Growth, photosynthesis and H\(^+\)-ATPase activity in two Jerusalem artichoke varieties under NaCl-induced stress

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In order to evaluate differential growth, photosynthesis and H\(^+\)-ATPase activity responses to salt-induced stress, two Jerusalem artichoke (Helianthus tuberosus L.) genotypes (Nanyu No. 1 and Qingyu No. 2) were used in sand-culture experiment with different concentrations of NaCl (0, 30, 60, 90, 120 and 150 mM). After 20 days of growth, the NaCl stress resulted in a decrease of biomass accumulation, relative leaf expansion rate and photosynthetic rate, but an increase of proline content in both genotypes. Compared with Qingyu No. 2, Nanyu No. 1 had lower biomass, photosynthetic rate, gas exchange and transpiration rate, but higher proline content, activities of plasma membrane H\(^+\)-ATPase (PM H\(^+\)-ATPase) and vacuolar membrane H\(^+\)-ATPase (VM H\(^+\)-ATPase). Hence, the NaCl adaptation strategy in Nanyu No. 1 was by lowering photosynthetic rate, stomatal conductance and transpiration rate while maintaining high H\(^+\)-ATPase activities, whereas the adjustment of Qingyu No. 2 was by keeping much higher rate of proline accumulation and concentration of chlorophyll. The differences in salt tolerance showed that different adaptation mechanisms existed between cultivars of Jerusalem artichoke. The findings offered the possibility of selecting salt-tolerant genotypes of Jerusalem artichoke.

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1. Introduction

According to the FAO report, more than 800 million hectares of land in the world (about 6% of the world’s total land area) are salt affected, with 32 million ha (2%) being affected by secondary salinity to varying degrees [1,2]. Both osmotic stress and ion toxicity caused by excess salt are harmful to plant growth. For example, salt stress causes stomatal closure [3], which decreases photosynthetic CO\(_2\) assimilation. High concentration of Na\(^+\) directly inhibits photosynthetic apparatus activity, referred to as Na\(^+\) toxicity. Previous studies showed existence of different salt adaptation mechanisms [3]. One of mechanisms is to enhance osmotic regulation, for example by accumulation of compatible solutes, such as proline, to increase the cellular osmolarity, which contributes to driving water influx and/or reducing water efflux [4,5]. As a result, the cells are kept turgid and can expand. A salt-tolerant mutant of Nicotiana plumbaginifolia accumulated higher proline content than wild-type [6], and the similar results were found in sunflower [7]. Another adaptation mechanism is to extrude excess Na\(^+\) from the cell by Na\(^+\)/H\(^+\) antiporters located in the plasma membrane [8]. The inward transport of H\(^+\) (driven by the electrochemical H\(^+\) gradient established by the plasma membrane (PM) H\(^+\)-ATPase) is coupled to outward transport of Na\(^+\). Another H\(^+\)-ATPase is located in the vacuolar membrane (VM), and is responsible for compartmentalizing Na\(^+\) from the cytoplasm into the vacuole. Both PM H\(^+\)-ATPase and VM H\(^+\)-ATPase are involved in alleviating Na\(^+\) toxicity. Wakeel et al. reported that salt-tolerant sugar beet (Beta vulgaris L.) kept a higher PM H\(^+\)-ATPase activity in shoot than salt-sensitive maize (Zea mays L.) [9]. Results from cucumber root VM H\(^+\)-ATPase showed that a slight increase in VM H\(^+\)-ATPase activity was associated with a survival mechanism [10]. Moreover, Ballesteros et al. reported that a salt tolerance mechanism based on PM H\(^+\)-ATPase may exist in sunflower roots [11].

Currently, the world faces the progressive depletion of non-renewable fuel resources. At the same time, energy consumption grows at increasing rates [12]. The solution to this problem depends on the development and implementation of technologies based on alternative sources of energy. Bioethanol is regarded as the best alternative to fossil fuels for reducing greenhouse gas emissions [13]. Bioethanol can be obtained from energy crops with a large yield of lignocellulosic biomass, such as corn, wheat and
sugar cane [14]. However, the production of commercial quanti-
ties of bioethanol from corn, wheat and sugar cane requires a
huge amount of arable land that are also needed for food produc-
tion. Hence, using marginal land unsuitable for food produc-
tion (e.g. saline or alkaline soils) for production of energy crops is an
important consideration.
Jerusalem artichoke (Helianthus tuberosus L.) has fallen out of
favour as an agricultural crop [15]. Nevertheless, the reintroduction
of H. tuberosus has been studied in many countries, particularly in
relation to its hardness and low production costs [16]. H. tuberosus
is of interest as a food for human consumption (including bever-
age alcohol and beer), feed and forage for animal [17]. It is also a
source of fructose syrups for the food industry, as well as fructans
for medical or dietetic purposes [18]. Moreover, H. tuberosus can
also be used as anti-erosion protection to fix terraces or unstable
sand, or as a barrier against fire in large forests [16]. At present, 
H. tuberosus is mainly grown as a potential biomass crop for ethanol
production, not only because it commonly yields around 7 and
potentially up to 14 tonnes of biomass per hectare (tha−1) [15], but
also because it has numerous industrial uses, such as paper pulp
or fuelwood from stems, methanol production from various plant
parts, acetone–butanol–ethanol production from tubers or whole
plants and hydroxymethylfurfural from tubers as a basic molecule
for the chemical industry [16,17,19].
In order to achieve low-cost raw materials for bioethanol pro-
duction, we proposed a new strategy for H. tuberosus cultivation in
marginal lands (saline and alkaline soils). This strategy could be
of particular importance in countries with scarce arable land
but high population, such as China. In saline environments, plants
need to combat the salinity stress by balancing the survival and
growth. Therefore, it is important to screen for H. tuberosus eco-
types that can tolerate salinity. Previous studies showed large
differences in salt tolerance among varieties of H. tuberosus. Long
et al. found that greater photosynthesis capacity, higher relative
growth rate, and relatively higher tissue Na⁺ accumulation at high
seawater concentrations appeared to be associated with seawa-
ter tolerance in H. tuberosus varieties [20]. Xue et al. investigated
salt-tolerant and salt-sensitive cultivars of H. tuberosus under salt
stress and suggested that the system for removing reactive oxygen
species is important for salt tolerance [21]. In the present study,
two genotypes of H. tuberosus most successfully grown in China
were used to evaluate the effect of NaCl stress on biomass accumula-
tion, leaf expansion rate, photosynthetic capacity and H⁺-ATPase
activity and assess the adaptation strategy of H. tuberosus under NaCl
stress.
2. Materials and methods
2.1. Plant material and growth conditions

Two H. tuberosus genotypes Nanyu No. 1 (developed by Liu Zhaopu and Long Xiaoqua at Nanjing Agricultural University, with white tuber surface, adapted to coastal area, the growth and development period of about 230 days, designated NY-1) and Qingyu No. 2 (selected by Qinghai Academy of Agricultural Science, with purple tuber surface, adapted to highland climate, the growth and development period of about 190 days, designated QY-2) [22] were vegetatively propagated by cutting approximately 2.0 g tuber slices containing one bud from the tuber after dorm-
ancy. Tuber slices were surface-sterilized with HgCl₂ (1.0 g/L) for 10 min, rinsed
thoroughly with deionized water and sprouted in plastic containers covered with
moist acid-washed quartz sand. The tuber slices were watered using 1/2 strength
Hoagland solution [23]. The nutrient solution was replaced every two days. Fif-
ten days later, plants of uniform size with three fully extended leaves and one
expanding leaf were transplanted to drained pots containing quartz sand. The pots
were placed in a plastic basin containing the same nutrient solution. Two days after
transplanting, the treatments started with 0, 30, 60, 90, 120 and 150 mM NaCl in
1/2 strength Hoagland solution. The treatment without exogenous NaCl was used as
the control. Treatment solutions were replaced every two days. The plants were
grown in a greenhouse with daily photoperiod of 12 h at a photon flux density of
392–415 μmol m⁻² s⁻¹ and maximum/minimum temperature of 25/18 °C and the
relative humidity of 65–75%.

2.2. Growth analysis

Plants were harvested 20 days after salt application. The fresh weights were
determined, and then the plant material was dried at 70 °C for 72 h and re-weighed
for dry weight.

The length and width of the third topmost expanding leaf were measured using
a ruler before commencement of treatments (Lt) and at harvest (Lh) [24]. The leaf
expansion rate (LER) was calculated as: LER (%) = (Lh − Lt)/Lt × 100. The relative
leaf expansion rate (RLE) was expressed as: RLE = LER × 100/LER cont × 100.

The pigments were extracted according to Moharek et al. [25], with some modifications: 0.2 g fresh sample was ground with a mortar and pestle with 2–3 mL of
95% (v/v) ethanol in dim light, and the homogenate was centrifuged at 5000 × g
(2521 rotor, Beckman Coulter Avant™ J-2 CentriFuge, Fullerton, CA, USA) at 4 °C for
5 min. The supernatant containing pigments was collected and diluted to a defined
template of 25 mL with 95% (v/v) ethanol. The pigment contents were determined
by measuring absorption at 470, 649 and 665 nm with a spectrophotometer (722N,
Shanghai Precision Scientific & Instrument Co., Ltd., China). The chlorophyll a (Ch),
chlorophyll b (Chb) and carotenoid contents (Ccar) were calculated according to Li
[26]:

\[
C_a = 13.5A_{645} - 6.8A_{663}
\]

\[
C_b = 24.9A_{663} - 7.3A_{645}
\]

\[
C_{car} = 1000A_{470} - 2.05 \times C_a - 114.8 \times C_b
\]

2.3. Photosynthetic parameters

At harvest, the net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) of new fully expanded leaves were measured by a Li-6400
portable photosynthesis-open system with 6400-028 LED source (LI-COR, USA).
The measurements were conducted on a sunny day, from 9:00 to 14:00 h with photo-
synthetic photon flux density (PPFD) of 1200 μmol m⁻² s⁻¹, temperature at 25 °C,
relative humidity of 60%, and CO₂ concentration in air at 370 μmol mol⁻¹.

2.4. Proline content determination

The proline content in roots was determined according to the method of Bates et
al. [27]. Briefly, roots were reacted with aqueous sulfosalicylic acid, and the filtrate
was used for the color reaction with acid ninhydrin and glacial acetic acid. The
reaction mixture was extracted with toluene. The absorbance was read at 520 nm.

2.5. Membrane vesicles isolation

Membrane vesicles were prepared using the method described by Wilson et al.
[28], with minor modification. Briefly, 15.0 g of fresh roots (dilat 2 cm only) were
rinsed with deionized water and homogenized in 30 mL of ice-cold buffer contain-
ing 30 mM Hepes-Tris (hydroxymethyltrisbasic, pH 7.5), 250 mM mannitol, 3 mM
EGTA (ethylene glycol tetraacetic acid), 3 mM EDTA (ethylene diamine tetraacetic
acid), 250 mM KCl, 1 mM PMSF [phenylmethylsulfonyl fluoride], 2 mM DTT (dithio-
treating), 10% (v/v) glycerin, 50 g/L PVF (polyvinylpyrrolidone), and 0.2% (w/v)
BSA (bovine serum albumin). The homogenate was filtered through four layers of
cheesecloth and centrifuged at 13,000 × g for 30 min. The supernatant was then centrifuged
at 50,000 × g (the rotor as above) for 30 min. The microsomal pellets were gently
washed with 2 mL of re-suspension buffer containing 2.5 mM-mes-tris (pH 7.5),
250 mM sorbitol, 1 mM EGTA, 1 mM PMSF, and 2 mM DTT. The microsomal mem-
brane preparation was laid over three sucrose step-gradients of 45%, 36% and 22%
(w/v). The gradient system was centrifuged at 100,000 × g (sw41 rotor, Beckman
Coulter Optima™ L-80 XP Ultracentrifuge, Fullerton, CA, USA) for 2 h. The vacuole
membrane vesicles were collected at the interface of 22/36%, and the plasma
membrane vesicles at the 36/45% interface. The whole process of membrane vesicles
isolation was conducted at 4 °C, and vesicles preparations were stored at −80 °C
for one week.

2.6. Hydrolytic activity of membrane vesicles H⁺-ATPase

The hydrolytic activity of PM H⁺-ATPase was assayed by adding 50 μL plasma
membrane vesicles into 500 μL reaction medium containing 30 mM Hepes-Tris (pH
6.5), 50 mM KNO₃, 3 mM MgSO₄, 0.1 mM (NH₄)₂MoO₄, 0.1 mM NaCl, and 0.01% (v/v)
Triton X-100. The reaction was initiated by adding 3 mM ATP-γ-Na. After 20 min of
reaction at 37 °C, the reaction was stopped by adding 50 μL 5% (v/v) trichloroacetic
acid. Inorganic phosphate hydrolyzed by the plasma membrane vesicles was deter-
mined according to Ohnishi et al. [29]. The protein content of membrane vesicles
was calculated from a BSA standard curve as described by Bradford [30].

The hydrolytic activity of VM H⁺-ATPase was assayed similarly to the PM H⁺-
ATPase assay, except that the reaction medium contained 30 mM Hepes-Tris (pH
7.5), 50 mM KCl, 3 mM MgSO₄, 0.1 mM (NH₄)₂MoO₄, 0.1 mM NaN₃, and 0.25 mM
Na₂VO₄. The hydrolytic activity was expressed as μmol Pi mg⁻¹ protein h⁻¹.
2.7. Statistical analysis

All data were analyzed by Microsoft Excel 2003 (Microsoft Corp., Redmond, WA, USA) and SPSS 16.0 (SPSS Corp., Chicago, IL, USA). Two-way analysis of variance (ANOVA) was used to determine the significant interaction between salt treatments and genotypes. Significant differences among means were tested using Duncan’s test.

3. Results

3.1. Growth response to salt stress

The NaCl treatment significantly reduced the fresh and dry weights of both NY-1 and QY-2 (Table 1). Compared to control, the whole plant fresh weight in the NaCl treatments decreased from 15.4 to 75.5% for NY-1 and from 13 to 56.8% for QY-2. The dry weight of QY-2 was higher than that of NY-1 under the same NaCl treatments. Leaf length and width expansion rates were decreased in the NaCl treatments (Table 2 and Fig. 1). Compared to control, the leaf length expansion rate significantly decreased at 30 mM NaCl for NY-1; however, for QY-2 no significant difference in leaf length expansion rate was found at NaCl concentration lower than 60 mM. Regarding the leaf width expansion rate, a significant decrease was found for QY-2 when treated with 30 mM NaCl, while there was no significant difference up to 90 mM NaCl for NY-1 (Table 2). The relative leaf width expansion rate was higher in QY-2 than NY-1 (Fig. 1).

3.2. Proline concentration increased during salt stress

The change in proline content was similar in the two genotypes and increased with an increase in NaCl treatment concentrations. NY-1 accumulated more proline in roots than QY-2. However, QY-2 kept a higher rate of proline content increase in roots than NY-1 under salt stress (Fig. 2).

3.3. Photosynthetic rate decreased during salt stress

The NaCl treatment significantly decreased C4 and C5 contents in NY-1. In contrast, in QY-2, NaCl stress from 30 to 120 mM increased the C4 content and from 30 to 90 mM increased C5 content compared to control, whereas C6 content increased in both NY-1 and QY-2. Compared to control, the values of C4/C5 increased for the two varieties when subjected to increasing NaCl stress. Under given severity of salinity stress, QY-2 kept higher C4 and C5 contents than NY-1 (Table 3).

In NY-1, NaCl stress decreased photosynthetic rate compared to control. However, in QY-2, no significant difference was noted with 30 and 60 mM NaCl compared to control. QY-2 showed a higher Pn in the NaCl treatments compared with NY-1 (Fig. 3).

With salt stress, no significant decrease was observed in stomatal conductance in QY-2 compared to control. For NY-1, however, NaCl stress caused a significant reduction in stomatal conductance under NaCl stress compared to control. The stomatal conductance was higher in QY-2 than NY-1 in the NaCl treatments (Fig. 3). NaCl stress (30 mM and higher) significantly decreased transpiration rate in NY-1. The reduction in transpiration rate in QY-2 was not observed when treated with 30 and 60 mM NaCl. Higher transpiration rate was measured in QY-2 than NY-1 when seedlings were exposed to the NaCl treatments (Fig. 3).

3.4. H+-ATPase activity under salt stress

Compared to control, the plasma membrane H+-ATPase activity significantly decreased in the 150 mM NaCl treatment in both NY-1 and QY-2. For QY-2, 30 mM NaCl increased the plasma membrane H+-ATPase activity; for NY-1, 60 and 90 mM NaCl still stimulated the H+-ATPase activity. Compared to QY-2, NY-1 kept a higher H+-ATPase activity under 0, 30, 60, 90 and 120 mM NaCl treatments (Fig. 4).

The vacuolar membrane H+-ATPase activity in NY-1 significantly increased in the 60 mM NaCl treatment. Compared to control, the H+-ATPase activity in QY-2 significantly increased at 30 and 60 mM NaCl. However, NY-1 kept a higher vacuolar membrane H+-ATPase activity than QY-2 when NaCl concentrations were higher than 60 mM.
Table 1
Effect of NaCl stress on fresh and dry weights of two *H. tuberosus* genotypes after 20 days of treatment.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>NaCl treatment (mM)</th>
<th>Fresh weight</th>
<th>Dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (g plant⁻¹)</td>
<td>Relative decrease (%)</td>
<td>Total (g plant⁻¹)</td>
</tr>
<tr>
<td>NY-1</td>
<td>0</td>
<td>1.34 ± 0.04a</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.14 ± 0.01b</td>
<td>14.93</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.00 ± 0.05c</td>
<td>25.75</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.87 ± 0.10c</td>
<td>35.45</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.82 ± 0.04c</td>
<td>38.81</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.81 ± 0.04c</td>
<td>38.81</td>
</tr>
<tr>
<td>QY-2</td>
<td>0</td>
<td>1.74 ± 0.05A</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.51 ± 0.06B</td>
<td>13.51</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.29 ± 0.04BC</td>
<td>25.86</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>1.27 ± 0.05C</td>
<td>27.01</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.99 ± 0.03D</td>
<td>43.39</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.92 ± 0.09D</td>
<td>47.13</td>
</tr>
</tbody>
</table>

Note: Values represent means ± SE of three independent experiments. Significant differences (P ≤ 0.05) between the NaCl treatments are indicated by different letters. Lower case letters and capital letters represent NY-1 and QY-2, respectively.

Table 2
Effect of NaCl stress on the leaf expansion rates (length and width) in two *H. tuberosus* genotypes after 20 days of treatments.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>NaCl treatment (mM)</th>
<th>Leaf length expansion rate (%)</th>
<th>Leaf width expansion rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NY-1</td>
<td>0</td>
<td>28.57 ± 1.99a</td>
<td>29.36 ± 5.24a</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>19.83 ± 2.39b</td>
<td>19.77 ± 1.48b</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>12.16 ± 0.04c</td>
<td>12.16 ± 0.04C</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>9.89 ± 0.92c</td>
<td>9.89 ± 0.92c</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>7.27 ± 1.94c</td>
<td>7.27 ± 1.94c</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>30.15 ± 5.47A</td>
<td>30.15 ± 5.47A</td>
</tr>
<tr>
<td>QY-2</td>
<td>0</td>
<td>21.18 ± 2.01AB</td>
<td>21.18 ± 2.01AB</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>16.44 ± 1.62BC</td>
<td>16.44 ± 1.62BC</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>11.27 ± 1.89C</td>
<td>11.27 ± 1.89C</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>7.29 ± 1.04C</td>
<td>7.29 ± 1.04C</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>25.54 ± 0.27B</td>
<td>25.54 ± 0.27B</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>26.33 ± 0.88B</td>
<td>26.33 ± 0.88B</td>
</tr>
</tbody>
</table>

Note: Values represent means ± SE of three independent experiments. Significant differences (P ≤ 0.05) between the NaCl treatments are indicated by different letters. Lower case letters and capital letters represent NY-1 and QY-2, respectively.

4. Discussion

This study demonstrated the genetic variation in *H. tuberosus* regarding responses to NaCl-induced stress, offering the possibility of identifying a *H. tuberosus* genotype superbly adapted to salinity. Although NY-1 and QY-2 both decreased fresh and dry weights under salt stress, QY-2 had higher fresh and dry weights than NY-1 with increasing concentrations of NaCl stress (Table 1). Compared to QY-2, a larger decrease in fresh weight of NY-1 indicated a slower growth rate. According to the theory of survival and growth by Munns et al. [31], a growth reduction would be a potential energy cost for salinity combat. The different fresh/dry weights decreasing rates in NY-1 and QY-2 showed different abilities to balance survival and growth.

Previous works on grass [33], maize [34] and rice [35] showed that salinity resulted in a lower water status and imbalanced osmotic adjustment, which led to the loss of cell turgidity and resulted in a small leaf size. The 20 days of NaCl stress induced a decrease in leaf expansion rate for NY-1 and QY-2 (Table 2), which was in line with previous finding by Munns et al. [32] in cereal varieties with salt stress lasting for more than 3 weeks. As a crucial indicator of crop growth [36], the leaf length and width expansion rates were higher in QY-2 than NY-1, which suggested a better capacity to maintain growth in QY-2 than NY-1 under salinity stress. The greater leaf area accompanied

Table 3
Effect of increasing NaCl stress on chlorophyll a, chlorophyll b and carotenoid contents in leaves of two *H. tuberosus* genotypes after 20 days of treatments.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment (mM)</th>
<th>Cₐ (mg g⁻¹ FW)</th>
<th>C₉ (mg g⁻¹ FW)</th>
<th>C₃b (mg g⁻¹ FW)</th>
<th>C₉/C₃b</th>
</tr>
</thead>
<tbody>
<tr>
<td>NY-1</td>
<td>0</td>
<td>1.031 ± 0.004a</td>
<td>0.831 ± 0.005a</td>
<td>0.027 ± 0.002d</td>
<td>0.015 ± 0.001c</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.957 ± 0.002b</td>
<td>0.762 ± 0.002b</td>
<td>0.036 ± 0.001c</td>
<td>0.021 ± 0.001b</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.934 ± 0.008c</td>
<td>0.754 ± 0.011bc</td>
<td>0.038 ± 0.005bc</td>
<td>0.023 ± 0.003b</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.895 ± 0.002d</td>
<td>0.742 ± 0.002c</td>
<td>0.038 ± 0.001bc</td>
<td>0.023 ± 0.001b</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.758 ± 0.001e</td>
<td>0.625 ± 0.002d</td>
<td>0.048 ± 0.001a</td>
<td>0.035 ± 0.001a</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.671 ± 0.003f</td>
<td>0.531 ± 0.002e</td>
<td>0.045 ± 0.001ab</td>
<td>0.038 ± 0.001a</td>
</tr>
<tr>
<td>QY-2</td>
<td>0</td>
<td>1.182 ± 0.001A</td>
<td>0.925 ± 0.006A</td>
<td>0.048 ± 0.003b</td>
<td>0.023 ± 0.002BC</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.139 ± 0.006B</td>
<td>0.919 ± 0.004A</td>
<td>0.046 ± 0.001A</td>
<td>0.022 ± 0.001BC</td>
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<td>60</td>
<td>1.134 ± 0.002B</td>
<td>0.894 ± 0.002B</td>
<td>0.044 ± 0.001A</td>
<td>0.022 ± 0.001BC</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>1.080 ± 0.003C</td>
<td>0.785 ± 0.001D</td>
<td>0.044 ± 0.001A</td>
<td>0.025 ± 0.001B</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.773 ± 0.001D</td>
<td>0.619 ± 0.002E</td>
<td>0.042 ± 0.001A</td>
<td>0.030 ± 0.001A</td>
</tr>
</tbody>
</table>

Note: Values represent means ± SE of three independent experiments. Significant differences (P ≤ 0.05) between the NaCl treatments are indicated by different letters. Lower case letters and capital letters represent NY-1 and QY-2, respectively.
by increased transpiration rate would exacerbate water loss and cause deleterious effects on plant growth under salt stress [31]. Hence, in comparison with QY-2, a lower leaf expansion rate in NY-1 appeared to be a water-saving mechanism under salt stress.

The C₄ content under stress was a result of chlorophyll degradation and chlorophyll synthesis [37]. Previous study on sunflower [38] showed that salt stress exerted a stronger influence on chlorophyll synthesis than chlorophyllase-mediated chlorophyll degradation. Moreover, the results showed that 21 days of 25 mM NaCl stress caused a higher C₄ content compared to control, whereas NaCl concentration higher than 25 mM caused a decrease in C₄ content [38]. In the study, compared with control, C₄ and C₅ content in QY-2 increased as NaCl concentration increased, potentially indicating a better capacity to adapt to salinity stress because a higher content of chlorophyll could decrease oxidative stress caused by excess radiation [25]. In addition, NaCl stress could increase carotenoid content, which could contribute to alleviating oxidative stress. The ratio of C₅/C₄₊b increased with increased severity of NaCl stress (Table 3), indicating a protective mechanism to prevent photooxidation [39].

According to the study of Aster tripolium L. and Sesuvium portulacastrum L. by Ramani et al. [40], decreased stomatal conductance and transpiration rate would represent a protective mechanism to decrease excess accumulation of toxic ions in shoots, thus minimizing ion toxicity. In the present study, lower rate of stomatal conductance and transpiration in NY-1 suggested greater capacity to alleviate ion toxicity compared with QY-2. However, a further investigation should be carried out to determine whether a decrease in photosynthetic rate was an adaptive response to stress or a deleterious effect of stress.

We found that both NY-1 and QY-2 increased proline content with an increase in NaCl concentration. However, QY-2 kept a higher rate of proline accumulation with increasing salinity levels than NY-1. A study on variation in salinity tolerance of sunflower showed that salt-tolerant accessions had a greater proline content than salt-sensitive accessions, which could be an important component of salt tolerance in sunflower [7]. The higher proline content in NY-1 may result in more osmotic regulation than in QY-2. However, QY-2 keeping a higher rate of proline accumulation indicated a stronger osmotic regulation mechanism to adapt to salt stress.

The vacuole of high plant cells is an important component of cellular physiology, acting as a storage compartment to maintain the cellular metabolism under ion toxicity. The plasma and vacuolar membranes contain H⁺-ATPase that plays a pivotal role in nutrient uptake and is responsible for generating an electrochemical potential gradient across the plasma and vacuolar membranes that drive ion transport. Therefore, H⁺-ATPase is crucial for plant adaptation to stress. Our results showed that relatively low NaCl concentrations would increase the H⁺-ATPase activity, with both

Fig. 4. Effects of NaCl stress on the activities of plasma membrane H⁺-ATPase (A) and vacuolar membrane H⁺-ATPase (B) isolated from roots of two H. tuberosus genotypes after 20 days of treatments. Values represent means ± SE of three independent experiments. Significant differences (P ≤ 0.05) between the NaCl treatments are indicated by different letters. Lower case letters and capital letters represent NY-1 and QY-2, respectively.
cultivars showing some capacity to maintain H+-ATPase activity with increasing stress levels. The same tendency was found in the vacuolar H+-ATPase activity in barley [41]. Our results showed that NY-1 kept a greater activity of H+-ATPase in the plasma and vacuolar membranes than QY-2, thus having better maintenance of an electrochemical potential gradient to drive nutrient uptake under salt stress.

5. Conclusions

Salinity stress significantly inhibited $H$. tuberosus growth, causing reduction in biomass, relative leaf expansion rate and photosynthetic rate. In contrast, the proline content in roots increased with increasing salt concentrations. After 20 days of NaCl stress, NY-1 showed lower biomass and photosynthetic rate but higher H+-ATPase hydrolysis activity in the plasma and vacuolar membranes compared with QY-2. Our data suggest that the adaptation strategy for NY-1 under salt stress was by keeping higher activity of H+-ATPase and lower Pn, Gs and Tr. However, in QY-2, the adjustment was by higher rate of proline accumulation and higher chlorophyll concentration under salt stress. Energy was allocated for combating salt stress, which resulted in a decreased growth rate. There may be different feedback mechanisms in NY-1 and QY-2 to balance survival and growth under stress.

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