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Full Length Research Paper

Calcium alleviation of sodium toxicity in salt-treated *Cyclocarya paliurus* seedlings

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Cyclocarya paliurus **seedlings were cultured in a greenhouse under saline conditions in 50 L black plastic boxes containing Hoagland-Arnon nutrient solution. Plants were treated with a nutrient solution plus 85 mM NaCl and 0, 6, 12 or 18 mM $Ca(NO_3)_2$. Vegetative growth, leaf and root Na^+ and Ca^{2+} concentrations were measured. Na^+ toxicity symptoms were observed in plants non-treated with Ca^{2+}. Shoot length was higher in Ca^{2+} treated plants, although shoot growth was reduced at 18 mM CaNO3, probably due to the high total ion concentration reached in the external solution. Ca^{2+} supply linearly increased leaf and root Ca^{2+} concentration and decreased leaf Na^+ concentration. However, there were no differences in root Na^+ concentration. Results indicate that Ca^{2+} may take part in the Na^+ exclusion mechanism, mainly preventing Na^+ transport to the shoot that may be an important ability for survival under saline conditions.**

Key words: *Cyclocarya paliurus*, NaCl, sodium exclusion, calcium.

INTRODUCTION

Cyclocarya paliurus (Batal) Iljinskaja, a native to China, is the sole species in its genus. It is a well-known multiple function plant in China. Particularly, a huge production of tender leaves from *C. paliurus* is required for the raw material of teas and medicinal use, thus increasing demands for new *C. paliurus* plantations are anticipated (Fang and Fu, 2007; Yao and Fang, 2009a). It was reported that there is a total of about 27×10^6 ha of saline soil in China, of which coastal land accounts for 8% (Yao and Fang, 2009a). *C. paliurus* naturally grows in the mountainous region (Yao and Fang, 2009b). It is much sensitive to salt condition compared to most of halophytes (Yao et al., 2009). To our knowledge, it grew well in medium with 17 mM NaCl, while there was a significant increase in seedlings mortality under 85 mM NaCl treatment (Yao and Fang, 2009a). The salinity for most of coastal lands is approximate 85 mM or so, thus it is necessary to improve the salt-tolerant ability of *C. paliurus* in order to effectively promote its planting in coastal or saline areas.

Ca^{2+} supply to the saline soil solution regulates Na^+ uptake by plants and can prevent the accumulation of toxic levels of Na^+ (Maas, 1993; Tattini and Traversi, 2009; Ding et al., 2010). However, effectiveness in alleviating the toxic effect of Na^+ depends on the Ca^{2+} and Na^+ concentration and on the species (Grattan and Grieve, 1999). Usually, plant salt tolerance is mainly associated to ion exclusion mechanisms located in the root (Benlloch et al., 1991; Tattini et al., 1995; Ben et al., 2009) and consisting in holding Na^+ and Cl^- at the root level and limiting the accumulation of these ions in the shoot. Cl^- uptake and transport to the shoot in *C. paliurus* is lower than for Na^+ (Li et al., 2007), without causing negative effects if concentrations are less or equal to 85 mM Cl^- in saline solution (Yao and Fang, 2009a). Although the mitigating effect of Ca^{2+} on the adverse NaCl effects has been reported in many plant species (La Haye and Epstein, 1969), the role of Ca^{2+} has not been sufficiently studied in *C. paliurus*. To enhance an understanding on the effect of Ca^{2+} alleviation on Na^+ toxicity in *C. paliurus* planted in saline conditions, the objective of the present study was to determine the effect of supplementary Ca^{2+} on Na^+ uptake and transportation

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Table 1. Effect of $Ca(NO_3)_2$ concentration on *Cyclocarya paliurus* seedlings growth 28 days after the beginning of the treatments.

$Ca(NO_3)_2$ concentration (mM)	Root length (cm)	Shoot length (cm)	Root DW (g)	Shoot DW(g)
0	10.21	7.74cC	0.09	0.47b
6	10.62	11.36bB	0.15	0.53b
12	12.33	19.25aA	0.28	0.91a
18	11.20	14.36bB	0.17	0.68ab

Data were analyzed by Duncan's multiple range test and means of at least ten replicates followed by identical letters were not statistically different, where small letters showed the differences in four $Ca(NO_3)_2$ concentration treatments at α=0.05 level and capital letters showed the differences at α=0.01 level.

in salt-treated *C. paliurus* seedlings.

MATERIALS AND METHODS

Seeds of *C. paliuru* were collected from good mother trees in Jiangxi Province of China and sowed in containers with mixed medium (perlite: vermiculite: peat soil=1:2:2) after dormancy were broken. When the height of seedlings reached about 7 cm, they were cultured in black plastic boxes with one-half-strength Hoagland-Arnon nutrient solution. After 7 days of culture, uniform seedlings were selected and cultured with normal Hoagland-Arnon nutrient solution (Hoagland and Arnon, 1950). The solution was aerated throughout the experiment, and the volume was maintained by adding distilled water to compensate for water loss by evaporation and transpiration. The nutrient solution was renewed every 3 days. The seedlings were grown in a controlled environment: 350-400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ daily photon flux density; 16 h, 25℃ (day)/8 h, 20℃ (night) regime; 60-70% relative humidity. Two-month-old *C. paliurus* seedlings cultured in normal Hoagland-Arnon nutrient solution were divided into 4 groups and exposed to 85 mM NaCl plus 0, 6, 12 or 18 $Ca(NO_3)_2$ solutions, respectively. There were 50 trees for each treatment.

After 28 days treatment, shoot and root length was measured, leaves formed during the experiment were sampled, and shoots and roots separated in all of plants. Roots were simultaneously divided into 3 groups. One of them was for the measurement of root growth. Another one and leaves were briefly rinsed with deionised water, oven-dried at 80℃ during 72 h. The samples were separately digested with a mixture of $HClO_4$ and HNO_3. The concentration of Na^+ and Ca^{2+} in the digested samples of roots and leaves was measured by an atomic absorption spectrophotometer (Thermo Element MKII-M6). The third group of roots was used for root X-ray microanalysis, which was measured in a JSM-6300 scanning electron microscope equipped with an energy-dispersive X-ray detector (Sigma) (Tomos et al., 1994). Counts per second of $[Ca^{2+}]$ and $[Na^+]$ were measured in roots from different treatments. Four transverse sections of each treatment were observed and three location spots of the same tissue of each section were analysed.

Means and standard errors were obtained for leaf Na^+ and Ca^{2+} concentration data. Data were subjected to analysis of variance to compare the effect of the treatments using SPSS 13.0 statistical software.

RESULTS

Shoot length significantly increased with $Ca(NO_3)_2$ concentration, showing a quadratic response that indicated a reduction in shoot growth at the highest $Ca(NO_3)_2$ concentrations (Table 1). Little growth was obtained when $Ca(NO_3)_2$ was not supplied to the medium. Non-significant differences were found either in root length or root dry weights among treatments.

Ca^{2+} deficient leaves and leaf symptoms, such as rolled leaf and leaf burn, defoliation and occasionally total death, were only observed in plants treated with 85 mM NaCl and 0 mM $Ca(NO_3)_2$. Otherwise, the rest of the treatments maintained leaf Ca^{2+} concentrations in the range of adequate levels. Leaf Na^+ concentration was above the toxicity level (>2 mg/g) in all treatments, regardless of $Ca(NO_3)_2$ concentration supplied in the saline solution (Figure 1). However, the leaf Na^+ concentration significantly decreased as $Ca(NO_3)_2$ increased from 0 to 12 mM. An increase of $Ca(NO_3)_2$ up to 18 mM seemed to affect leaf Na^+ concentration only slightly. A linear increase in leaf Ca^{2+} concentration was observed when Ca^{2+} concentration in the saline solution was raised, while there was non-significant difference in leaf Ca^{2+} concentration between 12 and 18 mM $Ca(NO_3)_2$ treatments in terms of variance analysis (Figure 1). Ca^{2+} was significantly increased in root with increased Ca^{2+} supply, but there were no differences in root Na^+ concentration (Figure 2). According to X- ray map-scanning images of Na^+ and Ca^{2+} distribution in roots, it was also clearly found that root Ca^{2+} distribution in plants treated with 12 mM $Ca(NO_3)_2$ was much more compare with that treated with 0 mM $Ca(NO_3)_2$, while root Na^{2+} distribution in plants treated with 0 or 12 mM $Ca(NO_3)_2$ was similar (Figure 3).

DISCUSSION

Plants untreated with $Ca(NO_3)_2$ grew less than plants treated with $Ca(NO_3)_2$, showing leaf symptoms associated with this growth response. Growth reduction following salt treatment in plants is generally attributed to excessive salt accumulation in growing tissues (Lewitt, 1980), although both Ca^{2+} deficiency and Na^+ toxicity could be involved in the appearance of leaf symptoms. Benlloch et al. (1991) reported that no leaf toxicity symptoms, just growth reduction, were observed at high Na^+ leaf concentrations in Ca^{2+} non-deficient plants. Our results showed that *C. paliurus* seedlings non-treated

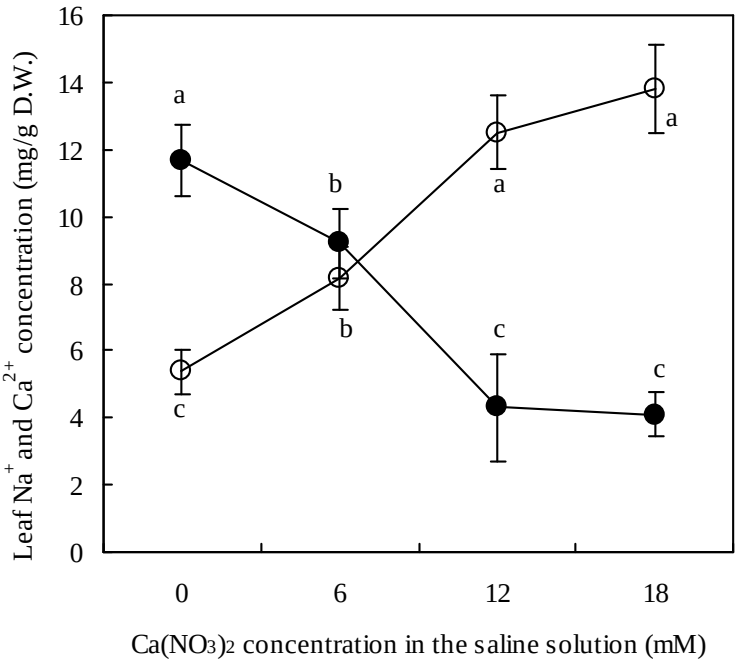

Figure 1. Effect of $Ca(NO_3)_2$ concentration on leaf Na^+ (●) and leaf Ca^{2+} (○) concentration in *Cyclocarya paliurus* seedlings treated with 85 mM NaCl. Leaves were sampled and analysed 28 days after the beginning of the treatments. Means of four replicates±standard error. Differences in treatments were analyzed by Duncan's multiple range test (α=0.05).

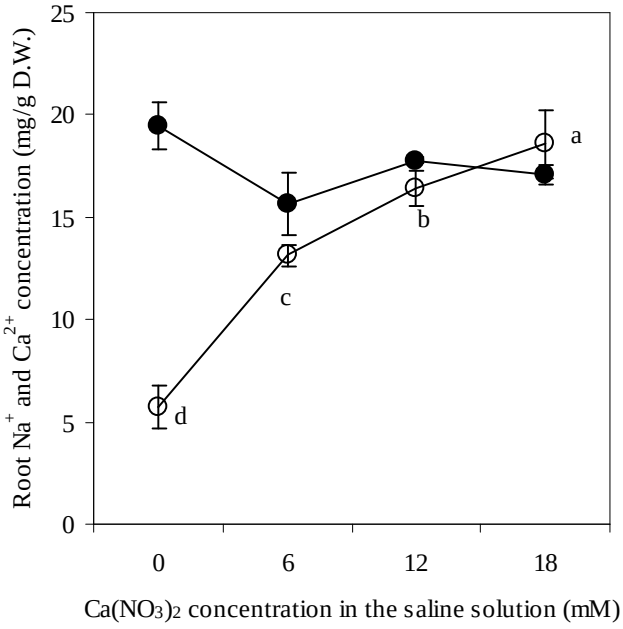

Figure 2. Effect of $Ca(NO_3)_2$ concentration on Root Na^+ (●) and leaf Ca^{2+} (○) concentration in *Cyclocarya paliurus* seedlings treated with 85 mM NaCl. Leaves were sampled and analyzed 28 days after the beginning of the treatments. Means of four replicates±standard error. Differences in treatments were analyzed by Duncan's multiple range test (α=0.05).

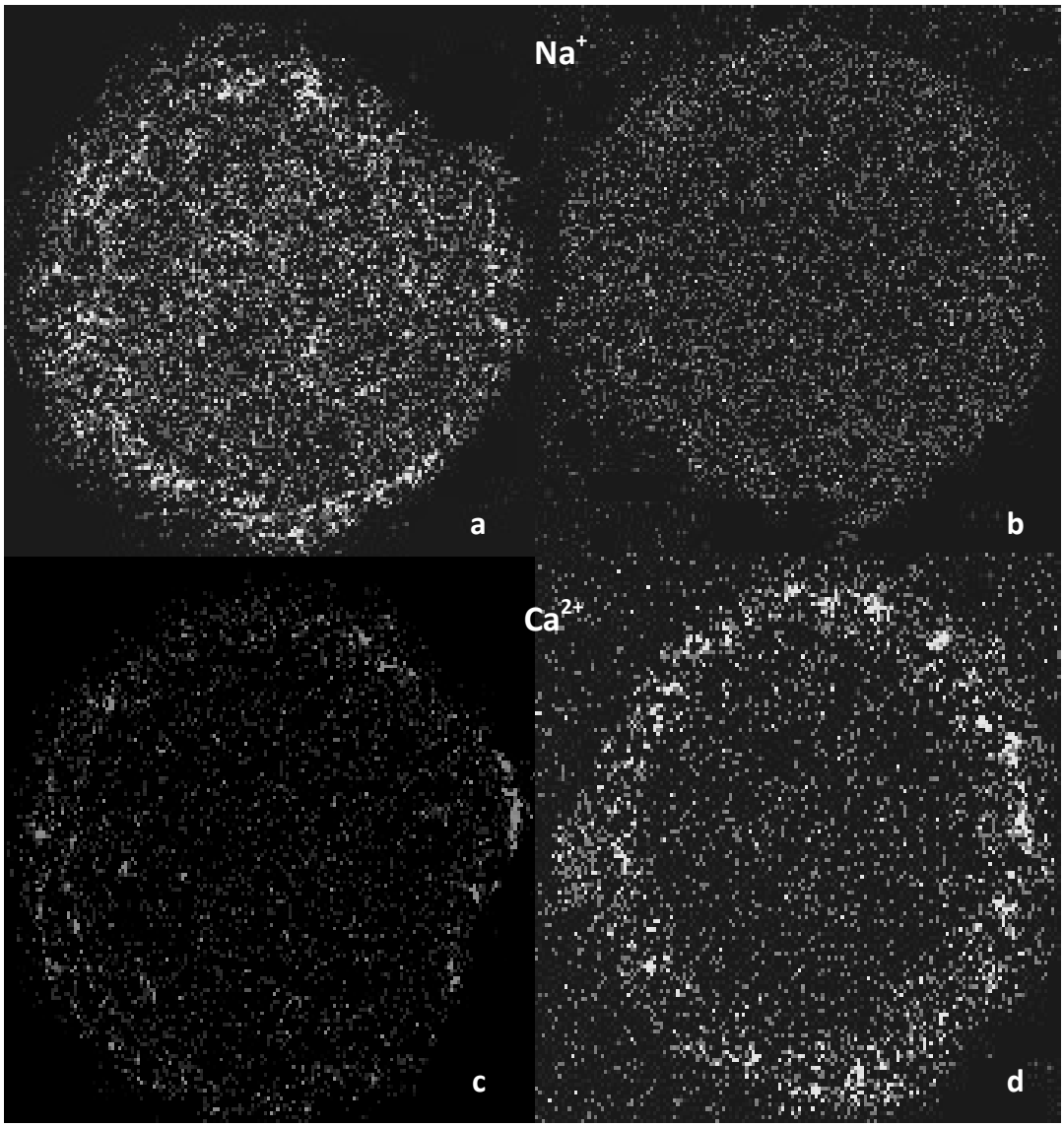

Figure 3. X-ray map-scanning images of Na^+ and Ca^{2+} distribution in roots of *Cyclocarya paliurus* seedlings treated with 85 mM NaCl plus 0 or 12 mM $Ca(NO_3)_2$, respectively. (a) Na^+ image of 85 mM NaCl; (b) Na^+ image of 85 mM NaCl+12 mM $Ca(NO_3)_2$; (c) Ca^{2+} image of 85 mM NaCl; (d) Ca^{2+} image of 85 mM NaCl+12 mM $Ca(NO_3)_2$.

with Ca^{2+} were Ca^{2+} deficient and suggest that leaf symptoms might not to be exclusively associated to Ca^{2+} deficiency because no damage was observed in apical meristems or young leaves. Probably, Na^+ toxicity is mainly shown when leaves are Ca^{2+} deficient what could explain the toxicity symptoms observed in the leaves.

Ca^{2+} supply to the saline solution and, consequently, the increase in the Ca^{2+}/Na^+ ratio enhanced plant growth. It is supposed that the growth reduction shown by *C. paliurus* seedlings treated with 18 mM $Ca(NO_3)_2$ was probably due to the water stress caused by the high total ion concentration in the external solution. Researchers also observed that although some halophytes are quite tolerant to salinity, they can be negatively affected by the total saline concentration, and growth reductions of them have been correlated with the accumulation of toxic ions in the shoot (Tattini et al., 1992; Chelli et al., 2010). Regulation of leaf Na^+ concentration could be influenced by the toxic ion exclusion capacity that plants show. This exclusion capacity could be higher in tolerant genotypes than in sensitive ones (Gucci and Tattini, 1997), which could partially explain the fact that the *C. paliurus* provenance from Anhui Province closed to coastal area of China is a much more salt-tolerant cultivar compared

with Jiangxi and Yunnan provenances (Yao and Fang, 2009a). Tolerant cultivars have the capacity for Na^+ retention in roots, whereas sensitive ones do not (Tattini, 1994; Kinraide et al., 2004). In this study, we found that there was no remarkable difference between leaf and root Na^+ concentration when $Ca(NO_3)_2$ was not supplied to the medium, while the root Na^+ concentration was significantly higher than the leaf Na^+ concentration using the supply of $Ca(NO_3)_2$. Thus this could supported in that Ca^{2+} could contribute to the improvement of salt-tolerant ability of *C. paliurus* seedlings under saline condition keeping the Na^+ retention in roots as many as possible.

We also found that leaf Ca^{2+} concentration was increased when Ca^{2+} rose in the saline solution, and this increase seemed to be related with a notable decrease in leaf Na^+ concentration that, consequently, may also be regulated by leaf Ca^{2+} concentration. The cytosolic Na^+ concentration could be kept at a low level minimizing the Na^+ influx into the cytosol (Blumwald et al., 2000), restricting Na^+ entry into plant cells by selective ion uptake. Selective cation channels (Maathuis and Sanders, 1995), and non-selective cation channels, NSCCs (Amtmann and Sanders, 1999; Demidchik et al., 2002), seem to be involved in mediating the toxic influx of Na^+, but recent findings suggest that the later ones are the dominant pathways for Na^+ influx into root cells (Roberts and Tester, 1997; Demidchik et al., 2002). The functions of NSCCs are inhibited by Ca^{2+} at 0.5 mM or higher concentrations (Amtmann and Sanders, 1999). However, in our experiment, Na^+ accumulation in roots, regardless of Ca^{2+} treatments and root Ca^{2+} concentrations, did not seem to be influenced by Ca^{2+} supply to the saline solution. According to Demiral (2005), an effective salt-exclusion mechanism is operating in the roots, and the control mechanism probably prevents salt translocation rather than salt absorption. In our experiment, therefore, root and leaf Na^+ and Ca^{2+} concentrations showed that Ca^{2+} may act mainly by inhibiting Na^+ transport from root to shoot. Thus, Ca^{2+} is supposed to be directly involved in Na^+ exclusion and retention mechanisms, regulating Na^+ transport, which may be an important ability for survival under saline conditions.

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