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Mycorrhizal symbiosis enhances tolerance to NaCl stress through selective absorption but not selective transport of K^+ over Na^+ in trifoliate orange

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ABSTRACT

Selectivity of potassium ion (K^+) over sodium ion (Na^+) is essential to understand plant's tolerance to salt stress, whereas information is limited whether arbuscular mycorrhizal fungi (AMF) increase selective absorption or transport of K^+ over Na^+ (SA_{K^+/Na^+} or ST_{K^+/Na^+}) in host plants. The 61-d-old trifoliate orange (*Poncirus trifoliata*) inoculated with or without an AM fungus *Funneliformis mosseae* was subjected to 45-day 100 mM NaCl stress. The AMF inoculation significantly increased plant growth (height, leaf number, stem diameter and biomass production), leaf relative water content (LRWC), and tissue K^+ absorption but decreased Na^+ absorption under no-NaCl or NaCl stress. Mycorrhization also significantly increased ratio of K^+/Na^+ in leaf, root and total plant under no-NaCl and NaCl stress. Meanwhile mycorrhizal seedlings showed higher SA_{K^+/Na^+} under no-NaCl and NaCl stress, and higher ST_{K^+/Na^+} under no-NaCl stress but lower ST_{K^+/Na^+} under NaCl stress. In addition, SA_{K^+/Na^+} significantly positively correlated with LRWC and almost all tested growth traits, whilst ST_{K^+/Na^+} only with leaf number and root biomass. These results suggested that it was the mycorrhizal-mediated increase of SA_{K^+/Na^+}, rather than ST_{K^+/Na^+}, under NaCl stress, that could enhance the plant's tolerance to NaCl stress, thus conferring a greater LRWC and plant growth in mycorrhizal citrus seedlings.

1. Introduction

Soil salinity, a major abiotic stress under the excess of sodium chloride (Na^+Cl^-), affects ~20% of the world's 230 million irrigated agricultural lands (see Munns and Tester, 2008). High Na^+ concentrations (>40 mM) have detrimental effects on crop growth mainly due to hyperosmotic stress (water deficit under strongly negative water potential), excessive absorption and ion imbalance (see Munns and Tester, 2008), particularly in semi-arid and arid regions where Na^+ is up to 50–100 mM in soil. Under salt stress, potassium ion (K^+) and sodium ion (Na^+) are the major ions contributing to osmotic pressure and ionic strength (Serrano and Rodriguez-Navarro, 2001). As K^+ and Na^+ have similar ionic radius (0.331 versus 0.358 nm), they may enter cytoplasm through the same ion channels, especially these are non-selective cation channels (Schaschtman and Liu, 1999). Salt-tolerant plants generally maintain intracellular K^+ and Na^+ homeostasis, which is important for the maintenance of plant growth and water status (Zhu, 2003). Therefore, the selectivity of K^+ over Na^+ is essential to understand the tolerance of plants to salt stress.

Arbuscular mycorrhiza (AM), a mutually symbiotic association between AM fungi (AMF) and roots of ~80% higher plants, enhances plant growth and tolerance to salt stress (Brundrett, 2009; Evelin et al., 2009). Complex mechanisms could explain mycorrhizal-enhanced salt tolerance: (1) greater nutrient and water absorption (Asghari, 2008; Sheng et al., 2008; Wu et al., 2009), (2) greater accumulation of soluble sugars, carbohydrates or proline (Feng et al., 2002; Zou and Wu, 2011), (3) greater antioxidant enzyme activities (He et al., 2007; Wu et al., 2010b; Zou and Wu, 2011), (4) better root system architecture (Echeverria et al., 2008; Wu et al., 2010a), and (5) ion balance (Giri and Mukerji, 2004; Giri et al., 2007; Wu et al., 2010a; Mardukhi et al., 2011). Among these mechanisms, an increased recent interest is how the ion balance could contribute plant tolerance to salt stress. For instance, a decrease of Na^+ absorption with a concomitant increase of Mg^{2+} absorption in two *Sesbania* species and an inhibition of Na^+ rather than

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Cl^- absorption in wheat contributed to salt tolerance (Giri and Mukerji, 2004; Mardukhi et al., 2011). Under salt stress both Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios were increased in the AM fungus *Paraglomus occultum* associated *Citrus tangerine*, whilst Ca^{2+}/Na^+ was decreased but Mg^{2+}/Na^{2+} did not change at all in the AM fungus *Funneliformis mosseae* associated *C. tangerine* (Wu et al., 2010a). On the other hand, an elevated K^+/Na^+ ratio also contributed to salt tolerance in the AM fungus *Glomus fasciculatum* associated *Acacia nilotica* plants (Giri et al., 2007). In contrast, under salt stress K^+/Na^+ ratio was decreased in *C. tangerine* seedlings associated with *F. mosseae* or *P. occultum* (Wu et al., 2010a). In general, under NaCl stress, ion selectivity is characterized by selective absorption (SA) and selective transport (ST) of K^+ over Na^+ (Roux et al., 2011). Herein, the SA_{K^+/Na^+} value indicates the root ability to absorb K^+ over Na^+, while the ST_{K^+/Na^+} signifies the root ability to transport K^+ over Na^+ from root to leaf (Wu and Wang, 2012). However, it is unclear whether the ionic balance of mycorrhizal plants under salt stress is ascribed to SA_{K^+/Na^+} or ST_{K^+/Na^+}.

Trifoliate orange (*Poncirus trifoliata* L. Raf.) is a close relative to *Citrus* and the most demanding citrus rootstock for the world's largest citrus plantation (2.4 million hectares in 2012) in China. The growth of trifoliate orange strongly depends on AM symbiosis but this plant is sensitive to NaCl stress (Wu et al., 2009; Sykes, 2011). Recently salt stress derived from irrigation particularly in the citrus orchards and rootstock nurseries of coastal regions in China has become an increasing concern. Our previous studies have shown that AMF colonization alleviated NaCl stress through greater antioxidant defense systems (catalase, ascorbate, and glutathione) and better root system architecture (total root length, root projected area, and root surface area) (Wu et al., 2010a,b; Zou and Wu, 2011). However, information about the roles of AMF in the selectivity of K^+ over Na^+ is limited under salt stress. The objectives of this study were therefore to address: (1) If AMF could enhance the selectivity of K^+ over Na^+ under NaCl stress, and (2) If the enhanced selectivity of K^+ over Na^+ could correlate with both the leaf relative water content (LRWC) and plant growth in the trifoliate orange. The expected outcomes might contribute to a better understanding of roles of mycorrhizal fungi in the plant ion selectivity and tolerance to NaCl stress.

2. Materials and methods

2.1. Experimental design

There were four treatments: (1) non-mycorrhizal and no-NaCl stress control ($AM^-/NaCl^-$), (2) mycorrhizal and no-NaCl stress ($AM^+/NaCl^-$), (3) non-mycorrhizal and 100 mM NaCl stress ($AM^-/NaCl^+$), and (4) mycorrhizal and 100 mM NaCl stress ($AM^+/NaCl^+$). With a completely randomized arrangement, each treatment had four replicates or pots for a total of 16 pots. Plants in the pots were grown in a campus glasshouse at the Yangtze University, Jingzhou, Hubei, China (see below for growth conditions).

2.2. Fungal inoculum

The AMF *F. mosseae* (T. H. Nicolson & Gerd.) C. Walker & A. Schüβler (BGC XJ02, commercially from Beijing Academy of Agriculture and Forestry Sciences, China) was used since trifoliate orange had greater salt tolerance with this species than a range of other AM species (Zou and Wu, 2011). The inoculums, propagated from spores of *F. mosseae* growing with 16-week-old *Sorghum vulgare* in pots (zeolite/river sand = 1/1, v/v; pH 8.8, 3.66 g organic matter, 0.96 mg Olsen-P and 720 mg potassium kg^{-1} substrate), were a mixture of root segments, spores (30 g^{-1} substrate), extraradical hyphae and substrates.

2.3. Plant growth

Seeds of trifoliate orange were surface-sterilized for 10 min with 70% ethanol and sown in plastic pots (15 cm diameter × 16.5 cm height) containing 2.8 kg autoclaved growth media (soil/vermiculte/sphagnum = 5/1/1 (v/v/v); pH 6.3, 9.8 g organic matter, 121.4 mg available nitrogen, 17.71 mg Olsen phosphorus and 52.1 mg available potassium per kg growth media). The soil is classified as ferralsols (FAO soil classification system). Fifteen grams autoclaved or non-autoclaved *F. mosseae* inoculums were well mixed with growth substrates. To minimise differences in other microbial communities, 2 mL inoculum filtrates (25 μm filters) were added to each pot for the AMF treatments. Plants were grown in a plastic greenhouse from March 27 to July 11, 2010 under 576–869 $\mu mol\ m^{-2}\ s^{-1}$ photo flux density, 18–35/14–30 °C (day/night) and 70–95% relative humidity. No external nutrients were supplied during the experiment.

Detrimental effects on plant growth are generally observed if Na^+ concentrations are over 40 mM, particularly in semi-arid and arid regions where Na^+ is up to 50–100 mM in soil (see Munns and Tester, 2008). Our preliminary study also showed a significant restriction of AM colonization in two-month-old trifoliate orange after 34 days of 100 mM NaCl stress (Zou et al., 2013). In parallel, in this study both AMF and non-AMF seedlings were subjected to 0 or 100 mM NaCl stress for 45 days after 61 days of AMF inoculation. To avoid osmotic shock and maintain salt strength during these 45 days of stress, seedlings were firstly gradually salinized by the gradient of 25 mM NaCl per day for 4 consecutive days to achieve 100 mM NaCl and then irrigated by 100 mM NaCl once every other 5 days till harvest.

2.4. Variable analysis

Fresh fine root segments (1 cm) were cleared with 10% KOH, acidified with 1% HCl for 15 min, stained with 0.05% trypan blue in lactophenol (w/v) for 5 min, and stored in lactophenol (Phillips and Hayman, 1970). Percentage of root mycorrhizal colonization was the ratio of infected root length against total observed root length.

Oven-dried (75 °C for 48 h) shoots, roots or growth media (randomized mixture) were grounded (0.9 mm) for Na^+ and K^+ determinations with an Atomic Absorption Spectrometer according to the company manual (AI 1200, Aurora Instruments Limited, Canada). The multiplication of tissue $[K^+]$ or $[Na^+]$ concentration by its biomass equalled to the amount of tissue K^+ or Na^+. Selective absorption (SA) and selective transport (ST) of K^+ over Na^+ were expressed as follows (Wu and Wang, 2012):

$$SA_{K^+/Na^+} = \frac{[K^+]/[Na^+]\ \text{in total plant}}{[K^+]/[Na^+]\ \text{in growth media}} \quad \text{and}$$

$$ST_{K^+/Na^+} = \frac{[K^+]/[Na^+]\ \text{in leaf}}{[K^+]/[Na^+]\ \text{in root}}$$

where $[K^+]$ or $[Na^+]$ in the total plant is the sum of $[K^+]$ or $[Na^+]$ in leaf and root.

Ratio of K^+/Na^+ was calculated in terms of the K^+ and Na^+ content.

Leaf relative water content (LRWC) was determined with the fourth fully expanded top leaf. Fresh leaves were immediately weighed before being immersed in distilled water for 24 h and then weighed again as saturated weight, and finally oven dried at 80 °C to constant weight. Calculation of LRWC was as follows (Bajji et al., 2001):

$$LRWC\,(\%) = \frac{\text{fresh weight} - \text{dry weight}}{\text{saturated weight} - \text{dry weight}} \times 100$$

2.5. Statistical analysis

Data (means ± SE, $n = 4$) were subjected to two-way ANOVA with the SAS 8.1 for Windows 7. Percentage of mycorrhizal colonization and LRWC were arcsine-transformed prior to statistical analysis. Duncan's multiple range test ($P < 0.05$) was used to compare differences in means within treatments. The Correlation (CORR) Procedure was used to analyze the Pearson correlation coefficient ($n = 16$).

3. Results

3.1. Root mycorrhizal colonization and plant growth characteristics

Root mycorrhizal colonization varied from 32% to 40% and was significantly decreased by the 100 mM NaCl stress (Table 1). Plant heights, stem diameters, leaf numbers and tissue biomass production were significantly higher in mycorrhizal than non-mycorrhizal plants grown in no-NaCl and NaCl stress (Table 1). In contrast, NaCl stress significantly decreased plant height, leaf numbers and tissue biomass production in non-mycorrhizal plants, but not in mycorrhizal plants.

3.2. Leaf relative water content (LRWC)

Compared with the non-AMF treatment, the AMF inoculation significantly increased LRWC, irrespectively of soil NaCl status (Fig. 1). Although the NaCl stress decreased LRWC, the differences were not significant, irrespectively of AM inoculation.

3.3. Na^+ and K^+ concentrations in leaf, root, and growth media

Soil salinity markedly increased Na^+ concentrations in leaf, root and growth media, regardless of the citrus seedlings inoculated with or without *F. mosseae*, and also significantly increased K^+ concentrations in the AMF root and non-AMF growth media (Fig. 2). Under no-NaCl stress, the AMF colonization significantly decreased Na^+ concentration only in leaf but not in root and growth media. On the other hand, the AMF colonization significantly increased K^+ concentration in leaf and root but decreased that in growth media. Under the NaCl stress, AMF inoculated treatment decreased Na^+ concentration in leaf and root by 31% and 50%, but increased K^+ concentration in leaf and root by 13% and 60%, respectively. In addition, AMs significantly decreased K^+ concentration in growth media by 53% under the NaCl stress.

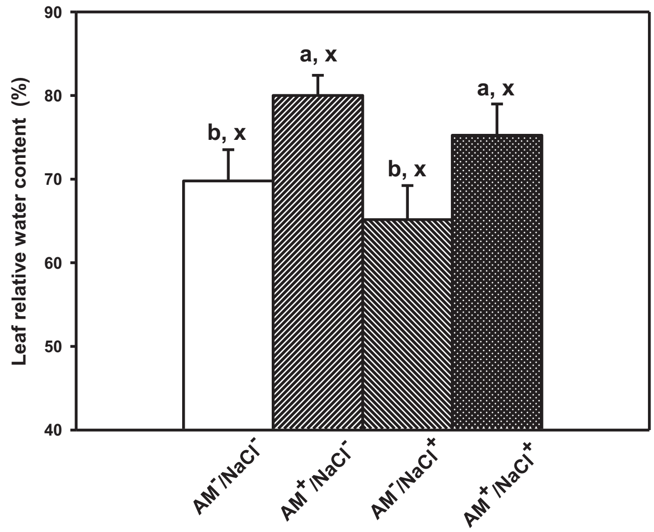

Fig. 1. Effects of *Funneliformis mosseae* inoculation and NaCl stress on leaf relative water content (LRWC) of four-month-old trifoliate orange (*Poncirus trfioliata*) seedlings. Data (means ± SE, $n = 4$) followed by different letters above the bars between AMF treatments under a given salt stress (a, b) or between salt treatments under a given AMF inoculation (x, y). Abbreviations: $AM^-/NaCl^-$, non-mycorrhizal and no-NaCl stress control; $AM^+/NaCl^-$, mycorrhizal and no-NaCl stress; $AM^-/NaCl^+$, non-mycorrhizal and 100 mM NaCl stress; and $AM^+/NaCl^+$, mycorrhizal and 100 mM NaCl stress.

3.4. Na^+ and K^+ contents in leaf, root, and total plant

Under no-NaCl stress, AMF colonization did not affect Na^+ contents in leaf, root, and total plant (Fig. 3). Under NaCl stress, compared to non-AMF treatment, effects of AMF colonization on Na^+ contents varied with plant tissues: significantly higher in leaf, significantly lower in root, but no differences in total plant (Fig. 3). Inoculation with AMF significantly increased K^+ contents in leaf, root, and total plant, irrespectively of soil salt status (Fig. 3).

3.5. Ratio of K^+/Na^+ in leaf, root, and total plant

Ratio of K^+/Na^+ in leaf, root, and total plant was significantly lower under NaCl stress than under no-NaCl stress, no matter whether the plants were inoculated with AMF (Fig. 4). Compared with non-mycorrhizal seedlings, mycorrhizal seedlings presented significantly higher ratio of K^+/Na^+ in leaf, root, and total plant, irrespectively of soil salt status (Fig. 4).

Table 1
Effects of NaCl stress and AMF (*Funneliformis mosseae*) inoculation on plant height, stem diameter, leaf numbers and tissue biomass production of four-month-old trifoliate orange (*Poncirus trfioliata*) seedlings.

Treatment	Mycorrhizal colonization (%)	Plant height (cm)	Stem diameter (cm)	Leaf number per plant	Dry weight (g)		
					Shoot	Root	Total
$AM^-/NaCl^-$	0.0 ± 0.0b,x	13.4 ± 1.1b,x	0.244 ± 0.008b,x	12.5 ± 0.8b,x	0.70 ± 0.02b,x	0.65 ± 0.02b,x	1.35 ± 0.03b,x
$AM^+/NaCl^-$	40.0 ± 2.7a,x	18.0 ± 1.3a,x	0.270 ± 0.004a,x	16.4 ± 0.7a,x	1.00 ± 0.08a,x	0.71 ± 0.05a,x	1.71 ± 0.13a,x
$AM^-/NaCl^+$	0.0 ± 0.0b,x	11.0 ± 1.1b,y	0.235 ± 0.012b,x	10.8 ± 1.2b,x	0.54 ± 0.07b,y	0.43 ± 0.03b,y	0.98 ± 0.07b,y
$AM^+/NaCl^+$	31.8 ± 2.3a,y	17.5 ± 1.2a,x	0.260 ± 0.008a,x	14.5 ± 0.9a,y	1.11 ± 0.10a,x	0.57 ± 0.03a,y	1.68 ± 0.12a,x
ANOVA							
NaCl stress	**	*	*	**	NS	**	**
AMF	**	**	**	**	**	**	**
NaCl stress × AMF	**	NS	NS	NS	**	*	**

Data (means ± SE, $n = 4$) followed by different letters indicate significant differences ($P < 0.05$) between AMF treatments under a given salt stress (a, b) or between salt treatments under a given AMF inoculation (x, y). NS-not significant. Abbreviations: $AM^-/NaCl^-$, non-mycorrhizal and no-NaCl stress control; $AM^+/NaCl^-$, mycorrhizal and no-NaCl stress; $AM^-/NaCl^+$, non-mycorrhizal and 100 mM NaCl stress; and $AM^+/NaCl^+$, mycorrhizal and 100 mM NaCl stress.

* $P < 0.05$.
** $P < 0.01$.

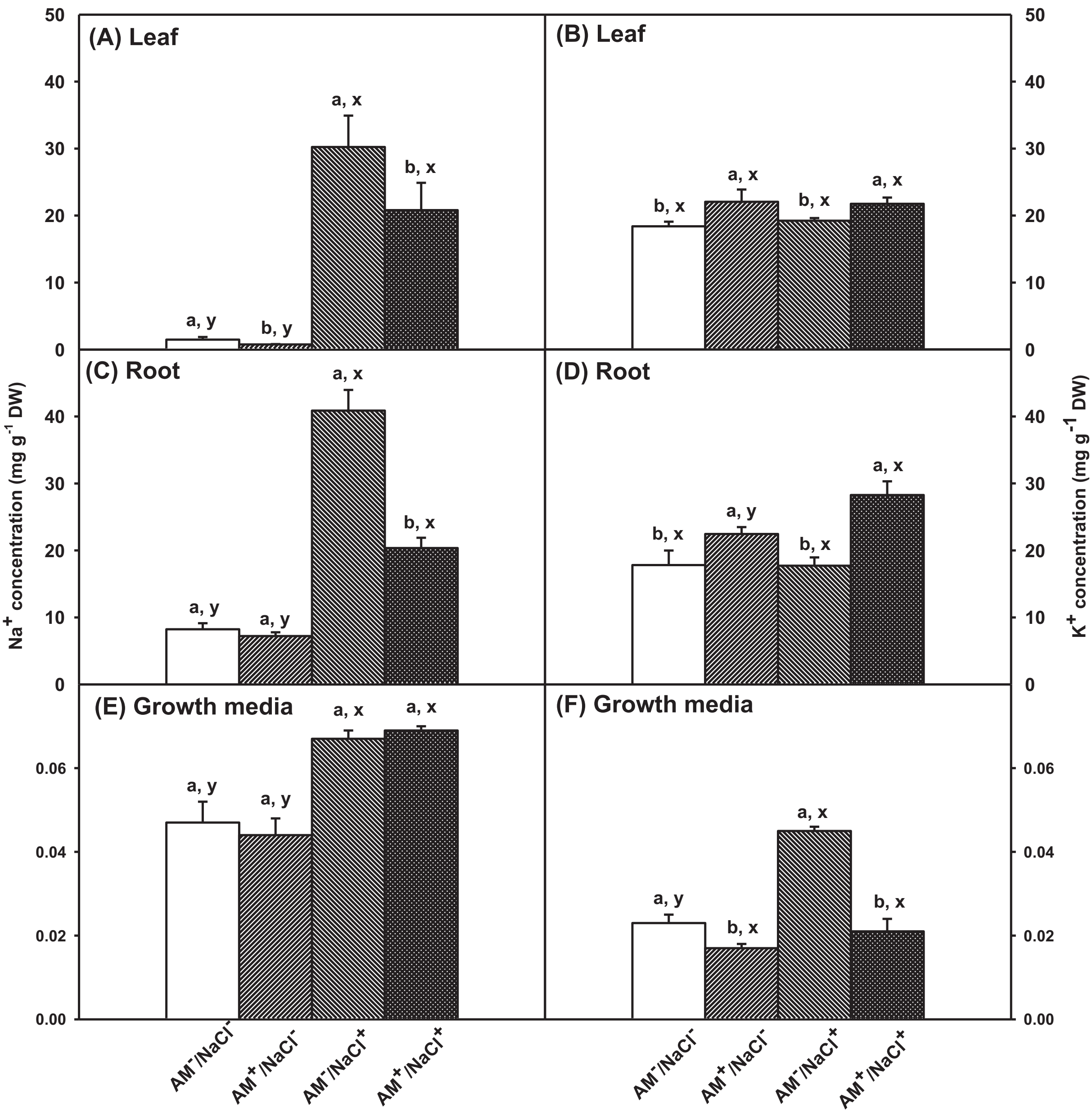

Fig. 2. Effects of *Funneliformis mosseae* inoculation and NaCl stress on Na^+ and K^+ concentrations in leaf, root, and growth media of four-month-old trifoliate orange (*Poncirus trfioliata*) seedlings. Data (means ± SE, *n* = 4) followed by different letters above the bars between AMF treatments under a given salt stress (a, b) or between salt treatments under a given AMF inoculation (x, y). Abbreviations: $AM^-/NaCl^-$, non-mycorrhizal and no-NaCl stress control; $AM^+/NaCl^-$, mycorrhizal and no-NaCl stress; $AM^-/NaCl^+$, non-mycorrhizal and 100 mM NaCl stress; and $AM^+/NaCl^+$, mycorrhizal and 100 mM NaCl stress.

3.6. Selectivity of K^+ over Na^+

Soil salinity significantly decreased both SA_{K^+/Na^+} and ST_{K^+/Na^+} in the AMF and non-AMF seedlings (Fig. 5). The AMF inoculated treatment notably increase SA_{K^+/Na^+} under the no-NaCl stress by 89% and under NaCl stress by 401% higher, respectively, compared to the AMF non-inoculated control (Fig. 5A). Inoculation with *F. mosseae* significantly increased ST_{K^+/Na^+} by 55% under no-NaCl stress but significantly decreased ST_{K^+/Na^+} by 48% under NaCl stress (Fig. 5B).

3.7. Correlation of SA_{K^+/Na^+} or ST_{K^+/Na^+} with growth variables or LRWC

The Pearson correlation analysis showed that SA_{K^+/Na^+} was highly positively correlated with plant height (Fig. 6A), stem diameter (Fig. 6C), leaf number (Fig. 6E), root dry weight (Fig. 6I), total dry weight (Fig. 6K), and LRWC (Fig. 6M), but not with shoot dry weight (Fig. 6G). In contrast, ST_{K^+/Na^+} was highly positively correlated with leaf number (Fig. 6F) and root dry weight (Fig. 6J) only, but not with plant height (Fig. 6B), stem diameter (Fig. 6D), shoot dry weight (Fig. 6H), total dry weight (Fig. 6L), and LRWC (Fig. 6N).

4. Discussion

4.1. NaCl stress restricted plant growth and root mycorrhizal colonization

In general, soil salinity affects not only host plants but also AM fungi (Murkute et al., 2006; Evelin et al., 2009). The present study showed that 100 mM NaCl stress had negative effects on plant growth and mycorrhizal colonization (Table 1). This is supported

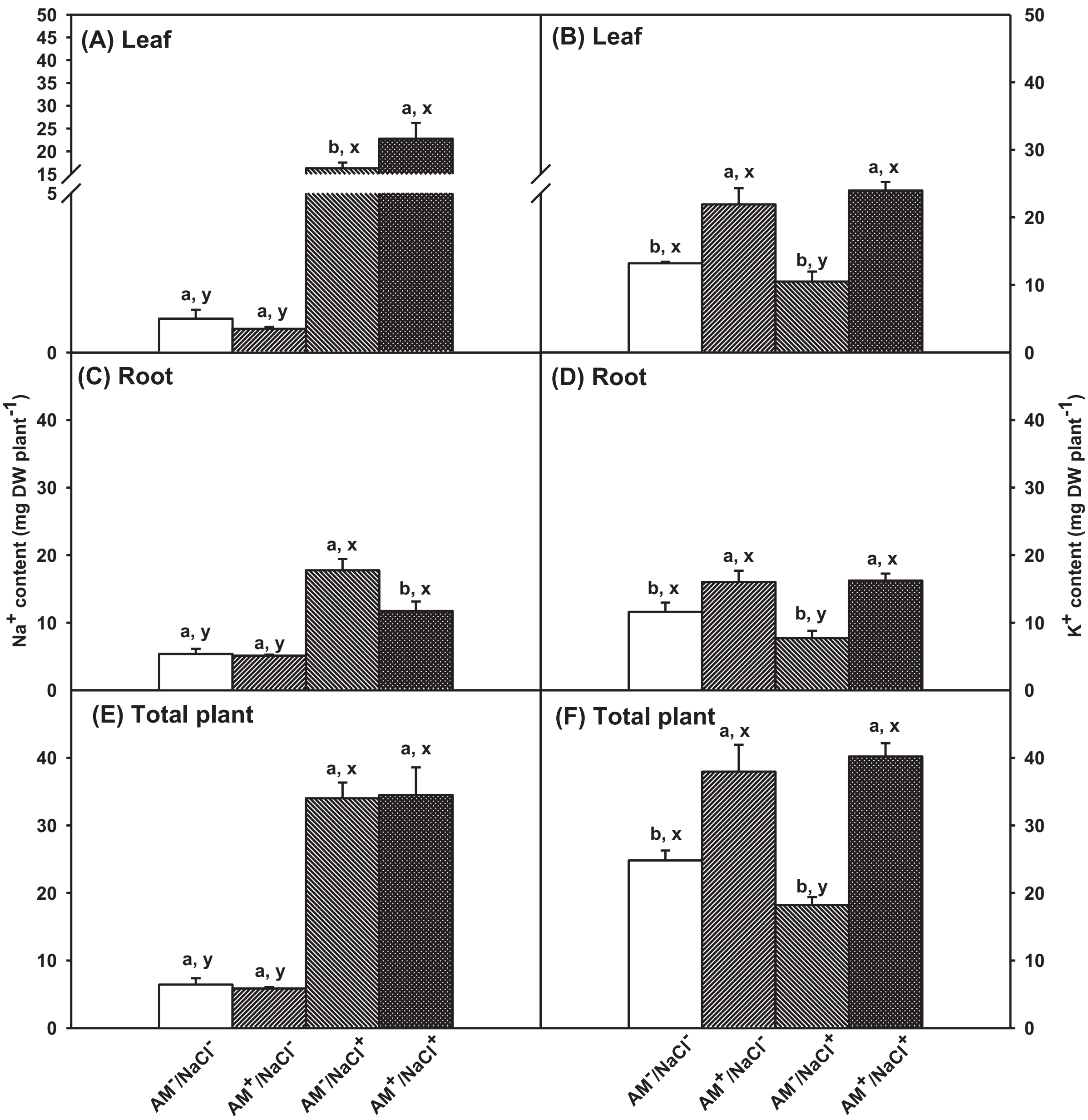

Fig. 3. Effects of *Funneliformis mosseae* inoculation and NaCl stress on Na^+ and K^+ contents in leaf, root, and total plant of four-month-old trifoliate orange (*Poncirus trfioliata*) seedlings. Data (means ± SE, *n* = 4) followed by different letters above the bars between AMF treatments under a given salt stress (a, b) or between salt treatments under a given AMF inoculation (x, y). Abbreviations: $AM^-/NaCl^-$, non-mycorrhizal and no-NaCl stress control; $AM^+/NaCl^-$, mycorrhizal and no-NaCl stress; $AM^-/NaCl^+$, non-mycorrhizal and 100 mM NaCl stress; and $AM^+/NaCl^+$, mycorrhizal and 100 mM NaCl stress.

by our previous study on *F. mosseae* colonized *C. tangerine* (Wu et al., 2010a). The negative effect of NaCl stress on root mycorrhizal colonization may be due to the inhibition of both spore germination and hyphal growth (McMillen et al., 1998; Juniper and Abbott, 2006). Meanwhile, the inoculation with *F. mosseae* increased the plant growth variables under NaCl stress than under no-NaCl stress (Table 1), suggesting that trifoliate orange plants might be more dependent on mycorrhizal symbiosis under NaCl stress than under no-NaCl stress (Wu et al., 2009).

4.2. Mycorrhizal inoculation decreased tissue Na^+ but increased K^+ under NaCl stress

As a toxic monovalent cation, Na^+ not only injures plant cells but also degrades soil structures (Moghaieb et al., 2004). As a major cationic inorganic nutrient and an osmotic regulator, K^+ is essential to plant cells. Both Na^+ and K^+ have a similar physicochemical structure, whilst Na^+ can compete K^+ at transport sites in the symplast or at K^+ binding sites in the cytoplasm (Maathuis and Amtmann, 1999). Therefore, NaCl stress often triggers a reduction of K^+ absorption, along with an increase of Na^+ accumulation in plant tissues, thereby leading to an imbalance of K^+/Na^+ (Nedjimi and Daoud, 2009). In the present study, the AMF inoculated treatment showed a significant increase of K^+ but a significant decrease of Na^+ concentration or content in leaf and root, compared to the non-AMF treatment (Figs. 2 and 3). Similar results were also found by studies with *A. nilotica*, *C. tangerine*, *Sesbania aegyptiaca* and *Sesbania grandiflora* (Giri and Mukerji, 2004; Giri et al., 2007; Wu et al., 2010a). The AMF colonization induced significantly higher K^+ and lower Na^+ concentration than the non-AMF control under NaCl stress, suggesting a preferential loading of K^+ rather than of Na^+ into the root xylem of the stressed AM plants (Hu and Schmidhalter, 2005).

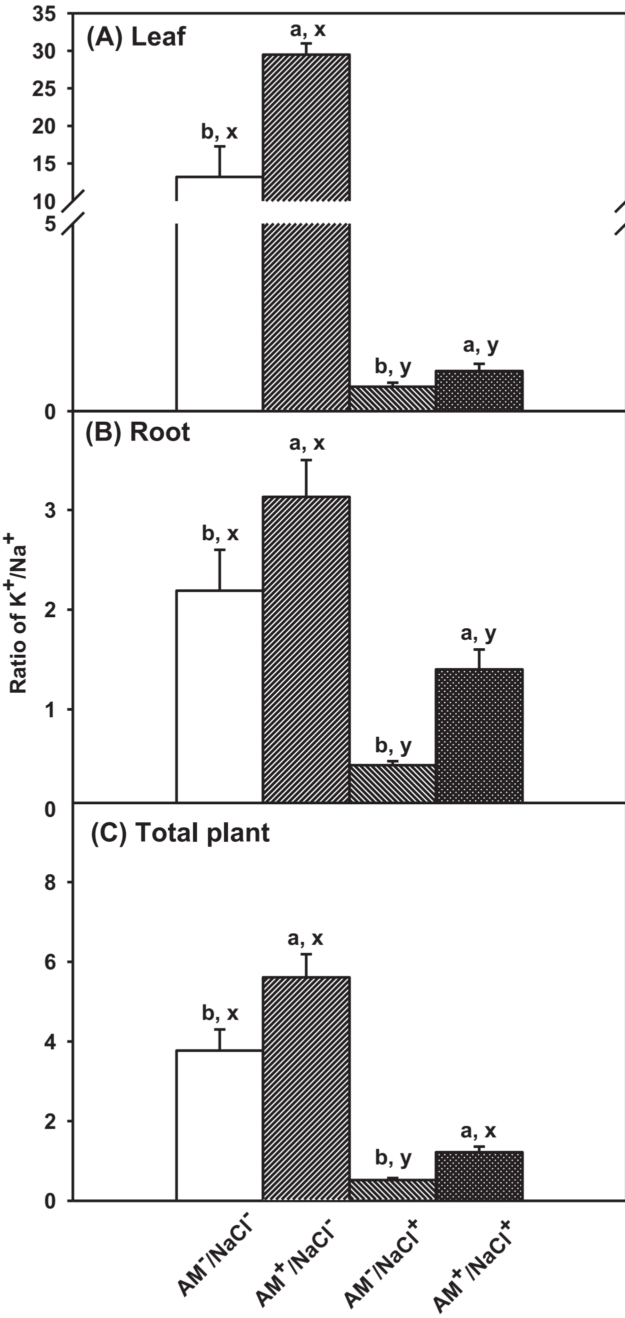

Fig. 4. Effects of *Funneliformis mosseae* inoculation and NaCl stress on K^+/Na^+ ratio in leaf, root, and total plant of four-month-old trifoliate orange (*Poncirus trfoliata*) seedlings. Data (means ± SE, *n* = 4) followed by different letters above the bars between AMF treatments under a given salt stress (a, b) or between salt treatments under a given AMF inoculation (x, y). Abbreviations: $AM^-/NaCl^-$, non-mycorrhizal and no-NaCl stress control; $AM^+/NaCl^-$, mycorrhizal and no-NaCl stress; $AM^-/NaCl^+$, non-mycorrhizal and 100 mM NaCl stress; and $AM^+/NaCl^+$, mycorrhizal and 100 mM NaCl stress.

As a result, it is reasonable that the roots of the NaCl-stressed AMF seedlings exhibited higher K^+/Na^+ ratio than those of the NaCl-stressed non-AMF seedlings (Fig. 4B). However, no significant differences of K^+/Na^+ ratio were observed between the no-NaCl stressed leaves inoculated with or without AMF (Fig. 4A), indicating that K^+ could accumulate more from substrates to roots, but might not able to transport more K^+ from roots to leaves. More accumulation of K^+ in the AM plants is vital for cytosolic enzymatic activities and for maintaining an appropriate osmotic pressure and membrane potential for cell regulation (Serrano and Rodriguez-Navarro, 2001; Zhu, 2003), thereby benefiting the mycorrhizal plants to tolerate NaCl stress.

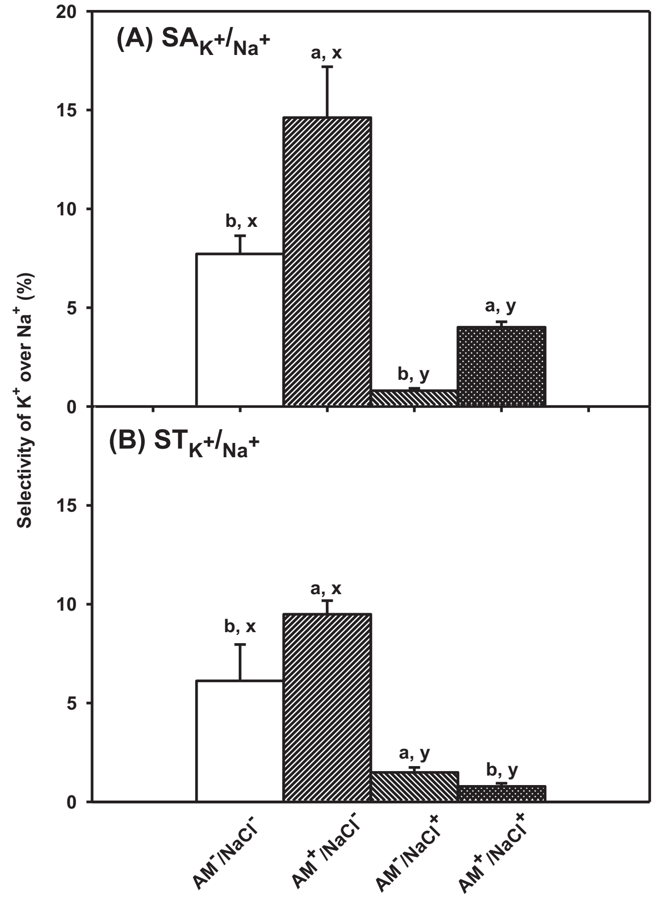

Fig. 5. Effects of *Funneliformis mosseae* inoculation and NaCl stress on selective absorption of K^+ over Na^+ (SA_{K^+/Na^+}) and selective transport of K^+ over Na^+ (ST_{K^+/Na^+}) of four-month-old trifoliate orange (*Poncirus trfioliata*) seedlings. Data (means ± SE, *n* = 4) followed by different letters above the bars between AMF treatments under a given salt stress (a, b) or between salt treatments under a given AMF inoculation (x, y): $AM^-/NaCl^-$, non-mycorrhizal and no-NaCl stress control; $AM^+/NaCl^-$, mycorrhizal and no-NaCl stress; $AM^-/NaCl^+$, non-mycorrhizal and 100 mM NaCl stress; and $AM^+/NaCl^+$, mycorrhizal and 100 mM NaCl stress.

4.3. *Mycorrhizal plants showed higher selective absorption and lower selective transport of K^+ over Na^+ under NaCl stress*

Since Na^+ competes with K^+ at transport sites and binding sites (Maathuis and Amtmann, 1999), selectivity of K^+ over Na^+ is very important for plants to tolerate NaCl stress. A higher SA_{K^+/Na^+} value indicates stronger ability to absorb K^+ over Na^+ from substrate to root (Wu and Wang, 2012). Our study indicated that SA_{K^+/Na^+} was significantly higher in the AMF seedlings than in the non-AMF seedlings under no-NaCl and NaCl stress (Fig. 5A), implying that AMs might enhance K^+ absorption but decreased Na^+ absorption from growth media to roots under NaCl stress. As reported by Evelin et al. (2009), mycorrhizal symbiosis may stimulate the host root to select more K^+ over Na^+ for maintaining ionic balance of the cytoplasm or preventing Na^+ influx.

A greater ST_{K^+/Na^+} indicates a stronger ability to transport K^+ over Na^+ from root to leaf (Wu and Wang, 2012). The present study showed that the *F. mosseae* inoculation significantly increased ST_{K^+/Na^+} under no-NaCl stress but decreased ST_{K^+/Na^+} under NaCl stress (Fig. 5B), implying that mycorrhizal effect on ST_{K^+/Na^+} might be related to salt status. A lower ST_{K^+/Na^+} in the NaCl-stressed

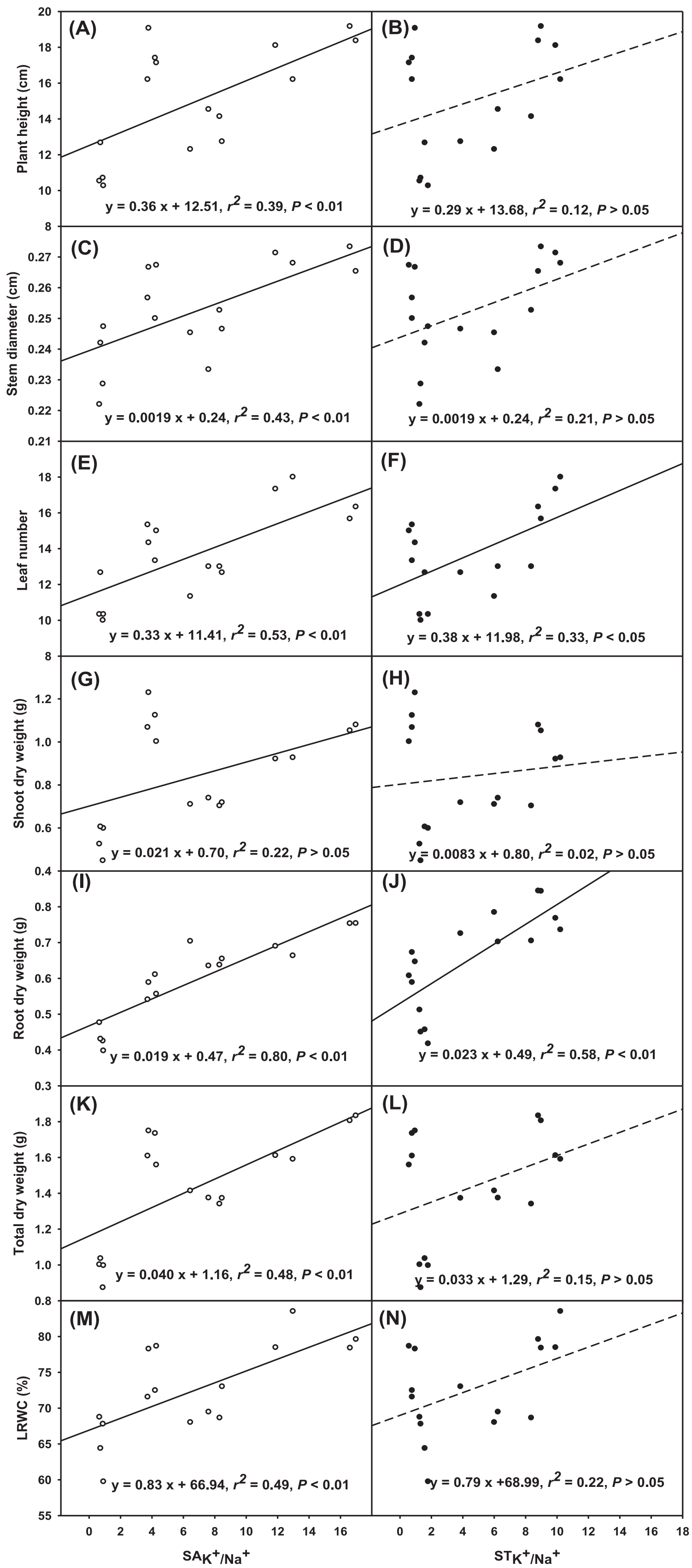

Fig. 6. Linear regression between selective absorption of K^+ over Na^+ (SA_{K^+/Na^+}) or selective transport of K^+ over Na^+ (ST_{K^+/Na^+}) and plant growth properties or leaf relative water content (LRWC) of four-month-old trifoliate orange (*Poncirus trfioliata*) seedlings ($n = 16$). Abbreviations: AM⁻/NaCl⁻, non-mycorrhizal and no-NaCl stress control; AM⁺/NaCl⁻, mycorrhizal and no-NaCl stress; AM⁻/NaCl⁺, non-mycorrhizal and 100 mM NaCl stress; and AM⁺/NaCl⁺, mycorrhizal and 100 mM NaCl stress.

mycorrhizal seedlings suggests that mycorrhizal plants may transport lower K^+ or higher Na^+ from root to leaf to compete with Na^+ under NaCl stress. Tissue Na^+ and K^+ distribution pattern also indicated that the mycorrhizal roots accumulated 34% plant total Na^+ and 40% plant total K^+, whereas the non-mycorrhizal roots accumulated 52% plant total Na^+ and 42% plant total K^+ under NaCl stress (Fig. 3). Interestingly, under NaCl stress AMF colonization did not affect Na^+ accumulation in the total plant as a result of a significant decrease of root Na^+ but a significant increase of leaf Na^+ content (Fig. 3). These results suggested that AMs did not alter the total amount of Na^+ absorption but strengthened transport of Na^+ from root to leaf.

4.4. Selective absorption of K^+ over Na^+, rather than selective transport of K^+ over Na^+, is important in improving both leaf water retention and plant growth

In the present study, mycorrhizal seedlings showed significantly higher LRWC than non-mycorrhizal seedlings grown in either no-NaCl or NaCl stress (Fig. 1), suggesting that *F. mosseae* improved the water absorption of mycorhizal trifoliate orange, which was also seen in *F. mosseae* colonized peanut (Al-Khaliel, 2010), pepper (Cekic et al., 2012), and trifoliate orange (Zou and Wu, 2011). Our results also showed that SA_{K^+/Na^+}, not ST_{K^+/Na^+}, was highly positively correlated with LRWC (Fig. 6M). An improved water availability of mycorrhizal plants from higher SA_{K^+/Na^+}, but not from ST_{K^+/Na^+}, might result in an improved root hydraulic conductivity, greater root system architecture, and higher stomatal conductance and photosynthetic rate (Jahromi et al., 2008; Kapoor et al., 2008; Wu et al., 2010a). In addition, SA_{K^+/Na^+} was also highly positively correlated with plant height (Fig. 6A), stem diameter (Fig. 6C), leaf number (Fig. 6E), root dry weight (Fig. 6I), and total dry weight (Fig. 6K), whilst ST_{K^+/Na^+} was only significantly positively correlated with leaf number (Fig. 6F) and root dry weight (Fig. 6J). Taken together, we concluded that SA_{K^+/Na^+}, rather than ST_{K^+/Na^+}, might be important in improving both leaf water status and plant growth.

5. Conclusion

In short, mycorrhizal colonization by *F. mosseae* improved tissue LRWC and plant growth of trifoliate orange seedlings under no-NaCl and NaCl stress. Leaf and root Na^+ concentrations and amounts under NaCl stress were significantly lower, whilst leaf and root K^+ concentrations and amounts under both no-NaCl and NaCl stress were significantly higher, in mycorrhizal than in non-mycorrhizal seedlings. Furthermore, *F. mosseae* inoculated seedlings exhibited significantly higher K^+/Na^+ ratio in roots but not in leaves, and higher SA_{K^+/Na^+}, but not ST_{K^+/Na^+}, under NaCl stress. Both leaf water retention and plant growth under mycorrhization were positively correlated with SA_{K^+/Na^+}, but not with ST_{K^+/Na^+}. All these results demonstrate that mycorrhizal trifoliate orange seedlings may have greater ability to tolerate NaCl stress than their non-mycorrhizal counterparts through SA_{K^+/Na^+} but not ST_{K^+/Na^+}.

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