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Research article

Distribution and re-transportation of sodium in three *Malus* species with different salt tolerance

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ABSTRACT

To further dissect the mechanism of salt tolerance in *Malus*, the comparison was made regarding the differences between the salt-tolerant and salt-sensitive species in sodium accumulation and extrusion capability in the roots and stem base as well as the sodium re-transportation from shoot to roots by using ^{22}Na labeling-based feeding of leaves and roots-split experiments. The results demonstrated that the salt-tolerant *Malus* species could accumulate more ^{22}Na in the main roots, lateral roots, stem base phloem and xylem, and extrude more sodium out than the salt-sensitive one. In addition, the salt-tolerant *Malus* species had the higher sodium re-transportation rate from shoot to roots. Altogether, it is concluded that the stronger sodium accumulation and extrusion in the roots and the stronger sodium re-transportation from shoot to roots in the salt-tolerant species play important roles in salt tolerance of *Malus* species.

1. Introduction

Under salt stress conditions, plants have envolved a series of mechanisms to survive in a way to tolerate the stress, such as salt dilution, salt excretion and salt exclusion (Levitt, 1980; Breckle, 1995). Further studies demonstrated that the salt tolerance greatly varies among the species and genotypes (Shen and Chen, 2001), suggesting the mechanisms vary among the species or genotypes. Significant efforts have been made to understand which mechanism(s) play more important roles in the specific species or genotypes of plants (Shen and Chen, 2001). For examples, some monocot plants such as wheat and maize, accumulate sodium in the roots contribute to their salt tolerance (Yeo et al., 1977; Lü and Wang, 1993; Yang et al., 2002; Läuchli et al., 2008). By contrast, in the dicot plants such as bean, sodium was mainly accumulated in the stem base (Jacoby, 1965), being similar to woody species. The significant capability differences among the plants differ the salt-tolerant from the salt-sensitive genotypes or species. Salt-tolerant fig cultivar intercepted more Na^+ in the roots and most transporting Na^+ of stem accumulated in the stem base, whereas the Na^+ content of stem apex was very low (Jiang et al., 1994). Much attentions were made on the sodium accumulation in the roots of barley (Nassery and Baker, 1972a) and the shoots of rice (Yeo and Flowers, 1982), as well as the sodium re-transportation and extrusion in barley (Nassery and Baker, 1972b), while relatively little information is available about the Na^+ exclusion in the *Malus* species.

Under salt stress, the roots and stem base serve as the main sodium exclusion localization, restricting upward transport of sodium and therefore playing important roles in salt tolerance in non-halophytes. However, the quantitative correlation between the capability of sodium accumulation in the sodium exclusion localization and the tolerance extent remains largely unknown. In previous study, we had determined the Na^+ exclusion capability in different salt-tolerant *Malus* species, and found that the main Na^+ exclusion localization was in the roots and stem base. The salt-tolerant *Malus* species accumulated more Na^+ in the old leaves, which could reduce the salt content of shoot through consenescence and falling off of old leaves (Yang et al., 2004). X-radial analysis of micro-region showed that the Na^+ of cytoplasm in leaf cells of salt-tolerant mangrove could be accumulated in the vacuole through compartmentalization or extruded to cell wall through plasmolemma. The Na^+ content of cytoplasm and chloroplast was reduced, which made the net photosynthetic rate of leaves descend less under salt stress (Li et al., 2008). Isotope was used in dynamic tracing of elements (Lin et al., 2017). In previous studies, the isotope tracer technology has been used to analyze the disciplinarian of water transport and nutrient element distribution in plants, and some results have been achieved (Amasheh et al., 2008; Luo et al., 2008; Zhang et al., 2008; Zheng et al., 2008). Metzner et al. (2010) applied stable isotope tracers of magnesium, potassium and calcium to study the transport mechanism of

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nutrient elements. Zhou et al. (2017) studied the uptake and distribution pattern of ^{15}N in two fruit species. However, the characteristics of sodium distribution in main Na^+ exclusion localization of *Malus* species are still unknown. In recent years, ^{22}Na isotopes have been used to study the transport mechanism of Na in animal cells (Yan et al., 2012) and the changes in air composition (Zhang et al., 2018). We use the ^{22}Na isotope tracer to treat roots and feed leaves to further understand the distribution disciplinarian of sodium in three *Malus* species, and roots-split experiment to analyze the re-transportation disciplinarian of sodium in three *Malus* species, and the results provide some theoretical foundations for salt-tolerant mechanism in woody plants.

2. Materials and methods

2.1. Plant culture

Salt-sensitive species *Malus baccata* (L.) Borkh., middle salt-tolerant species *Malus xiaojinensis* Cheng et Jiang and salt-tolerant species *Malus zumi* Mats of *Malus* species were used as experimental materials. After seeds germination, the *Malus* seedlings were cultivated in the Hoagland nutrient solution, which were ventilated continuously, and the Hoagland nutrient solution was replaced every 5 days. Then the *Malus* seedlings were transferred to a greenhouse with temperature at 22 °C–28 °C daily and at 15 °C–20 °C night in the same growth medium. Relative humidity range was at 45%–50% daytime and at 60%–70% night. Light intensity was about 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 12/12 with light/darkness period.

2.2. ^{22}Na treatment of roots

Malus seedlings with 10 cm in height were treated with 1 mM NaCl labeled with 1 μCi/mL of ^{22}Na for 10, 30, 120 and 240 min, respectively, and then they were put into unlabelled 1 mM NaCl for an extra 240 min. During the time course, the main roots, lateral roots, stem base phloem, stem base xylem, young leaves, mature leaves and old leaves were collected for standby. Each treatment was replicated three times.

2.3. ^{22}Na feeding of leaves

Comparably same site leaves of *Malus* seedlings were daubed for three times with 1 mM NaCl labeled with 1 μCi/mL of ^{22}Na and mixed with little Tween 80. Six hours later, the feeding leaves were cut off, and the other side leaves, stem base, main roots and lateral roots were taken for standby (considering this taking material time as 0 h), then at 4 h, 8 h and 12 h, the materials were taken the same way. Each treatment was replicated three times.

2.4. Roots-split experiment

Roots of *Malus* seedlings were separated into two similar parts and let them grow in two test tubes. One containing the Hoagland nutrient solution labeled with 0.6 μCi/mL of ^{22}Na, and the other containing the Hoagland nutrient solution without ^{22}Na. In addition, one containing the NaCl solution with the concentration of 50, 100 and 150 mM (NaCl solution was prepared with Hoagland nutrient solution) in one side of the test tube, and the other containing the Hoagland nutrient solution without NaCl. After 1 day, the roots and solution opposite the test tube and the stem base were taken for reserve. The whole recirculation amount of ^{22}Na was the sum of ^{22}Na in the roots and solution opposite the test tube and the stem base, and that of Na^+ was also the sum of Na^+ in the roots and solution opposite the test tube and the stem base. Each treatment was replicated three times.

2.5. Measurement of ^{22}Na radioactivity

Measurement of ^{22}Na radioactivity was referred to the method of Yu et al. (1997). The materials (main roots, lateral roots, stem base phloem, stem base xylem, young leaves, mature leaves and old leaves) was killed in oven at 110 °C for 10 min, dried at 70 °C–80 °C and then weighed. After crushing, 50 mg of dry materials was taken to measure the radioactivity on low background measuring device, and 1 mL of liquid sample was sampled and dripped into the measuring plate. After drying, the radioactivity was measured. Radioactivity was determined by a radioactivity meter Capintec CRC-25R (America). The radioactivity of measured data is corrected to the time of application when the efficiency and half-life are corrected.

3. Results

3.1. ^{22}Na dynamic accumulation in different organs of three Malus species

The three *Malus* species including two salt-tolerant species and one salt-sensitive species were treated with 1 mM NaCl labeled with 1 μCi/mL of ^{22}Na in the culture medium with time course 30–240 min. As shown in Fig. 1, the longer exposure to ^{22}Na, the more ^{22}Na accumulation in main and lateral roots for all the three *Malus* species tested. At the same time point within 240 min, significant difference was detected in ^{22}Na accumulation among the three *Malus* species with the accumulation capability order of *M. zumi* > *M. xiaojinensis* > *M. baccata*. Especially in lateral roots, this tendency was more obviously. However, after 240 min, ^{22}Na accumulation was significantly decreased in three *Malus* species, and both *M. zumi* and *M. xiaojinensis* were decreased more rapidly than *M. baccata* did, suggesting that the re-transportation capability of ^{22}Na in *M. zumi* and *M. xiaojinensis* is higher than that in *M. baccata* (Fig. 1).

Similar ^{22}Na accumulation tendency was found in the stem base

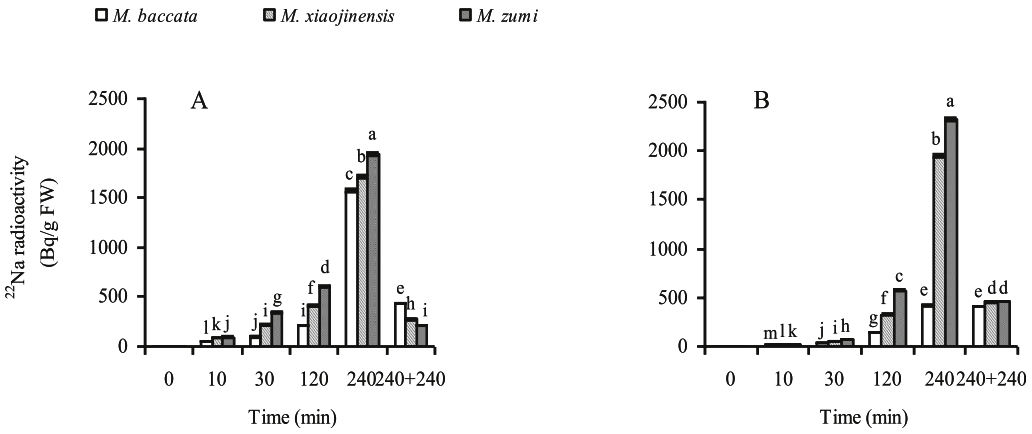

Fig. 1. ^{22}Na content in three *Malus* species. (A) main roots; (B) lateral roots. For the first 240 min, the seedlings were treated with 1 mM NaCl labeled with 1 μCi/mL of ^{22}Na, and then they were put into unlabelled 1 mM NaCl for an extra 240 min (the same as in Figs. 2, 3 and 11). Error bars represent SD (n = 3). The values followed by different letters are significantly different at the 0.05 probability level. The same as below.

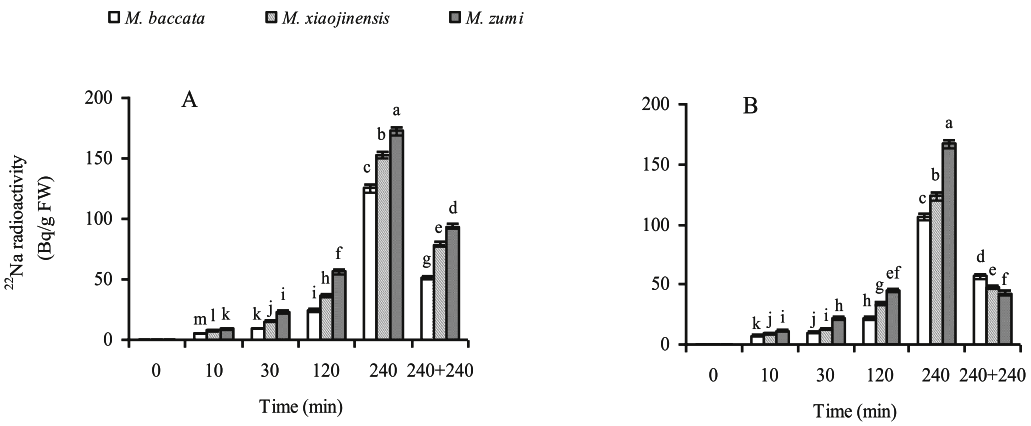

Fig. 2. ^{22}Na content in three *Malus* species. (A) stem base phloem; (B) stem base xylem. Error bars represent SD (n = 3).

phloem and xylem in three *Malus* species as shown in Fig. 2. After 240 min, although the ^{22}Na accumulation decelerated in three *Malus* species, significant difference was found in the decrease extent among the three *Malus* species. The difference was found between xylem and phloem. The ^{22}Na accumulation in the xylem decreased more rapidly in both *M. zumi* and *M. xiaojinensis* than that in *M. baccata*, while the salt-tolerant species of *M. zumi* and *M. xiaojinensis* retained more ^{22}Na than the salt-sensitive species of *M. baccata* in the phloem, suggesting that more ^{22}Na accumulation in the phloem of salt-tolerant species may account for the less toxicity of ^{22}Na to the seedlings and hence lead to more salt-tolerant.

Furthermore, ^{22}Na accumulation was compared in the young, mature and old leaves in three *Malus* species during the time course. Similar to in roots, the ^{22}Na accumulation in xylem and phloem was increased in three *Malus* species tested with 30–240 min and after 240 min the accumulation was decreased in the leaves of three *Malus* species. However, in all the specific time points, significant difference of the ^{22}Na accumulation was found in the young and mature leaves of the salt-tolerant species. The young and mature leaves in *M. zumi* could accumulate less ^{22}Na relative to the moderate salt-tolerant species of *M. xiaojinensis* and the salt-sensitive species of *M. baccata*. And the most significant differences were found at the time point of 240 min. By contrast, ^{22}Na accumulation of old leaves in the salt-tolerant species of *M. zumi* was significantly higher than that in the moderate salt-tolerant and the salt-sensitive species of *M. xiaojinensis* and *M. baccata* (Fig. 3). After 240 min, although the ^{22}Na content was more dramatically decreased in the young and mature leaves of three *Malus* species, the salt-tolerant species of *M. zumi* was accumulated the lowest level of ^{22}Na, while the salt-sensitive species of *M. baccata* hold the highest level of ^{22}Na. However, relative to the moderate salt-tolerant and salt-sensitive species, the ^{22}Na content of old leaves in the salt-tolerant species of *M. zumi* was significantly higher (Fig. 3). All together, these data indicated that the lower sodium accumulation in the young and mature leaves and higher sodium accumulation in the old leaves could confer the salt tolerance of the species of *M. zumi*.

3.2. Effect of ^{22}Na feeding in leaves on ^{22}Na absorption and re-transportation of three Malus species

^{22}Na feeding treatment of leaves (Fig. 4) showed ^{22}Na absorption and re-transportation in leaves. The whole amount of ^{22}Na absorption in *M. zumi* and *M. xiaojinensis* was higher than that in *M. baccata.* With the treatment time increasing, ^{22}Na contents of leaves in three *Malus* species were all quickly decreased. ^{22}Na content of leaves in *M. zumi* was reduced the most and preserved the least level. ^{22}Na content of stem base in *M. baccata* was continuously decreased, and a little increasing was found during 4 h and then decreased in *M. zumi* and *M. xiaojinensis*. At the same treatment time, ^{22}Na content of stem base in *M. zumi* and *M. xiaojinensis* was higher than that in *M. baccata*. The ^{22}Na content of main roots and lateral roots in *M. zumi* and *M. xiaojinensis* was obviously increased. The ^{22}Na content of *M. xiaojinensis* in the main roots was obviously higher than that in the lateral roots, however, that of *M. zumi* in the main roots was similar with that in the lateral roots at first and then that of *M. zumi* in the lateral roots was obviously higher than that in the main roots at last, which showed an obviously ^{22}Na re-transportation from main roots to lateral roots. ^{22}Na content of main roots and lateral roots in *M. baccata* was similar and increased less compared with that in *M. zumi* and *M. xiaojinensis*.

3.3. ^{22}Na extrusion of roots in three Malus species

As shown in Fig. 5, the quantitative ^{22}Na extrusion capabilities were compared among the salt-tolerant, moderate salt-tolerant and salt-sensitive *Malus* species. The salt-sensitive species of *M. baccata*

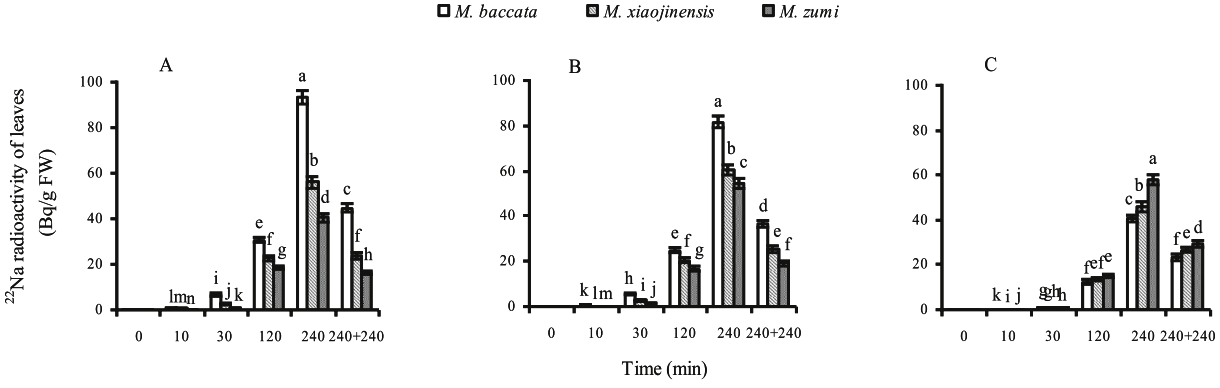

Fig. 3. ^{22}Na content in three *Malus* species. (A) young leaves; (B) mature leaves; (C) old leaves. Error bars represent SD (n = 3).

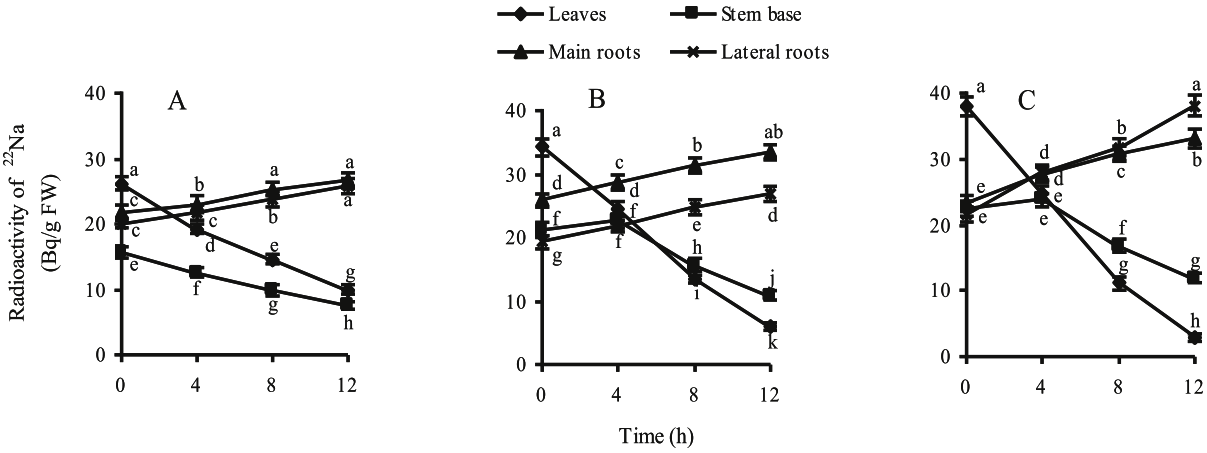

Fig. 4. ^{22}Na absorption and re-transportation in three *Malus* species. (A) *M. baccata*; (B) *M. xiaojinensis*; (C) *M. zumi*. Error bars represent SD (n = 3).

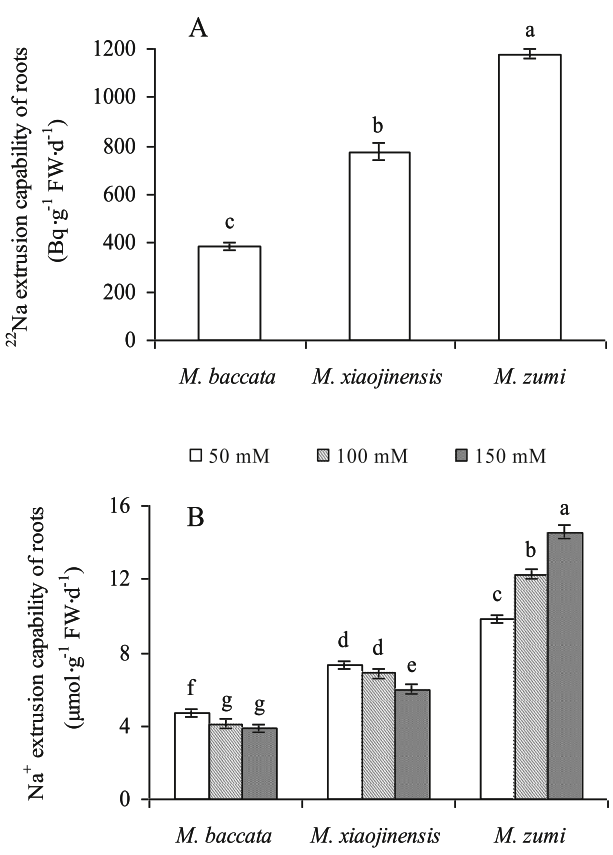

Fig. 5. Na extrusion capability of roots in three *Malus* species. (A) ^{22}Na treatment. (B) NaCl treatment. Error bars represent SD (n = 3).

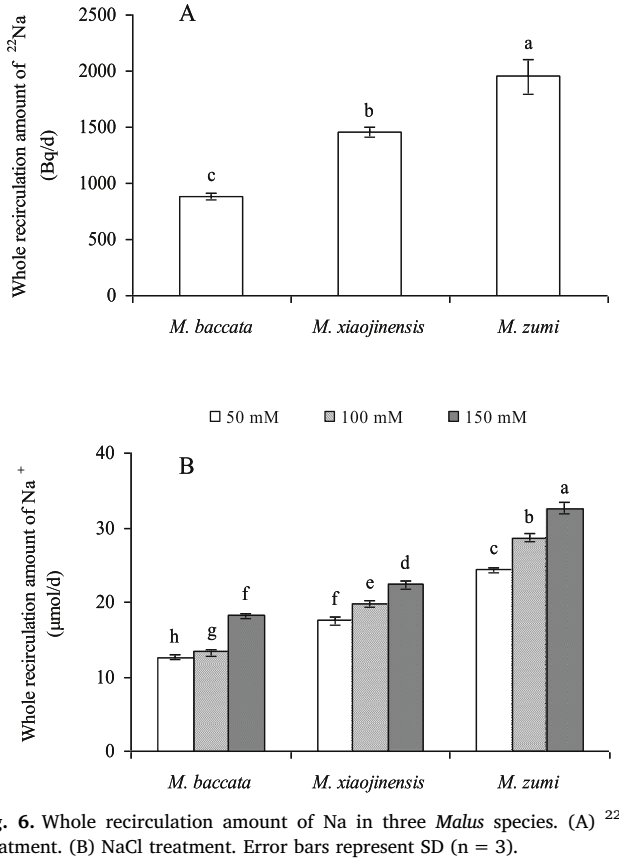

Fig. 6. Whole recirculation amount of Na in three *Malus* species. (A) ^{22}Na treatment. (B) NaCl treatment. Error bars represent SD (n = 3).

performed the lowest ^{22}Na extrusion capability compared to the other two species. By contrast, more than doubled or tripled ^{22}Na extrusion capability was found in the moderate salt-tolerant species of *M. xiaojinensis* and the salt-tolerant species of *M. zumi*, respectively, suggesting that the ^{22}Na extrusion capability of roots significantly contributes to salt tolerance. With the increase of NaCl concentration, the Na^+ extrusion capability of roots in *M. zumi* was obviously increased, while that in *M. baccata* and *M. xiaojinensis* was obviously decreased under NaCl treatment of 100 and 150 mM, respectively. The average Na^+ extrusion capabilities of roots in *M. zumi*, *M. xiaojinensis* and *M. baccata* were 12.21, 6.72 and 4.26 μmol·g^{-1} FW·d^{-1}, respectively, and that in *M. xiaojinensis* was 1.58 folds that in *M. baccata*, and that in *M. zumi* was 2.87 folds that in *M. baccata*.

3.4. Recirculation of ^{22}Na and salt tolerance in three Malus species

Roots-split experiment showed the whole recirculation amount of ^{22}Na (Fig. 6) and the ^{22}Na extrusion percent of whole recirculation of roots (Fig. 7). Significant differences were detected in the whole recirculation amount of ^{22}Na among the three *Malus* species with the recirculation capability order of *M. zumi* > *M. xiaojinensis* > *M. baccata* (Fig. 6). The whole recirculation amount of ^{22}Na in *M. xiaojinensis* was 1.64 folds that in *M. baccata*, and that in *M. zumi* was 2.20 folds that in *M. baccata*, suggesting that the recirculation capability of sodium in salt-tolerant species significantly contributes to salt tolerance. With the increase of NaCl concentration, the whole recirculation amount of Na^+ in three *Malus* species showed an increasing trend, and the largest increase was in *M. zumi*. The average whole recirculation amount of Na^+ in *M. zumi*, *M. xiaojinensis* and *M. baccata* were 28.48,

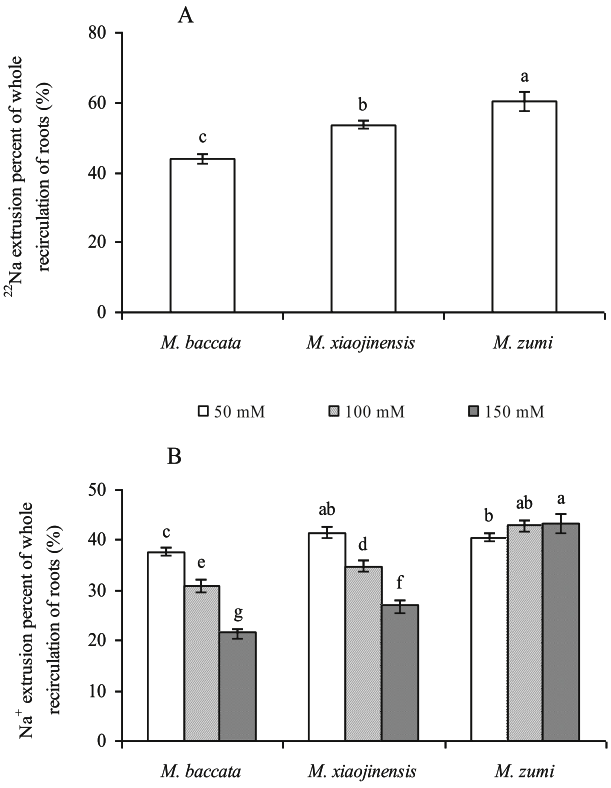

Fig. 7. Na extrusion percent of whole recirculation of roots in three *Malus* species. (A) ^{22}Na treatment. (B) NaCl treatment. Error bars represent SD (n = 3).

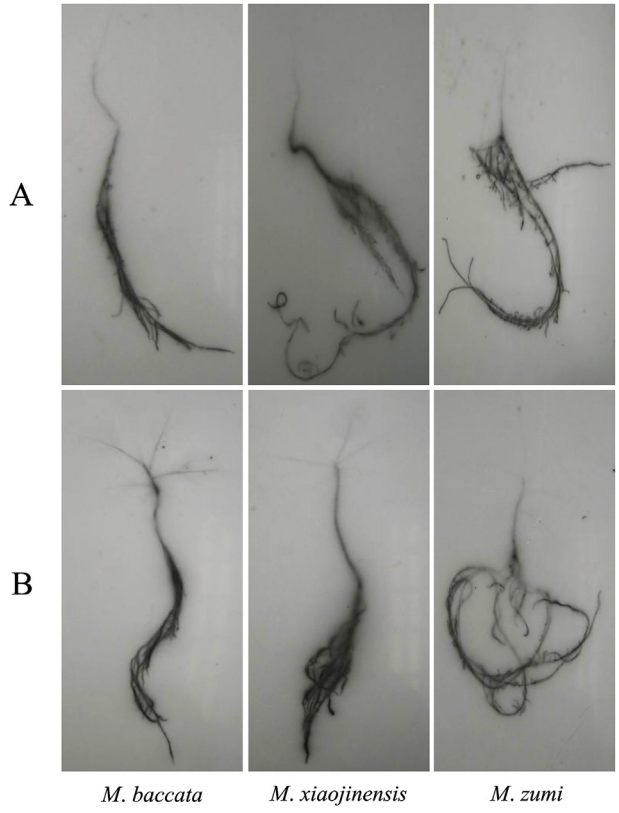

Fig. 8. Autoradiograph of three *Malus* species grown under 1 mM NaCl labeled with 1 μCi/mL of ^{22}Na. (A) Treated for 10 min; (B) Treated for 30 min.

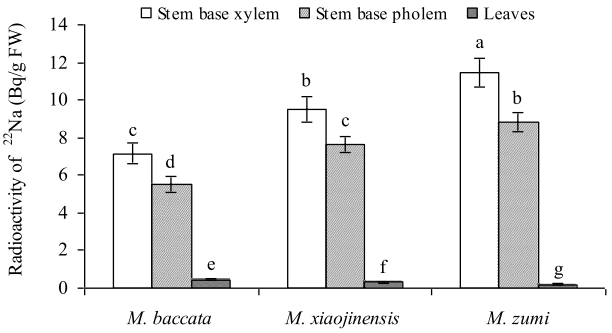

Fig. 9. Comparison of ^{22}Na content of leaves, stem base phloem and xylem in three *Malus* species. Error bars represent SD (n = 3).

19.86 and 14.71 μmol/d, respectively. The whole recirculation amount of Na in three *Malus* species showed similar changes under the treatment of isotope ^{22}Na and NaCl, however, that in the salt-tolerant species was obviously higher than that in the salt-sensitive one.

Fig. 7 showed the ^{22}Na extrusion percent of whole recirculation of roots in three *Malus* species the same tendency as Fig. 6. Significant differences were detected in the ^{22}Na extrusion percent of whole recirculation of roots among the three *Malus* species with the order of *M. zumi* (60.49) > *M. xiaojinensis* (53.60) > *M. baccata* (43.81), suggesting that the amount of sodium extrusion is an important trait of salt exclusion in salt-tolerant *Malus* species. With the increase of NaCl concentration, the Na^+ extrusion percent of whole recirculation of roots in *M. baccata* and *M. xiaojinensis* was obviously decreased, while that in *M. zumi* was obviously increased. The average Na^+ extrusion percent of whole recirculation of roots in three *Malus* species was as follows: *M. zumi* (42.23) > *M. xiaojinensis* (34.41) > *M. baccata* (30.03). The Na extrusion percent of whole recirculation of roots in three *Malus* species under ^{22}Na treatment was obviously higher than that under NaCl treatment, indicating that the increase of Na concentration was a major factor in reducing the Na extrusion percent of whole recirculation.

4. Discussion

Reed plants might be provided with a mechanism for transferring Na^+ from the xylem to the phloem within the shoot base region (Matsushita and Matoh, 1991) and the transfer-cells of *Phaseolus coccineus* had been implicated in the process of transferring Na^+ from the xylem to the phloem (Kramer et al., 1977). Seeing from Fig. 8, in the ^{22}Na treatment of roots, the ^{22}Na of *M. baccata* and *M. xiaojinensis* had reached the stem apex and that of *M. zumi* remained mainly in the roots and the stem base at 10 min. The ^{22}Na content of leaves in three *Malus* species was very low at 10 min (Fig. 9), meanwhile, that of stem base xylem and phloem was very high. The higher ^{22}Na content of stem base phloem could not transport from the shoot leaves, however, it could be transported from the stem base xylem, indicating that horizontal transport of ^{22}Na in stem base plays an important role in sodium retransportation of salt-tolerant *Malus* species. At 30 min (Fig. 8), the ^{22}Na of *M. baccata* had reached the whole leaves and that of *M. xiaojinensis* reached the part leaves, however, that of *M. zumi* reached only the leafstalks. It showed that the salt-tolerant *Malus* species had greater sodium exclusion capability to restrict the sodium from transporting to shoot and was more salt-tolerant.

The ^{22}Na transport rate of stem base phloem in three *Malus* species was the highest at 10 min, and then decreased. This might be because that the ^{22}Na accumulation in the phloem resulted in the reduction of ^{22}Na transport rate of stem base phloem. The ^{22}Na transport rate of stem base phloem was reduced to the lowest at 120 min and then increased quickly. The ^{22}Na recirculation from shoot leaves to stem base phloem resulted in the increasing of ^{22}Na transport rate in stem base phloem (Fig. 10). At the same time of ^{22}Na treatment in the roots, the

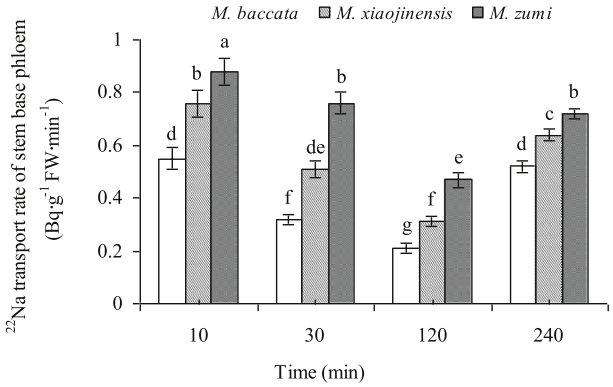

Fig. 10. Comparison of ^{22}Na transport rate of stem base phloem in three *Malus* species. Error bars represent SD (n = 3).

^{22}Na transport rate of stem base phloem in *M. zumi* was obviously higher than that in *M. xiaojinensis*. In *M. xiaojinensis* it is obviously higher than in *M. baccata*, indicating that the ^{22}Na transport rate of stem base phloem is positively correlated with the salt tolerance of *Malus* species.

Wang et al. (2006) reported that *Thellungiella halophila* secures low net Na accumulation under low concentration saline conditions, while *Arabidopsis thaliana* accumulates more Na than *Thellungiella halophila*. Figs. 1 and 2 showed that the three *Malus* species all accumulated ^{22}Na in the main roots, lateral roots, stem base phloem and xylem. The salt-tolerant species of *M. zumi* accumulated the most ^{22}Na which was similar to *Arabidopsis thaliana*, and the salt-sensitive species of *M. baccata* accumulated the least ^{22}Na which was similar to *Thellungiella halophila*. Since *Malus* and *Arabidopsis thaliana* are all non-halophytes, they may have some similarities in sodium exclusion mechanisms. In addition, whether volume and weight, the stem base phloem and xylem occupied the relatively little proportion in contrast with the main roots and lateral roots. The whole ^{22}Na contents of main roots, lateral roots, stem base phloem and xylem were showed in Fig. 11. We could see that the ^{22}Na accumulation in the main roots, lateral roots, stem base phloem and xylem was similar with Figs. 1 and 2, but the whole accumulated ^{22}Na of roots (especially lateral roots) was obviously higher than that of stem base. Since the downward Na^+ movement must occur in the phloem (Jacoby, 1979; Lessani and Marschner, 1987), as one of main sodium exclusion localization, the stem base accumulated limited sodium and it might play more important role in re-transporting sodium from stem base phloem to roots. This re-transportion of sodium from stem base phloem to roots could enable the stem base to continue intercepting sodium from upward movement, and the salt exclusion properties are presented.

Sodium was gradually accumulated in the old leaves of rice or *Malus* to protect the young leaves (Yeo and Flowers, 1982; Yang et al., 2004). The old leaves could die as a result of a fast increase of the salt concentrations in the cell wall or cytoplasm when vacuoles could no longer sequester incoming salts (Munns, 1993). From Fig. 3, the salt-tolerant species of *M. zumi* preserved the lowest ^{22}Na content in the young leaves and mature leaves and the highest ^{22}Na content in the old leaves. We think that more sodium accumulated in the old leaves is one of the salt tolerant mechanism in salt-tolerant *Malus* species.

Yu et al. (2003) reported that the salt-tolerant *Glycine soja* BB52 absorbed more ^{22}Na and transported more ^{22}Na to roots than the salt-sensitive *Glycine soja* N23232 did after 10 h ^{22}Na feeding of leaves. In this ^{22}Na feeding experiment of leaves, the salt-tolerant *Malus* species of *M. zumi* could transport more ^{22}Na to roots than the salt-sensitive one of *M. baccata* and had less ^{22}Na level in the leaves. It indicated that the salt-tolerant *Malus* species had greater re-transportation capability than the salt-sensitive one and was more salt-tolerant. In roots-split experiment, Jacoby (1979) indicated that about half of ^{22}Na of whole recirculation in *Phaseolus vulgaris* lost to the medium. From Fig. 7, the salt-sensitive *Malus* species of *M. baccata* lost about 43% of ^{22}Na to the

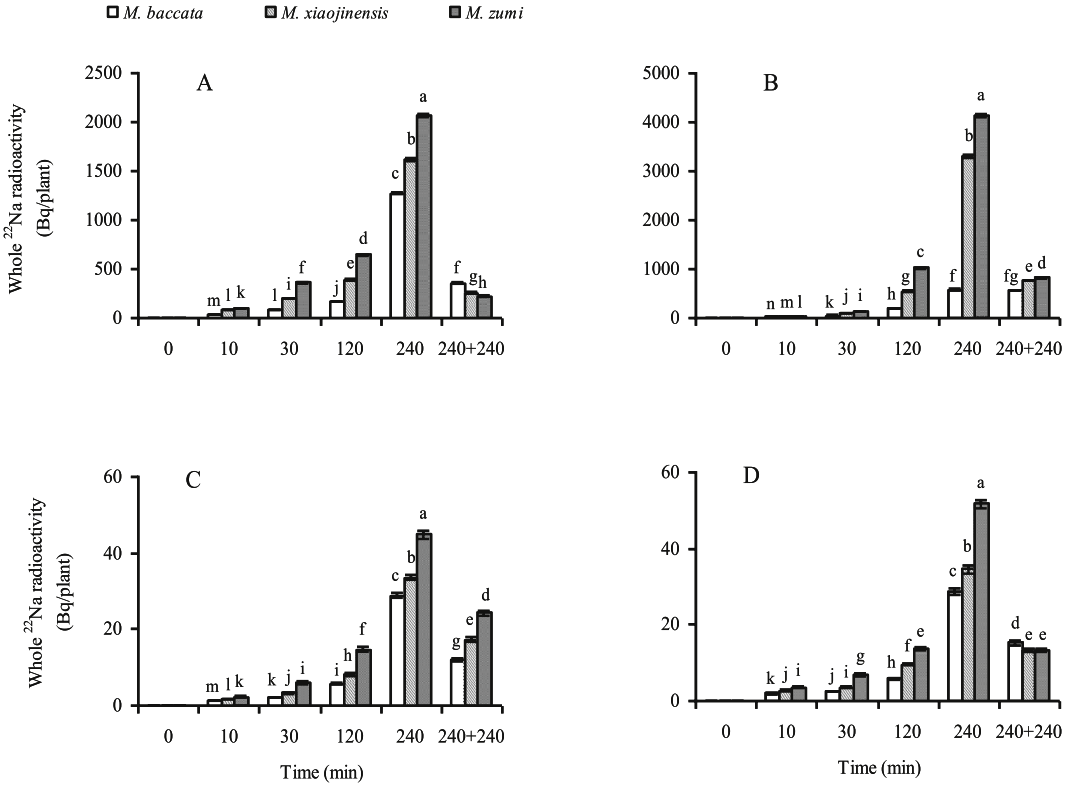

Fig. 11. Whole ^{22}Na content of different parts in three *Malus* species. (A) main roots; (B) lateral roots; (C) stem base phloem; (D) stem base xylem. Error bars represent SD (n = 3).

medium, while the salt-tolerant *Malus* species of *M. xiaojinensis* and *M. zumi* lost about 53% and 60% of ^{22}Na to the medium respectively. Meanwhile, the ^{22}Na extrusion capability of roots and the whole recirculation amount of ^{22}Na in *M. zumi* was the highest in three *Malus* species, which made it more salt-tolerant.

Through accumulating Na^+ in the roots (Yang et al., 2002; Läuchli et al., 2008) or in the stem base (Jacoby, 1965), the salt-tolerant plants have lower Na^+ content in the shoot, which makes leaves have relatively higher net photosynthetic rate under salt stress (Li et al., 2008). On a whole, the salt-tolerant *Malus* species of *M. zumi* and *M. xiaojinensis* accumulated more ^{22}Na in the roots (especially lateral roots) than the salt-sensitive one of *M. baccata* did. More sodium accumulation in the main Na^+-exclusion localization (roots and stem base) and higher stem base phloem sodium transport rate of salt-tolerant *Malus* species could restrict upward transport of sodium. The salt-tolerant *Malus* species preserved lower ^{22}Na content in the young leaves and mature leaves and higher ^{22}Na content in the old leaves in contrast with the salt-sensitive one did. It also represented a higher sodium re-transportation capability of leaves in salt-tolerant *Malus* species. In addition, higher sodium extrusion capability of roots and whole recirculation amount of sodium in salt-tolerant *Malus* species also efficiently reduced the sodium content of leaves.

Contribution

The first author Yang Hong-Bing did the experiment and wrote the manuscript.

Yu Yan-Chong and Wang Yi cultivated the plants and treated.

Xu Xue-Feng analyzed the experimental data.

The corresponding author Han Zhen-Hai designed the experiment and revised the manuscript.

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