<td>Disease spot distribute in leaf apex of old leaf</td>
</tr>
<tr>
<td>400</td>
<td>32.032 ± 0.364 d</td>
<td>4.586 ± 0.652 d</td>
<td>Slow</td>
<td>Disease spot distribute in leaf apex of old leaf</td>
</tr>
<tr>
<td>300*</td>
<td>50.215 ± 0.211 a</td>
<td>8.215 ± 0.211 bc</td>
<td>Slow</td>
<td>Disease spot distribute in leaf apex of old leaf</td>
</tr>
</tbody>
</table>

The data are expressed as means ± SE of 10 replications. Different letters show significant differences (P < 0.05). * stands for processing salinity of the salt spray group, and no mark stands for processing salinity of the substrate salinity group.

Table 1. Effects of salt stress on the growth of N. tazetta seedlings.

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positive correlation on the mass concentrations of Na⁺ and Cl⁻ in *N. tazetta* seedlings (Pearson correlation coefficient was 2.647, *P* < 0.01). There were extremely significant weak positive correlations between the mass concentrations of Na⁺ and ash, Ca²⁺ and ash, and Cl⁻ and ash in *N. tazetta* seedlings (Pearson correlation coefficients of 0.547, 0.613, and 0.571, respectively; *P* < 0.01). There were extremely significant weak negative correlations between the mass concentrations of Na⁺ and K⁺, K⁺ and Cl⁻, Na⁺ and Ca²⁺, Na⁺ and Mg²⁺, Ca²⁺ and Cl⁻, and K⁺ and Ca²⁺ in *N. tazetta* seedlings (Pearson correlation coefficients of –0.652, –0.312, –0.543, –0.203, –0.456, and –0.129, respectively; *P* < 0.01). There were significant weak negative correlations between the mass concentrations of K⁺ and Ca²⁺ in *N. tazetta* seedlings (Pearson correlation coefficient of –2.482, *P* < 0.05).

Under substrate salinity and salt spray, the ratios of K⁺/Na⁺ in the different leaf parts and age groups were significantly lower than those in the control group (Tables 2 and 3; *P* < 0.05). The ratio of K⁺/Na⁺ decreased with increasing salinity. The pattern of K⁺/Na⁺ ratios in the distinct parts of the leaf was as follows: leaf middle > leaf margin > leaf apex. Under substrate salinity, the ratios of K⁺/Na⁺ in the different age groups of leaves decreased as follows: young leaf > mature leaf > old leaf, whereas under salt spray, the ratios of K⁺/Na⁺ in the different age groups of leaves decreased as follows: mature leaf > old leaf > young leaf. When they were treated with 300 mM NaCl, the ratio of K⁺/Na⁺ was lower under substrate salinity than that under salt spray.

**Discussion**

Salt spray affects the growth of the plants by affecting the absorption ability of the leaf, and substrate salinity affects the growth of the plants through permeability and transportation in the roots. The mass concentrations of Ca²⁺, Mg²⁺, Na⁺, K⁺, and Cl⁻ in *N. tazetta* L. seedlings changed with the salt stress treatments. However, the salt redistribution in plants manifests in different ways depending on whether the stress is from salt spray or substrate salinity. Under substrate salinity, the salt was absorbed by the roots from the culture media. The salt was then transported from the stem to leaf through the xylem; therefore, a large amount of Na⁺ is deposited at the apex of the leaf by transpiration (Tavakkoli et al., 2011). Under salt spray stress, leaves absorb the salt from the salt fog. The apex of the leaf acts as the absorption point, and the salt in the seedling leaves is therefore more concentrated in the leaf apex than in the margin or the middle. Therefore, the salt injury symptoms first begin to appear in the leaf apex under substrate salinity and salt spray. This is consistent with previous studies (Munns and Tester, 2008). In the young
leaves, Na+ and Cl– were the most concentrated under the salt spray treatment, and these ions were mostly concentrated in the old leaves under the substrate salinity treatments. This demonstrates that the young leaves were the most sensitive to salt under the salt spray treatment, whereas the old leaves were most sensitive to salt under substrate salinity. This is consistent with previous studies (Sun et al., 2010).

The key ion related to resistance to salt stress is K+. (Shinozaki and Dennis, 2003). It is the most abundant cation in higher plants, and it also plays important roles in physiological functions, such as regulation of ion balance, osmotic regulation, protein synthesis, maintenance of cell turgor pressure, and photosynthesis. The ionic radii of K+ and Na+ are similar to that of hydrated energy, and K+ and Na+ have competition with absorption sites and active sites, thus inhibiting the enzyme activity and metabolic process of K+. (Shabala and Cuin, 2008). Higher K+ content and a higher ratio of K+/Na+ can reduce the injury due to salt stress (Maathuis and Amtmann, 1999). We found differences in the ratios of K+/Na+ in the different age groups and parts of the leaf. These differences are due to ion selectivity. In the leaf apex of *N. tazetta* L. seedlings under both salt stress treatments, the ratio of K+/Na+ in the leaf apex of young leaves under the substrate salinity treatments, and in the young leaves under the salt spray treatment, the ratio of K+/Na+ is smaller, the selectivity of K+ is less, the selectivity of Na+ is greater, and the ion balance ability of K+/Na+ is weak. Therefore, the salinity is mainly distributed in the leaf apex of old leaves under the substrate salinity treatments; the salinity is mainly distributed in the leaf apex of young leaves under the salt spray treatments. The ratio of K+/Na+ in the different parts of the leaf was greater under the salt spray treatment than in the substrate salinity treatments at the 300 mM NaCl treatment level. Therefore, the ion toxicity on *N. tazetta* L. seedlings was more serious under substrate salinity than under salt spray treatments. This is not consistent with previous studies (Mahlooji et al., 2017; Megan, 2006; Ogura and Yura, 2008).

Salt levels that exceed a certain concentration can cause injury to the plant, but low salt levels can promote plant growth (Zolla et al., 2010). There was a significant positive correlation between the mass concentrations of Na+ and Cl– in the experimental treatments of *N. tazetta* L. seedlings, which is consistent with previous studies (Amini et al., 2017; Munns and Tester, 2008). The mass concentrations of Na+ and Cl– exhibited a very high similarity among the different ages and parts of *N. tazetta* L. seedlings. Wang and Lin (1999) studied the distribution of plant nutrients in *Kandelia candel*, *Bruguiera gymnorrhiza*, and *Rhizophora stylosa* during the process of plant leaf senescence. The mass concentrations of Cl– in three mangrove plant leaves were similar to that of Na+, and there was a significantly positive linear correlation between the mass concentrations of Na+ and Cl– (Wang and Lin, 1999). Under substrate salinity and salt spray treatments, the mass concentrations of Na+ and Cl– in *N. tazetta* L. seedlings increased significantly, whereas other elements were not notably affected. Thus, the mass fraction of ash in the seedlings under salt stress was also increased. This may be an adaptation to salt stress via the production of more organic osmoregulation substances. The mass concentrations of Na+ and Cl– were significantly decreased by the two methods of salt stress, causing ion poison. In terms of ion poisoning, salt injury is mainly an Na+ effect or a Cl– effect. However, the determination of the dominant

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**Table 2. Effects of salt stress on the ratios of K+/Na+ in the different parts of leaf.**

<table>
<thead>
<tr>
<th>Leaf Part</th>
<th>Control 100 mM</th>
<th>200 mM</th>
<th>300 mM</th>
<th>300 mM*</th>
<th>400 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf margin</td>
<td>7.7811 ± 0.35 a</td>
<td>1.0519 ± 0.17 d</td>
<td>0.8625 ± 0.12 f</td>
<td>0.8464 ± 0.13 f</td>
<td>0.8892 ± 0.08 f</td>
</tr>
<tr>
<td>Leaf middle</td>
<td>6.5803 ± 0.43 b</td>
<td>1.0917 ± 0.22 d</td>
<td>0.9364 ± 0.18 ef</td>
<td>0.9171 ± 0.17 ef</td>
<td>0.9473 ± 0.13 ef</td>
</tr>
<tr>
<td>Leaf apex</td>
<td>4.0532 ± 0.32 c</td>
<td>0.9861 ± 0.18 e</td>
<td>0.8473 ± 0.13 f</td>
<td>0.7172 ± 0.12 gh</td>
<td>0.8574 ± 0.09 f</td>
</tr>
</tbody>
</table>

The data are expressed as means ±SE of three replications. Different letters show significant differences (P < 0.05). * stands for processing salinity of the salt spray group, and no mark stands for processing salinity of the substrate salinity group.

**Table 3. Effects of salt stress on the ratios of K+/Na+ in the different ages of leaf.**

<table>
<thead>
<tr>
<th>Leaf Age</th>
<th>Control 100 mM</th>
<th>200 mM</th>
<th>300 mM</th>
<th>300 mM*</th>
<th>400 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young leaf</td>
<td>6.5275 ± 0.38 c</td>
<td>1.0613 ± 0.19 e</td>
<td>0.9381 ± 0.16 f</td>
<td>0.9132 ± 0.12 fg</td>
<td>0.8539 ± 0.03 gh</td>
</tr>
<tr>
<td>Mature leaf</td>
<td>7.3243 ± 0.41 b</td>
<td>1.3422 ± 0.20 d</td>
<td>0.9431 ± 0.26 fg</td>
<td>0.9045 ± 0.11 gh</td>
<td>0.9434 ± 0.04 f</td>
</tr>
<tr>
<td>Old leaf</td>
<td>7.7516 ± 0.28 a</td>
<td>1.0592 ± 0.23 e</td>
<td>0.9164 ± 0.27 gh</td>
<td>0.7208 ± 0.22 i</td>
<td>0.8872 ± 0.02 g</td>
</tr>
</tbody>
</table>

The data are expressed as means ±SE of three replications. Different letters show significant differences (P < 0.05). * stands for processing salinity of the salt spray group, and no mark stands for processing salinity of the substrate salinity group.
ion in this process is still an open research question.

**Conclusion**

Salinity could be a major influencing factor on leaf growth in *N. tazetta* L. seedlings. Under substrate salinity and salt spray, the leaf disease areas of *N. tazetta* L. seedlings are more concentrated in the leaf apex. Growth in the salinity treatment groups is generally worse than that in the control group. The mass fraction of ash and the mass concentrations of Ca$^{2+}$, Mg$^{2+}$, Na$^+$, K$^+$, and Cl$^-$ were generally greater in the salinity treatments of all five ions (Ca$^{2+}$, Mg$^{2+}$, Na$^+$, K$^+$, and Cl$^-$) than in the control. The concentrations of all five ions (Ca$^{2+}$, Mg$^{2+}$, Na$^+$, K$^+$, and Cl$^-$) also increased with increasing salinity, especially in the leaf apex. Meanwhile, under the same salinity, the ion concentrations are lower in the salt spray treatments than in the substrate salinity treatments. This indicates that substrate salinity could induce more injury to the leaf. The results of this investigation suggest that salinity may induce injury and limit the growth of *N. tazetta* L. seedlings. These findings should benefit the artificial cultivation of this plant.

**Literature Cited**


