RESPONSES IN GROWTH, PHOTOSYNTHESIS, AND WATER UPTAKE OF A HALOPHYTE (*TETRAGONIA TETRAGONOIDES*) UNDER HALOPHILISM CONDITION

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Abstract - Halophytes not only tolerate to salinity, but also promote their growth under a suitable saline condition (referred to as halophilism). *Tetagonia tetragonioides* is a halophyte that its growth is promoted by NaCl salinity up to 100 mM, but the mechanism of halophilism has not been elucidated. The present study examined responses in the plant's growth, photosynthesis, and water uptake under the halophilism condition. The results the fresh and dry biomass were increased by 35.86 and 37.04% compared to the plant grown without salt, respectively. The growth enhancement of plant was contributed by the growth of both shoots and roots. Leaf chlorophyll content was also increased 1.10 times under the halophilism condition. Relative water content was not significantly increased, but proline content was increased 15.93 – 52.31 times with increasing salinity levels. These results suggest mechanisms that regulated photosynthesis and water uptake for the halophilism.

Key words - *Tetagonia tetragonioides*; halophyte; photosynthesis; water uptake; halophilism in plant.

1. Introduction

Saline soils account for over 6% of the world's agricultural arable land and it tends to increase [1], [2]. However, most of agricultural crops are very sensitive to salinity which may cause about 10% of decrease in yield of crops if soil salinity increases by 4 - 8 dS/m [3]. Increasing soil salinization will lead to a decline in agricultural productivity and a threat to food security. Improving salt tolerance of crops and applying salt - tolerant plants as new crops are considered as effective and feasible strategies for adapting to salinization [3].

Salinity, caused mainly by NaCl, imposes both osmotic stress and ion toxicity to plant cells at high concentrations in soils. Osmotic stress leads to inhibition of water and mineral absorption of plant roots, meanwhile presence of Na^+ and Cl^- are detrimental to intercellular metabolisms and physiological processes such as photosynthesis and respiration. Besides that, ions also induce formation of radical oxygen species that involved in oxidative stress [1], [3]. As a result, the growth of plants is inhibited. To cope with salinity, salt-tolerant plants evolve various mechanisms by means of which negative effects of salt are mitigated [4 - 7].

Halophytes are plants that can not only survive under extremely saline conditions; but also obtain growth promotion, also called halophilism, at certain saline conditions that inhibit growth of other plants. Because of this salt-loving trait, halophytes become to be considered as potential new crops for saline-affected soils. A number of halophytes have been cultivated for purposes of food, forage, biofuel and pharmaceutical [8]. There have been numerous understandings on mechanisms of salt tolerance of halophytes, but information on halophilism has been limited so far. There have been a few reports that described different responses of growth and physiology of halophytes [19]. It is proposed that understanding on mechanisms and factors related to halophilism are useful for improving crop productivity in saline agriculture.

Tetragonia tetragonioides (commonly called New Zealand spinach), a flowering halophyte belonging to Aizoaceae family, is native to eastern Asia, Australia, and New Zealand, and is distributed mostly in sandy coastal areas. Nowadays it has been introduced in many countries and cultivated for food, ground - cover cum ornamental and medicinal uses [10]. It was reported that growth of *T. tetragonioides* is promoted by salt at concentrations of up to 100 mM and the growth tends to be inhibited at salt concentrations above 200 mM [6], [9], [10]. The plant can survive in saline condition with salt concentration of up to 500 mM, but the growth is significantly decreased. Ion intercellular sequestration, osmotic adjustment, and elimination of radical oxygen species induced by salt are tolerance mechanisms to high salinity of *T. tetragonioides* [6], [10]. But the mechanism of halophilism of the plant is still unclear.

The present study examined growth and physiological parameters associated with photosynthesis and water uptake of *T. tetragonioides* grown under saline condition suitable for growth enhancement. Thereby, growth and physiological responses of the plant were elucidated. For the purpose, the plants were grown with two salt treatments including 50 mM NaCl (growth - promoting concentration), 200 mM NaCl (growth - inhibiting concentration) and without salt treatment. The content of photosynthetic pigments including chlorophyll *a* (chl *a*), chlorophyll b (chl *b*), chl (*a*+*b*), and carotenoids; as well as the relative water content and proline of the leaves from the salt - treated plants were analyzed.

2. Material and Methods

2.1. Plant material

T. tetragonioides seeds, collected from the plants grown in a green house of the Faculty of Biology and Environmental Science (The University of Danang - University of Science and Education), were sown in 0.5 L volumetric plastic pots containing a potting mixture of coconut coir: vermiculite: perlite with ratio of 2: 1: 1. The seedlings were maintained for 8 weeks in a growth chamber (CMP6010 – Conviron, Canada) set up with conditions of 28ºC for 14 h lighting and 25ºC for 10 h darkness, and light density of 10.000 lux. A half concentration of Hoagland nutrients with pH = 6.0 - 6.5 (½ Hoagland solution) was applied to seedlings for growth. The eight-week-old seedlings having 5 pairs of leaves and 5 cm height were used for salt treatments.

2.2. Salt treatment

Salt treatments were carried out following a method as described by Sahin et al. [11]. The 8 – week- old seedlings were irrigated by ½ Hoagland solutions without or with 50 and 200 mM NaCl every two days. In each irrigation, the solutions were applied continuously to the mixture until leaking the solution at bottom for several minutes. In case of 200 mM NaCl treatment, the plants were irrigated with the solutions with increased salt concentrations to limit osmotic shock, which salt concentration was gradually increased from 50, 100 to 200 mM with an irrigation interval of one day. The treatments were maintained for one week and two weeks for determining physiological and growth parameters respectively.

2.3. Determination of growth parameters

Aerial part (assigned as shoots) and roots were separated for weighing fresh weight (FW). Then, the shoots and roots were dried at 70°C for 48 h in an oven before measuring dry weight [10]. In addition, the number of leaves per plant, shoot height and root length were determined.

2.4. Determination of photosynthetic pigment contents

The content of photosynthetic pigments of leaves including chl *a*, chl *b*, and carotenoids were determined by a method as described by Lichtenthaler and Wellburn (1983) [12]. Leaf samples (0.1 g) were homogenized with 10 mL of 80% acetone solution (v/v) using a mortar and pestle, and incubated at 4ºC overnight. The extract was then centrifuged at 5.000x g for 10 min. The absorbance (A) of the supernatant was determined at wavelengths of 645, 663 and 470 nm using a Jasco V730 UV-VIS spectrometer (Shimadzu, Japan) with acetone blank. The content of chl *a*, chl *b*, and carotenoids was calculated according to the formula:

$$\text{Chl } a \text{ (mg/g FW)} = 11.75 \times A_{663} - 2.35 \times A_{645}$$

$$\text{Chl } b \text{ (mg/g FW)} = 18.61 \times A_{645} - 3.96 \times A_{663}$$

$$\text{Carotenoids (mg/g FW)} = 4.69 \times A_{470} - 0.268 \times (20.2 \times A_{645} + 8.02 \times A_{663}).$$

2.5. Determination of relative water content

The relative water content (RWC) of the leaves was determined as described by Gonzalez *at. al* [13]. Leaf samples were rapidly weighed to determine fresh weight (FW), and then placed in a petri dish containing distilled water and incubated at 4°C for at least 4 h prior to determining turgid weight of leaf sample (TW). Thereafter, the dried weight of leaf samples (DW) was determined after drying at 70ºC for 48 h. The RW was calculated using the formula:

$$\text{RWC (\%)} = [(\text{FW - DW})/ (\text{TW - DW})] \times 100$$

2.6. Determination of proline content

The proline content of leaves was determined according to a method described by Bates et al. [14]. Fresh leaf samples (0.5 g) were ground in 10 mL of sulfosalicylic acid, and then centrifuged at 12.000 rpm for 15 min at 4°C. Reaction mixture consisting of 2 mL supernatant + 2 mL ninhydrin acid + 2 mL acetic acid was incubated at 95 - 100°C for 60 min. The reaction mixture was rapidly cooled to stop the reaction, and then 4 mL of toluene was added to the mixture. The solvent fraction with a colored complex containing toluene was measured for absorbance at 520 nm using a Jasco V730 UV-VIS spectrometer (Shimadzu, Japan). Toluene was used as a blank and the proline content was determined by mean of a standard curve.

2.7. Experiment design and data analysis

The salt treatments were completely randomized with three plants per treatment and replicated four times (n = 12). Data were statistically analyzed and difference between three treatments was analyzed according to Duncan's test with p-value < 0.05 using R software.

3. Results and Discussion

3.1. Characteristics in salt-promoted growth of T. tetragonioides

The characteristics of salt-promoted growth of *T. tetragonioides* were examined for growth parameters between the treatments, including plant, shoot, and root FW and DW, shoot and root length, and leaf number. After 7 days of the treatment, the plants began to show changes, but unclear in the plant growth. The different change was obvious at 14 days after the onset of treatments (Figure 1; Figure 2).

At day 14 after the onset of treatment, both fresh and dried weights of whole plants treated with 50 mM NaCl were significantly higher than that without the salt treatment. The fresh and dried weights were 15.647 g and 1.100 g, which increased 35.86 and 37.04% compared with that of the salt-untreated plants (11.517 g FW and 0.801 g DW), respectively (Figure 1A). The increment may be higher in case of that the plants were maintained with longer time with the treatments. Meanwhile, the FW of plants treated with 200 mM NaCl was decreased by 7.41% (10.663g) compared to the salt-untreated plants, but the DW was not significantly changed (Figure 1A). This result indicates that NaCl promoted both the growth of fresh and dry biomass of *T. tetragonioides* under halophilism condition, but inhibited the plant growth under salt stress. A similar increment in the growth of *T. tetragonioides* under salinity was also reported by Yousif et al. [10] and Atzori et al. [17]. These results also suggest that the experimental platform established in the present study was representable for studying on the halophilism characteristics of *T. tetragonioides*.

A similar expression was observed in the growth of shoots after 14 days of the treatment. The shoot FW and DW of the 50 mM NaCl-treated plants were increased 36.14% (15.151 g) and 37.08% (1.033 g) compared with that of the salt-untreated plants (Figure 1B). This result suggests that the growth enhancement of shoots was involved into the salt-promoted growth of plants.

Remarkably, data showed that there was no significant difference in leaf number and shoot length between the 50 mM NaCl-treated plants with the salt-untreated plants (Figure 1D, E), suggesting that the growth enhancement of shoots was mainly due to the increase of biomass and size of the leaves rather than formation of new leaves and stem elongation. In fact, the leaves of the plants grown under halophilism condition were dark green and clearly larger in size than those of the salt-untreated plants (Figure 2). This phenomenon was reported in case of halophytes such as *Guettarda speciosa* [15] and *Atriplex patula* [16]. These results suggest that the salt-promoted growth of plants could be result of promoting cell elongation and division in halophytes [19]. Leaves are photosynthetic apparatus of plants, determining biomass and yield in many species. Thus, increasing leaf size by salinity will lead to an enhancement in photosynthetic efficiency that may contribute to the growth enhancement of halophytes.

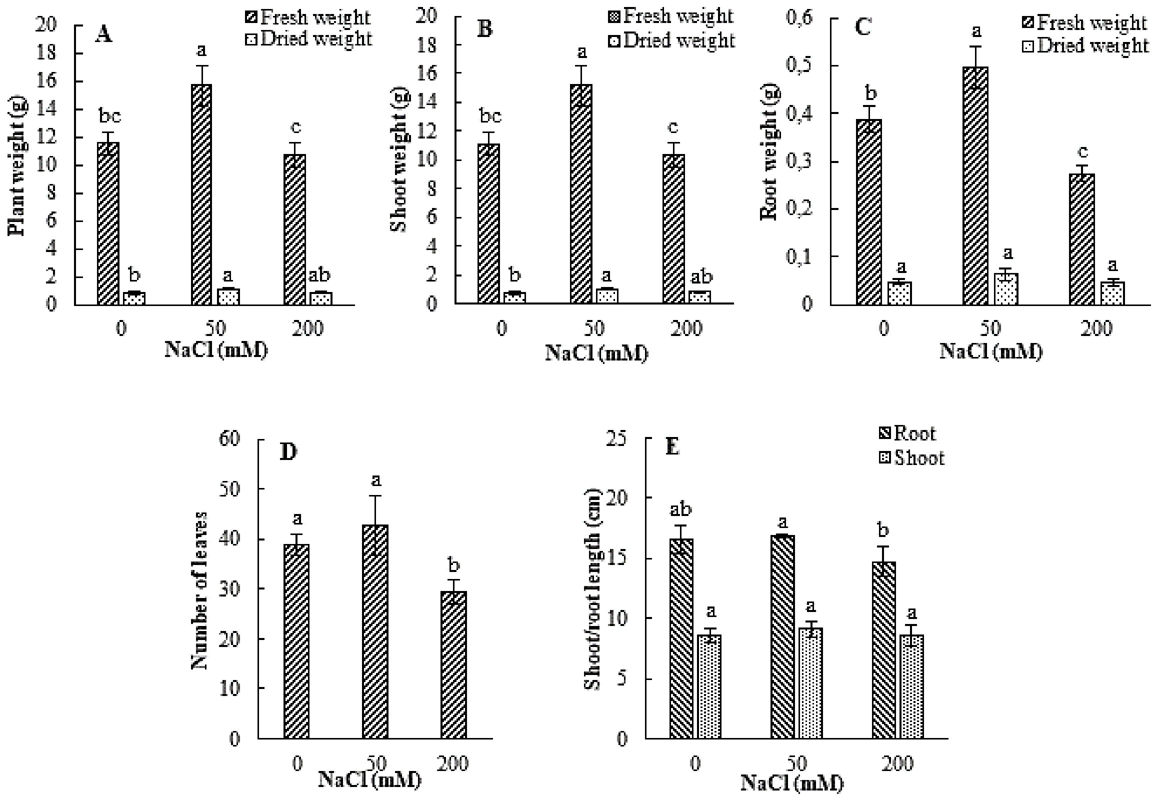

Figure 1. *Growth parameters of T. tetragonioides at 14 days after the onset of treatments. Fresh and dried weight: A, whole plant; B, shoots; C, roots; D, number of leaves; E, shoot and root length. Different letters in each parameter represent statistically significant differences between the treatments according to Duncan's test with p-value < 0.05*

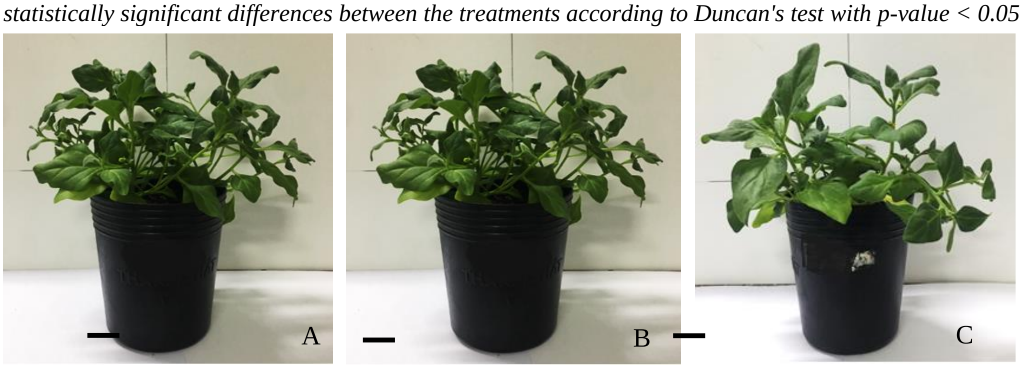

Figure 2. *T. tetragonioides plants at 14 days after the onset of treatments. A, 0 mM; B, 50 mM; C, 200 mM NaCl-treated plants; bars = 2 cm*

Differing from the 50 mM NaCl-treated plants, the limitation of shoot growth occurred in the plants treated with 200 mM NaCl although the decrease was not statistically significant. The plant FW and shoot FW were slightly decreased by 7.41% (10.663 g) – and 6.67% (10.388 g) compared to the salt-untreated plants (Figure 1A, B). In addition, the leaf number and shoot length were also reduced compared with the salt-untreated plants, although the reduction of shoot length was not statistically significant (Figure 1D, E). Inhibition of shoot growth is a typical growth response of plants under high salinity [1].

A similar growth enhancement of roots was also observed in the 50 mM-treated plants. The root FW and DW of the 50 mM NaCl-treated plants were 0.496 g and 0.064 g, respectively, increasing 27.96 and 29.12% compared to that of the salt-untreated plants (Figure 1C). Also, the salt-treated roots had a higher length than the salt-untreated roots although a statistically significant difference was not obtained (Figure 1E). These results indicate that NaCl promoted not only the growth of *T. tetragonioides* shoots, but also the growth of roots. It is reported that there may be a mechanism that enhances cell elongation of roots in order to efficiency of water and nutrient uptake for growth enhancement of plants under halophilism condition. But the root biomass of the 200 mM NaCl-treated plants was significantly reduced compared to that of the salt-untreated plants (Figure 1C). Because the roots are directly exposed to salt, the root cells may be more affected than shoots. It is noted that although the growth of halophytes under salt stress condition is limited to a certain extent, maintenance in water and nutrient uptake capacity, and reducing salt effects on the shoots are tolerance mechanisms to salinity stress [1].

3.2. Characteristics in physiological responses in salt-promoted growth of T. tetragonioides

3.2.1. Accumulation of photosynthetic pigments

Chlorophylls and carotenoids are the main photosynthetic pigments, which play important roles in plant photosynthesis, absorbing energy from sunlight. Thus, the content of these pigments in leaves is related to the efficiency of photosynthesis activity in plant cells [10]. Thus, the effects of environmental stress on photosynthesis can be assessed through the content of these pigments. It is also reported that the photosynthetic activity in plants is affected by salinity at the extent that depends on the salt concentration [1], [15].

In the present study, data showed that chl (*a*+*b*) content in leaves of the salt-treated plants was increased compared to the salt-untreated plants although statistically significant differences were not obtained (Figure 3A). The chl (*a*+*b*) content in the plants treated with 50 and 200 mM NaCl was increased by 1.10 (0.2810 mg/g FW) and 1.13 (0.2898 mg/g FW) times, respectively (Figure 3A). Similarly, both chl *a* and chl *b* contents were also increased with the salt treatments (Figure 3B, C). The leaf color of the salt-treated plants became more dark-green than that of the salt-untreated plants (Figure 2). These results suggest that the accumulation of chlorophylls in *T. tetragonioides* leaves was enhanced by salinity. Meanwhile, carotenoid content was slightly enhanced, but not statistically significant in the salt-treated plants (Figure 3D).

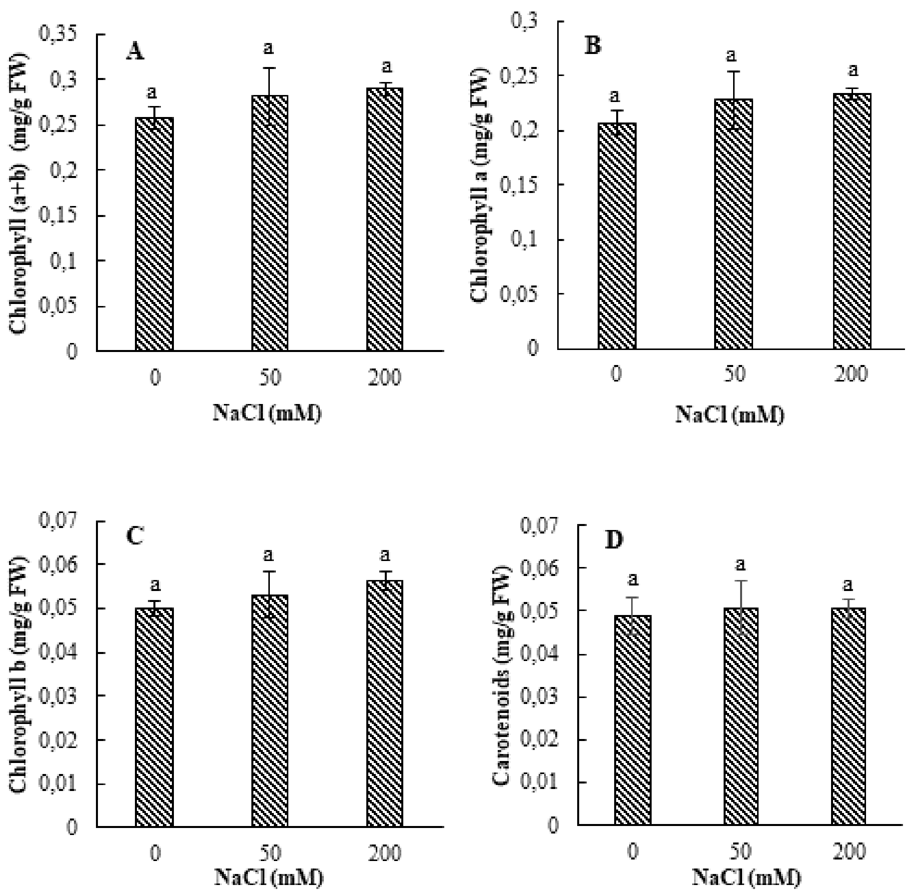

Figure 3. *Content of photosynthetic pigments of leaves at 7 days after the onset of treatment.*
A, Chl (a+b); B, Chl a; C, Chl b; D, Carotenoids (D). Different letters represent statistically significant difference between the treatments according to Duncan's test with p-value <0.05

It is reported that many salt-tolerant plants enhanced chlorophyll accumulation under salinity stress, which is considered to increase photosynthetic efficiency for contributing to salt tolerance of plants [1], [15], [18]. The enhancement of chlorophyll content in *T. tetragonioides* grown under salinity stress was reported in a previous study [10]. Here, the chlorophyll accumulation was increased not only in the salt-stressed plants, but also in the plants treated with 50 mM NaCl, suggesting that chlorophyll accumulation was required for halophilism of *T. tetragonioides*. Li et al. reported that accumulation of chlorophylls is a factor associated with stimulating carbon uptake and increasing the photosynthetic efficiency of a halophyte *Sesuvium portulacastrum* grown in saline conditions [18]. These results suggested that halophytes enhance photosynthetic activity, not only under salinity stress, but also under halophilism conditions. It is also suggested presence of a salt-enhanced photosynthesis mechanism contributes to the growth enhancement of halophytes under salinity.

3.2.2. Water uptake and proline accumulation

Salinity increases osmotic potential of extracellular environment, which can induce physiological drought that inhibiting ability of water uptake of plant cells. Leaf RWC is an indicator of water status and uptake capacity. In the present study, data showed that RWC in leaves of the plants treated with 200 mM NaCl was significantly reduced compared to salt-untreated plants. Whereas, the RWC of plants treated with 50 mM NaCl tended to be slightly increased, although the increase was not statistically significant (Figure 4A). This result indicated that *T. tetragonioides* leaf cells had the ability to enhance water uptake under halophilism conditions. Similar results were observed in many halophytes. It is suggested that enhancement in water uptake by salt may be associated with cell elongation, which is a factor contributing to the growth enhancement of halophytes under salinity [19].

Proline not only plays an important role in growth and development, but also contributes greatly to tolerance of plants to various abiotic stresses [14]. Proline can be accumulated with a high content in cytoplasm of plant cells and acts as an osmolyte that decreases cellular water potential for water uptake. As a result, increased accumulation of proline is reported in salt-tolerant plants grown under salinity, and is considered as a factor that helps plant cells resist osmotic stress caused by high salinity [1], [3].

A similar characteristic was observed in the present study. The proline content increased with increasing salt treatments (Figure 4B). At 50 mM NaCl concentration, the proline content reached 23.65 µmol/g FW, increased 15.93 times compared to the salt-untreated plants (2.58 µmol/g FW). Whereas, the proline content was increased 52.31 times (135.47 µmol/g FW) in the 200 mM NaCl-treated plants (Figure 4B). This result indicates that increased proline accumulation was not only associated with salt tolerance, but also the halophilism of *T. tetragonioides*. The proline accumulation may contribute to the increased water uptake of plant (Figure 4A). Proline accumulation under salinity was also observed in a halophyte *S. portulacastrum* [18]. It is suggested that accumulation of salt ions and compatible solutes were key factors contributing to water uptake enhancement that involved in the halophilism of ice plant (*Mesembryanthemum crystallinum*) [19].

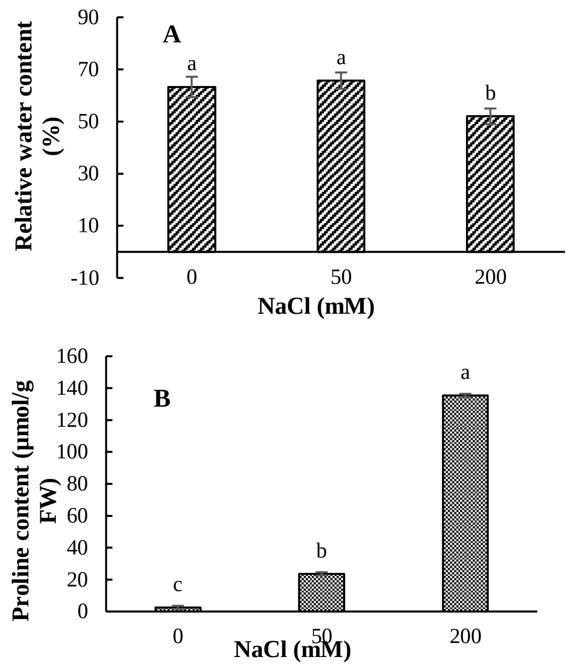

Figure 4. *Water uptake and proline accumulation of T. tetragonioides at 7 days after the onset of treatments. A, relative water content; B, proline content. Different letters represent statistically significant difference between the treatments according to Duncan's test with p-value < 0.05*

4. Conclusions

The present study showed that *T. tetragonioides* exhibited typical responses in growth and physiology under the halophilism condition. Plant biomass was increased by 35.86 - 37.04% with 50 mM NaCl. The growth enhancement was contributed by both increase in fresh and dry biomass of shoots and roots. In addition, the chlorophyll contents were increased, but carotenoid content was unchanged with increasing salinity levels. Also, the RWC was enhanced with 50 mM NaCl treatment, while proline content was increased with increasing salinity levels. The enhanced accumulation of chlorophylls, proline, and RWC suggests that *T. tetragonioides* had mechanisms that involved photosynthesis and water uptake for the halophilism. Further studies are needed to elucidate mechanisms and factors associated with the halophilism.

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