Salt Stress Treatment and Salt Exposure time Altered Physiological Characteristics in Thai Rice (*Oryza sativa* L. subsp. *indica*)

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Abstract

The objective of this study was to investigate physiological responses in Thai rice (*Oryza sativa* L. subsp. *indica*) varieties. Two Thai rice varieties (Homjan and Pathumthani 1), three NaCl concentrations (0, 171, 342 mM) and three salt exposure times (2, 4, 8 days) were designed in a 2×3×3 Factorial in Completely Randomized Design (CRD) with 4 replications. This study showed that the root and leaf osmotic potential in HJ and PT1 seedlings were severely reduced when NaCl concentration and salt exposure time were increased. The photosynthetic pigment concentrations and chlorophyll *a* fluorescence parameters were significantly decreased with increasing the salt stress treatment and salt exposure time. Moreover, these results exhibited that the decrease in photosynthetic pigment concentrations and chlorophyll *a* fluorescence parameters in PT1 salt-stressed seedlings were higher than that in HJ salt-stressed seedlings. The reduction of photosynthetic pigment concentrations and chlorophyll *a* fluorescence parameters in both HJ and PT1 salt-stressed seedlings resulted in the growth reduction. These results demonstrated that the differences of physiological responses in rice may be further used in identifying salt tolerance ability in Thai rice.

Keywords: Salt stress, osmotic potential, photosynthesis, pigments, rice

1. Introduction

Rice (*Oryza sativa* L.) was a chief food for feeding and providing the daily calories for three billions of people [1]. However, the growth and yield of rice were decreased more than fifty percentages in worldwide by abiotic extreme conditions particularly salt stress [2, 3, 4]. Salt stress was the major factor to limit plant growth and productivity in many areas of the world [5]. Most cultivation area for rice production was affected by salinity soil [6]. Since salt affected soil area in the world was approximately 10^8-10^9 ha that it was three times more than the agricultural area [7]. The rice crop was identified as a salt sensitive plant that was negatively affected at seedling and reproductive stages [8, 9, 10, 11]. Many previous researches exhibited that the rice growth and development were reduced when subjected the salt stress [8, 12, 13, 14]. Salt stress was initiated by accumulation of salt in the soil solution, especially NaCl that induced osmotic stress and ionic stress [15, 16]. Enhancement of salt accumulation disturbed physiological characteristics in plants such as ion accumulation [14, 17, 18, 19], ion selectivity [20, 21, 22, 23] and photosynthesis [16, 25]. Photosynthetic efficiency was limited by photosynthetic pigment concentration and chlorophyll *a* fluorescence parameters.

The photosynthetic pigments played important role on the photosynthesis including; chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (TC) and total carotenoid (C_{x+c}). The photosynthetic pigments contained in the chloroplast that were composed of light harvesting complexes namely photosynthetic apparatus. In addition, the chlorophyll *a* fluorescence parameters such as maximum quantum yield (F_v/F_m), photosystem II quantum efficiency (Φ_{PSII}), photochemical quenching (qP) and non-photochemical quenching (NPQ) exhibited the water oxidation and photosynthetic electron transport in photosystem II (PSII) under stress condition [26, 27].

Under salt stress, the excess salt particularly sodium ion (Na^+) and chloride ion (Cl^-) damaged chloroplast that was sensitive to salt ion, leading to destroy the chloroplast ultrastructure, resulting in dysfuntion in the light harvesting complexes [18, 28]. Many researches showed salt stress treatment reduced photosynthetic pigment concentrations and chlorophyll *a* fluorescence parameters in rice [13], castor bean [29], raspberry [30], sunflower [31] and

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citrus [32]. Therefore, the aim of this research was to investigate the physiological characteristics and growth performances in Thai rice response to various salt stress treatments and salt stress periods.

2. Materials and mehods

2.1 Plant material and growth condition

Seeds of Homjan (HJ) and Pathumthani1 (PT1) rice (*Oryza sativa* L. subsp. *indica*) were obtained from Pathumthani Rice Research Center, Rice Research Institute, Department of Agriculture, Ministry of Agriculture and Cooperative, Pathumthani, Thailand. The seeds were dehusked and rinsed with 70% (v/v) ethanol for 1 min, disinfected once in 5% (v/v) Clorox® (5.25% (w/v) sodium hypochlorite solution, Clorox Co. Ltd., Oakland, CA, USA) with 0.1% (v/v) Tween-20® (Merck, Germany) for 12 h, once in 25% (v/v) Clorox® for 30 min, and then rinsed with sterile distilled water. Surface sterilized seeds were then germinated on MS semi-solid medium [33] supplemented with 3% (w/v) sucrose and adjusted to pH 5.7. Cultures of HJ and PT1 seeds were incubated under 25±2°C air temperature, 60±5% relative humidity (RH), 60±5 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF) provided by fluorescent lamps (TLD 36 W/84 Cool-White 3350 Im, Philips, Thailand) for 16 h d^{-1} photoperiod for 14 days.

2.2 Salt stress treatments

Fourteen-day-old HJ and PT1 seedlings were aseptically transferred to 60 mL MS sugar-free liquid medium by using vermiculite as supporting material for 7 days. Air-exchange rate in the glass vessels was adjusted to 2.32 μmol CO_2 h^{-1} by punching a hole in the plastic cap (Ø 1 cm) and covering the hole with a gas-permeable microporous polypropylene film (0.22 μm pore size, Nihon Millipore Ltd., Tokyo, Japan). Consequently, the culture medium was adjusted to 0, 171 and 342 mM NaCl. After 2, 4 and 8 days of salt treatments, osmotic potential of roots and leaves, photosynthetic pigment concentrations, chlorophyll *a* fluorescence parameters and growth were measured.

2.3 Physiological response measurements

2.3.1 Osmotic potential

Root and leaf osmolalities were measured according to [34] by using a vapor pressure osmometer (5520 Vapro®, Wescor, Inc., USA). The osmolality was converted to the osmotic potential according to Kozai *et al.* [35] equation;

$$Osmotic\ Potenyial\ (bar) = 4.6153 \times (273.16 + t) \times \rho_w \times \ln(55.509 / (55.509 + a))$$

where, t was temperature (°C)
ρ_w was density of water at t °C (g cm^{-3})
a was osmolality (mol kg^{-1})

$$Osmotic\ Potenyial\ (MPa) = \frac{1}{10} \times Osmotic\ Potenyial\ (bar)$$

2.3.2 Photosynthetic pigment concentrations

Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (TC) and total carotenoids (C_{x+c}) concentrations were determined by following Shabala *et al.* [36] and Lichtenthaler [37]. One hundred milligrams of leaf tissues were placed in the glass vessel (Opticlear®; KIMBLE, Vineland, New Jersey, USA), 10 mL 95.5% (v/v) acetone was added prior to blending with a homogenizer (T25 Basic ULTRA-TURRAX®; IKA, Kuala Lumpur, Malaysia). To prevent evaporation, the glass vessels were sealed with parafilm and then stored at 4°C for 48 h. The Chl *a*, Chl *b*, TC and C_{x+c} concentrations were measured by using an UV-visible spectrophotometer (DR/4000; HACH, Loveland, Colorado, USA) at 662, 644 and 470 nm. An acetone solution was used as a blank. The Chl *a*, Chl *b*, TC and C_{x+c} concentrations in the leaves were calculated according to the following equations;

$$\text{Chl}\,a = 9.784D_{662} - 0.99D_{644}$$
$$\text{Chl}\,b = 21.42D_{644} - 4.65D_{662}$$
$$\text{TC} = \frac{1000D_{470} - 1.90\text{Chl}\,a - 63.14\text{Chl}\,b}{214}$$

where, D_i was an optical density at the wavelength i

2.3.3 Chl a fluorescence parameters

Chl *a* fluorescence emission of adaxial leaf surface was monitored by Fluorescence Monitoring System (FMS 2; Hansatech Instruments Ltd., Norfolk, UK) in the pulse amplitude modulation mode, as previously described by [38]. The maximum quantum yield of PSII (F_v/F_m), quantum efficiency of PSII (Φ_{PSII}), photochemical quenching (qP) and non-photochemical quenching (NPQ) in the leaf tissues were evaluated by FMS software for Windows (Fluorescence Monitoring System Software; Hansatech Instruments Ltd., Norfolk, UK), and were calculated as described by Maxwell and Johnson [26].

2.3.4 Growth

To compare growth of seedlings among the control and the NaCl-treated plants, fresh weight (FW) and dry weight (DW) were measured. The FW of roots and shoots were immediately weighted after the seedlings were exposed to salt stress at 2, 4 and 8 days. For DW measurement, the roots and shoots were dried at 110°C in a hot-air oven (Memmert, Model 500, Germany) for 48 h prior to cooling down in a desiccator and measuring the DW.

2.3.5 Experimental design and statistical analysis

The experiment was designed as 2×3×3 factorials in a Completely Randomized Design (CRD), respectively. Four replicates (n = 4) and five seedlings per replication were used. Significant level was determined by one-way analysis of variance (ANOVA) by using the SPSS software (SPSS for Windows, SPSS Inc., USA). Mean values were

compared by the Duncan's Multiple Range Test (DMRT).

3. Results and Discussions

Physiological responses in term of the osmotic potential, photosynthetic pigment concentrations, chl a fluorescence parameters and growth in HJ and PT1 seedlings exposed to 0 , 171 and 342 mM NaCl for 2, 4 and 8 days were observed. In HJ and PT1 salt-stressed seedlings, the osmotic potential in roots was reduced when NaCl concentration was increased. Two days after salt treatments, the root osmotic potential in HJ and PT1 rice under 342 mM NaCl was decreased by 1.8 and 2.2 times, respectively when compared to the control. There was slightly changed during 4 and 8 days after salt treatments (Fig. 1A and 1B) whereas root osmotic potential in both HJ and PT1 rice exposed to 171 mM NaCl was not significant from the control at all salt exposure times (Fig. 1A and 1B). In addition, the leaf osmotic potential in HJ and PT1 salt-stressed seedlings was rapidly decreased with the increasing of NaCl concentration (171 and 342 mM) and salt exposure time (2, 4 and 8 days) (Fig. 1C and 1D). The highest reduction of the leaf osmotic potential was found in the HJ and PT1 rice exposed to 342 mM NaCl for 2 days that reduced by 4.4 and 4.3 times, respectively when compared to the control (Fig. 1C and 1D).

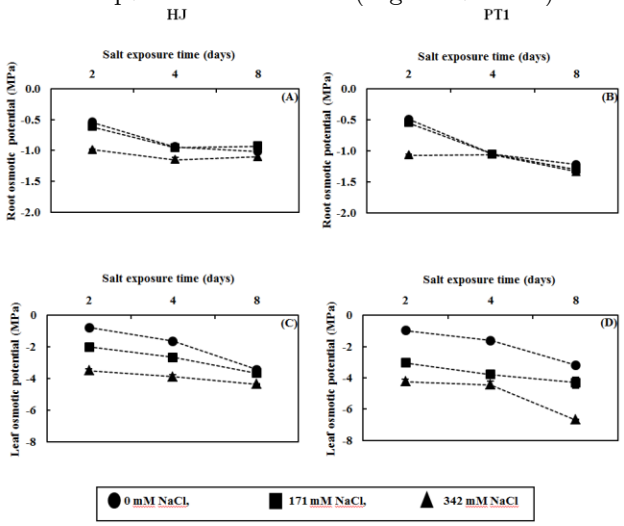

Figure 1 Change of the root and leaf osmotic potentials in HJ (A and C) and PT1 (B and D) seedlings when exposed to 0, 171 and 342 mM NaCl for 2, 4 and 8 days.

Reduction of the osmotic potential in the salt-stressed plants depended mainly on varieties [39], organ [40], salt concentration [41] and salt exposure time [42]. From the result in Fig. 1 the osmotic potential in roots and leaves of the control treatment was reduced at 4 and 8 days. Because, the seedlings were cultured in the closed system (glass vessels) without changing or refilling the medium, seedlings may accumulate more ions and lost the water from the seedlings via transpiration.

Increasing of the NaCl concentration reduced the osmotic potential in the culture medium which led to water deficit condition [43, 44]. In order to uptake water from the medium, the seedlings have to adjust itself by lowering osmotic potential [16, 45]. This result confirmed the reduction of the osmotic potential in HJ and PT1 under salt stress, especially leaf tissues. This fact was supported by Cha-um *et al.* [40] and Giaveno *et al.* [46] who reported that the osmotic potential in salt-stressed leaves was lower than that in the roots.

In this study, the leaf osmotic potential in PT1 salt-stressed seedlings was severely decreased more than those in HJ salt-stressed seedlings. It was possible that the reduction of osmotic potential was directly related to the increasing of Na^+ accumulation in leaves which concurred to the report of rice [14, 47, 48]. Moreover, relationship between NaCl concentration and osmotic potential reduction was found in the other plants such as barley [49], *Rumex* [50], red raspberry [30] and safflower [51].

In current study, chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (TC) and total carotenoids (C_{x+c}) were decreased after exposed to salt stress, except Chl *a* and TC in HJ at 171 mM NaCl (Fig. 2A and Fig. 3A). The Chl *a* and Chl *b* in HJ and PT1 salt-stressed seedlings were not significant difference when compared to the control after two days of salt treatments. There were significant reduction of Chl *a* and Chl *b* in HJ and PT1 rice when exposed to 171 and 342 mM NaCl for 4 and 8 days. The Chl *a* and Chl *b* in PT1 salt-stressed seedlings exposed to 171 and 342 mM NaCl were reduced more than those in HJ salt-stressed seedlings (Fig. 2). The TC in HJ and PT1 salt-stressed seedlings was severely reduced in comparing to the control at all salt exposure times. Eight days after salt treatments, the reduction of TC in HJ and PT1 rice exposed to 342 mM NaCl was severely reduced by 5.0 and 22.2 times, respectively when compared to the non-stressed seedlings (Fig. 3A and 3B).

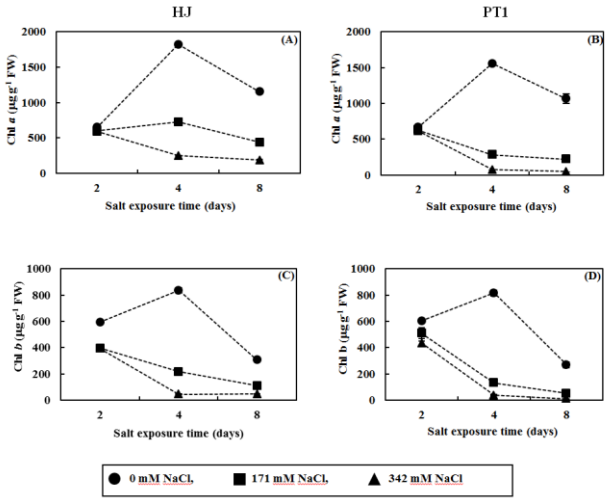

Figure 2 Change of the Chl *a* and Chl *b* in HJ (A and C) and PT1 (B and D) seedlings when exposed to 0, 171 and 342 mM NaCl for 2, 4 and 8 days

After 4 and 8 days of salt stress induction, the C_{x+c} in HJ and PT1 seedlings was decreased (Fig. 3C

and 3D). However, the decreasing of C_{x+c} in both varieties was different. At 171 mM NaCl, the reduction of C_{x+c} in PT1 was more than in HJ. By comparing NaCl concentration, the C_{x+c} in the both seedlings under higher NaCl concentration decreased more than that under lower NaCl concentration. The C_{x+c} was slightly changed at the early salt exposure time (2 days) while it changed quickly when the NaCl concentration was increased

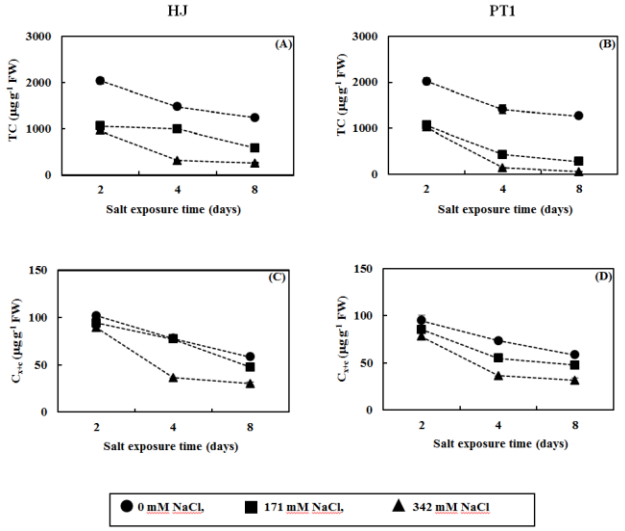

Figure 3 Change of the TC and C_{x+c} in HJ (A and C) and PT1 (B and D) seedlings when exposed to 0, 171 and 342 mM NaCl for 2, 4 and 8 days.

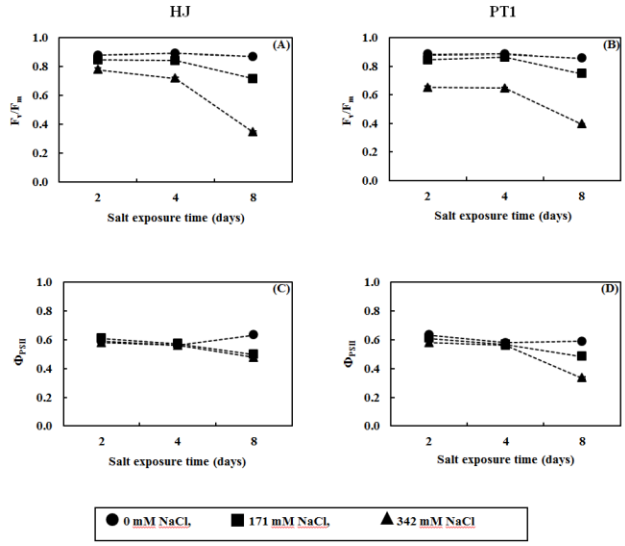

Figure 4 Change of the F_v/F_m and Φ_{PSII} in HJ (A and C) and PT1 (B and D) seedlings when exposed to 0, 171 and 342 mM NaCl for 2, 4 and 8 days.

Beside the reduction of osmotic potential, toxicity of Na^+ and Cl^- was reported [30, 49, 50, 51]. This ionic effect disturbed plant metabolism, especially photosynthesis which affected plant growth and development [16]. In this study, the increase of NaCl concentration to 342 mM reduced photosynthetic pigment concentrations i.e. chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (TC) and total carotenoids (C_{x+c}) in HJ and PT1 seedlings. However, the photosynthetic pigment concentrations in HJ salt-stressed seedlings were higher than those in PT1 salt-stressed seedlings (Fig. 2 and Fig. 3). Boriboonkaset [52] reported that the chloroplast ultrastructure in salt-sensitive (IR29 and PT1) was severely disordered more than that in salt-tolerant (Pok and HJ) seedlings after exposing to 342 mM NaCl.

At 171 mM NaCl, the increase of Chl *a* and TC in HJ might relate to the higher salt tolerance ability of HJ than PT1. Similar result was reported by Wanichananan [53] who demonstrated that the chlorophyll index (CI) in salt-sensitive (KDML105) seedlings was declined by 4 times more than that in salt-tolerant (Hawm Naipon) seedlings when subjected to 513 mM NaCl for 8 days. There are many researches showed that the increasing of salt concentration reduced the photosynthetic pigments in plants such as *Argyranthemum coronopifolium* [42], canola [54] and rice [40]. Moreover, the degradation of photosynthetic pigments depended on lipid protein ratio of pigment-protein complexes alteration and increasing of chlorophyllase activity [55, 56]. Notably, the photosynthetic pigments in the control treatment increased in the first four days because the growth of seedlings was increased, however the pigment contents were sharply decreased after eight days. Transpiration increased the loss of water of seedlings and nutrient concentration of the culture medium which led to the reduction of the osmotic potential of the nutrient and resulted in the reduction of photosynthetic pigments and growth.

NaCl reduced the C_{x+c} in HJ and PT1 seedlings, however the reduction of C_{x+c} in HJ salt-tolerant seedlings at 171 mM NaCl was less than that in PT1 salt-sensitive seedlings (Fig. 3C). It was reported that the salt stress generated reactive oxygen species which caused lipid peroxidation and photo-oxidation of chlorophyll [14, 31]. The higher C_{x+c} in HJ salt-stressed seedlings prevented chlorophyll degrdation (Fig. 3 and Fig. 4) and resulted in the increasing of salt tolerance in HJ more than in PT1 seedlings. This result was similar to Cha-um *et al.* [57] who reported that the C_{x+c} in KDML105 rice which is a salt-sensitive variety, was reduced by 12 times in comparing to the control when subjected to 342 mM NaCl for 8 days.

Chl *a* fluorescence parameters *i.e.* maximum quantum yield of PSII (F_v/F_m), quantum efficiency of PSII (Φ_{PSII}), photochemical quenching (qP) and non-photochemical quenching (NPQ) are used to indicate the water oxidation in photosystemII (PSII). The F_v/F_m in HJ and PT1 seedlings exposed to 342 mM NaCl for 8 days was approximately reduced 2.5 and 2.2 times, respectively when compared to the control (Fig. 3A and 3B). There was no difference of the F_v/F_m in both salt-stressed seedlings exposed to 171 mM NaCl at all salt exposure times (Fig. 4A and 4B). Additionally, the Φ_{PSII} in HJ and PT1 salt-stressed seedlings was not significant during 2 and 4 days after salt treatments. However, after 8 days of salt treatments, the Φ_{PSII} in PT1 seedlings was severely reduced while Φ_{PSII} in HJ salt-stressed seedlings was

not different between 171 and 342 mM NaCl (Fig. 4C and 4D).

The qP in HJ and PT1 salt-stressed seedlings was similar to the reduction of the PSII. In early salt exposure time (2 and 4 days), the qP was not affected by NaCl concentration. At 342 mM NaCl, the qP in HJ and PT1 seedlings was critically reduced after 8 days of salt treatments (Fig. 5A and 5B). In addition, the NPQ represented protective role of the anti-oxidative system. The increasing of NaCl concentration decreased NPQ at all salt exposure times, especially at 342 mM NaCl. Significant difference of the NPQ reduction was found in the HJ and PT1 seedlings which subjected to 171 and 342 mM NaCl for 2 days after salt treatments (Fig. 5C and 5D). The protective role of the anti-oxidative system in PT1 salt-stressed seedlings were significantly reduced more than those in HJ salt-stressed seedlings.

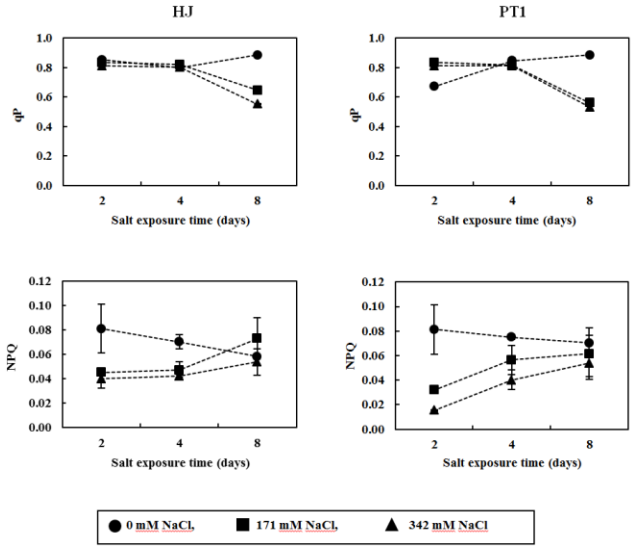

Figure 5 Change of the qP and NPQ in HJ (A and C) and PT1 (B and D) seedlings when exposed to 0, 171 and 342 mM NaCl for 2, 4 and 8 days.

Reduction of the photosynthetic pigments affected the light reaction of photosynthesis. The water oxidation in the light reaction and overall photosynthesis are monitored by Chl *a* fluorescence parameters [26, 58]. The water oxidation in the light reaction was used as a tool to indicate the sensitivity to stress conditions such as chilling [59], heat [60, 61], drought [61], light intensity [62], submergence [63] and salt [48, 64, 65, 66]. In HJ and PT1 seedlings, the 171 mM NaCl was slightly changed F_v/F_m, PSII and qP. However, the F_v/F_m was severely affected by 342 mM NaCl (Fig. 4 and 5). At 342 mM NaCl, the F_v/F_m in HJ was higher than that in PT1. This might play a role on higher salt tolerance of HJ than PT1. The increase of salt ions diminished the water oxidation in photosystemII (PSII). These ions disturbed oxygen-evolving complex activity that resulted in free radical oxygen induction. The free radical oxygen damaged the photosynthetic pigments (chlorophyll), lipid and protein that compromised of the light harvesting center in the light reaction, and resulted in reduction the photosynthesis [67, 68, 69].

This result agreed with the report of Cha-um *et al.* [13] who demonstrated that the stabilization of photosynthetic pigments, especially chlorophyll in salt-tolerant (GS No. 4371) and salt-sensitive (GS No. 7032) rice enhanced the water oxidation in the light reaction when exposed to 342 mM NaCl for 8 days. As well as, the water oxidation in the light reaction in sunflower [31], rice [48], canola [54], olive [58], rose [70], sorghum [71], sweet almond [72], cucumber [73], *Ramonda serbica* [74] and barley [75] were severely reduced by salt stress. The NPQ is a non-photochemical quenching which plays an important role on anti-oxidative system. After salt treatment, the NPQ in HJ and PT1 seedlings was decreased, but the decreasing of the NPQ in HJ was less than in PT1. This higher NPQ is correlated with the better salt tolerance ability in HJ. This result was similar to Cha-um *et al.* [13] who reported that the NPQ in salt-sensitive (GS No. 7032) and salt-tolerant (GS No. 4371) rice lines was reduced more than 50% in comparing to the control when subjected to 342 mM NaCl for 8 days. Moreover, this research showed the higher correlation between photosynthetic pigment concentration and chlorophyll *a* fluorescence parameters when subjected to salt stress treatment for 2, 4 and 8 days (Table 1-6).

Reduction of the photosynthetic pigment concentrations and Chl *a* fluorescence parameters caused the reduction of the growth in term of fresh weight (FW) and dry weight (DW) in HJ and PT1 salt-stressed seedlings particularly shoot growth when NaCl concentration and salt exposure time were increased (Table 7). The root and shoot growths in HJ and PT1 salt-stressed seedlings was not significantly different from the non-stressed seedlings while shoot growth among the two varieties was affected by rice varieties, NaCl concentration, salt exposure time and combination of the factors (Table 7). The increase of salt concentration and salt exposure time reduced growth of both rice varieties. The reduction of growth resulted from the reduction of the leaf osmotic potential, photosynthetic pigment concentrations and Chl *a* fluorescence parameters.

Table 1 Correlation coefficients of the physiological responses in HJ salt-stressed seedlings exposed to salt stress for 2 days.

Parameters	LOP	Chl *a*	Chl *b*	TC	C_{x+c}	F_v/F_m	Φ_{PSII}	qP	NPQ
LOP	1	-	-	-	-	-	-	-	-
Chl *a*	0.760^{**}	1	-	-	-	-	-	-	-
Chl *b*	0.839^{**}	0.816^{**}	1	-	-	-	-	-	-

TC	0.868^{**}	0.751^{**}	0.969^{**}	1	-	-	-	-	-
C_{x+c}	0.649^{*}	0.552^{ns}	0.676^{*}	0.566^{ns}	1	-	-	-	-
F_v/F_m	0.938^{**}	0.652^{*}	0.743^{**}	0.757^{**}	0.590^{*}	1	-	-	-
Φ_{PSII}	0.744^{**}	0.548^{ns}	0.750^{**}	0.734^{**}	0.599^{*}	0.825^{**}	1	-	-
qP	0.948^{**}	0.705^{*}	0.818^{**}	0.823^{**}	0.640^{*}	0.978^{**}	0.833^{**}	1	-
NPQ	0.600^{*}	0.714^{**}	0.657^{*}	0.572^{ns}	0.548^{ns}	0.475^{ns}	0.287^{ns}	0.573^{ns}	1

Significant level at $p \leq 0.01$ (**), $p \leq 0.05$ (*) and non-significant (ns) using by Pearson's correlation coefficients.

Table 2 Correlation coefficients of the physiological responses in PT1 salt-stressed seedlings exposed to salt stress for 2 days.

Parameters	**LOP**	**Chl *a***	**Chl *b***	**TC**	C_{x+c}	F_v/F_m	Φ_{PSII}	**qP**	**NPQ**
LOP	1	-	-	-	-	-	-	-	-
Chl *a*	0.502^{ns}	1	-	-	-	-	-	-	-
Chl *b*	0.851^{**}	0.763^{**}	1	-	-	-	-	-	-
TC	0.899^{**}	0.642^{*}	0.911^{**}	1	-	-	-	-	-
C_{x+c}	0.726^{**}	0.430^{ns}	0.643^{*}	0.683^{*}	1	-	-	-	-
F_v/F_m	0.861^{**}	0.405^{ns}	0.708^{**}	0.646^{*}	0.671^{*}	1	-	-	-
Φ_{PSII}	0.851^{**}	0.741^{**}	0.836^{**}	0.799^{**}	0.609^{*}	0.878^{**}	1	-	-
qP	0.841^{**}	0.408^{ns}	0.709^{**}	0.626^{*}	0.629^{*}	0.995^{**}	0.879^{**}	1	-
NPQ	0.797^{**}	0.531^{ns}	0.718^{**}	0.800^{**}	0.876^{**}	0.658^{*}	0.715^{**}	0.640^{*}	1

Significant level at $p \leq 0.01$ (**), $p \leq 0.05$ (*) and non-significant (ns) using by Pearson's correlation coefficients.

Table 3 Correlation coefficients of the physiological responses in HJ salt-stressed seedlings exposed to salt stress for 4 days.

Parameters	**LOP**	**Chl *a***	**Chl *b***	**TC**	C_{x+c}	F_v/F_m	Φ_{PSII}	**qP**	**NPQ**
LOP	1	-	-	-	-	-	-	-	-
Chl *a*	0.940^{**}	1	-	-	-	-	-	-	-
Chl *b*	0.915^{**}	0.995^{**}	1	-	-	-	-	-	-
TC	0.984^{**}	0.940^{**}	0.913^{**}	1	-	-	-	-	-
C_{x+c}	0.887^{**}	0.722^{*}	0.668^{*}	0.910^{**}	1	-	-	-	-
F_v/F_m	0.957^{**}	0.887^{**}	0.848^{**}	0.983^{**}	0.939^{**}	1	-	-	-
Φ_{PSII}	0.565^{*}	0.678^{*}	0.705^{*}	0.595^{*}	0.360^{ns}	0.553^{*}	1	-	-
qP	0.697^{*}	0.799^{**}	0.817^{**}	0.713^{**}	0.494^{ns}	0.640^{*}	0.378^{ns}	1	-
NPQ	0.747^{**}	0.769^{**}	0.802^{**}	0.731^{**}	0.553^{*}	0.652^{*}	0.513^{*}	0.844^{**}	1

Significant level at $p \leq 0.01$ (**), $p \leq 0.05$ (*) and non-significant (ns) using by Pearson's correlation coefficients.

Table 4 Correlation coefficients of the physiological responses in PT1 salt-stressed seedlings exposed to salt stress for 4 days.

Parameters	**LOP**	**Chl *a***	**Chl *b***	**TC**	C_{x+c}	F_v/F_m	Φ_{PSII}	**qP**	**NPQ**
LOP	1	-	-	-	-	-	-	-	-
Chl *a*	0.975^{**}	1	-	-	-	-	-	-	-
Chl *b*	0.972^{**}	0.995^{**}	1	-	-	-	-	-	-
TC	0.967^{**}	0.987^{**}	0.973^{**}	1	-	-	-	-	-
C_{x+c}	0.929^{**}	0.910^{**}	0.889^{**}	0.923^{**}	1	-	-	-	-
F_v/F_m	0.724^{*}	0.684^{*}	0.661^{*}	0.728^{*}	0.887^{**}	1	-	-	-
Φ_{PSII}	0.771^{**}	0.794^{**}	0.783^{**}	0.784^{**}	0.794^{**}	0.688^{*}	1	-	-

qP	0.945**	0.937**	0.932**	0.921**	0.874**	0.642*	0.808**	1	-
NPQ	0.752**	0.668*	0.650*	0.687*	0.752**	0.610*	0.421ns	0.808**	1

Significant level at $p \leq 0.01$ (**), $p \leq 0.05$ (*) and non-significant (ns) using by Pearson's correlation coefficients.

Table 5 Correlation coefficients of the physiological responses in HJ salt-stressed seedlings exposed to salt stress for 8 days.

Parameters	**LOP**	**Chl *a***	**Chl *b***	**TC**	C_{x+c}	F_v/F_m	Φ_{PSII}	**qP**	**NPQ**
LOP	1	-	-	-	-	-	-	-	-
Chl *a*	0.794**	1	-	-	-	-	-	-	-
Chl *b*	0.796**	0.999**	1	-	-	-	-	-	-
TC	0.816**	0.997**	0.995**	1	-	-	-	-	-
C_{x+c}	0.894**	0.916**	0.912**	0.941**	1	-	-	-	-
F_v/F_m	0.908**	0.876**	0.874**	0.907**	0.982**	1	-	-	-
Φ_{PSII}	0.697*	0.985**	0.984**	0.972**	0.855**	0.805**	1	-	-
qP	0.780**	0.996**	0.995**	0.993**	0.919**	0.885**	0.988**	1	-
NPQ	0.547*	0.412ns	0.428ns	0.385ns	0.374ns	0.315ns	0.383ns	0.384ns	1

Significant level at $p \leq 0.01$ (**), $p \leq 0.05$ (*) and non-significant (ns) using by Pearson's correlation coefficients.

Table 6 Correlation coefficients of the physiological responses in PT1 salt-stressed seedlings exposed to salt stress for 8 days.

Parameters	**LOP**	**Chl *a***	**Chl *b***	**TC**	C_{x+c}	F_v/F_m	Φ_{PSII}	**qP**	**NPQ**
LOP	1	-	-	-	-	-	-	-	-
Chl *a*	0.813**	1	-	-	-	-	-	-	-
Chl *b*	0.803**	0.998**	1	-	-	-	-	-	-
TC	0.831**	0.991**	0.985**	1	-	-	-	-	-
C_{x+c}	0.951**	0.860**	0.850**	0.876**	1	-	-	-	-
F_v/F_m	0.959**	0.777**	0.765**	0.794**	0.963**	1	-	-	-
Φ_{PSII}	0.962**	0.886**	0.876**	0.900**	0.989**	0.977**	1	-	-
qP	0.774**	0.986**	0.979**	0.992**	0.820**	0.731**	0.849**	1	-
NPQ	0.419*	0.287ns	0.295ns	0.266ns	0.323ns	0.219ns	0.284ns	0.232ns	1

Significant level at $p \leq 0.01$ (**), $p \leq 0.05$ (*) and non-significant (ns) using by Pearson's correlation coefficients.

Table 7 Fresh weight (FW) and dry weight (DW) in HJ and PT1 roots after cultured in liquid MS medium for 7 days and subsequently exposed to 0, 171 and 342 mM NaCl for 2, 4 and 8 days.

Rice	NaCl (mM)	Salt exposure time (days)	Roots		Shoot	
			FW (mg)	DW (mg)	FW (mg)	DW (mg)
HJ	0	2	53.5	6.5	194.7 ab	35.6 bcd
		4	67.2	4.6	216.6 a	43.1 b
		8	56.9	4.2	149.9 cd	61.9 a
	171	2	47.5	5.5	178.7 abc	31.5 bcd
		4	54.5	3.7	205.9 a	34.6 bcd
		8	41.5	2.6	141.3 cde	27.0 cde
	342	2	37.9	4.7	178.0 abc	28.1 cde
		4	34.2	3.1	98.2 fg	24.9 de
		8	34.1	2.4	92.3 fg	25.8 cde
PT1	0	2	56.3	6.6	196.8 ab	38.2 bc

		4	44.2	2.4	101.1 fg	18.1 e
		8	52.9	4.1	129.4 def	32.8 bcd
	171	2	44.8	5.8	189.7 ab	32.4 bcd
		4	40.5	2.3	82.5 g	17.9 e
		8	41.6	3.3	129.2 def	31.9 bcd
	342	2	41.5	5.0	163.9 bcd	29.2 cde
		4	39.7	2.0	82.3 g	17.2 e
		8	41.0	2.7	106.7 efg	28.6 cde
Significant level						
Rice			ns	*	**	**
NaCl			**	**	**	**
Salt exposure time			ns	**	**	**
Rice × NaCl			*	ns	*	**
Rice × Salt exposure time			*	**	**	**
NaCl × Salt exposure time			ns	ns	ns	ns
Rice × NaCl × Salt exposure time			ns	ns	**	*

Means with the different letters in each column show significant difference at $p \leq 0.01$ (**), $p \leq 0.05$ (*) and non-significant (ns) by Duncan's New Multiple Range Test (DMRT).

In this study, the shoot growth in HJ and PT1 seedlings were severely reduced when NaCl concentration was increased. However, the shoot growth in HJ salt-stressed seedlings was higher than that in PT1 salt-stressed seedlings whereas salt did not affect the root growth (Table 7). Similarly, the report of Cha-um *et al.* [76] who demonstrated that the photosynthetic pigment concentrations and Chl *a* fluorescence parameters in salt-tolerant (KML, KDML, POK, HJ, DPY, CMJ1, CMJ2, UR1, and CH) seedlings were higher than those in salt sensitive (R258, PT1, IR29, and UR2) seedlings that resulted in maintaining the shoot growth ability when subjected to 200 mM NaCl for 14 days. Furthermore, an increase of salt concentration was progressively reduced shoot growth of sweet sorghum [77], tomato [78], cotton [79] and quinoa [80].

4. Conclusions

NaCl reduced shoot growth in both HJ and PT1. Leaf osmotic potential, photosynthetic pigment concentrations and Chl *a* fluorescence parameters played important roles on shoot growth under salt stress. Salt ion accumulation reduced leaf osmotic potential and meanwhile the ions damaged photosynthetic pigment apparatus.

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