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ION CONTENTS, RELATIVE ELECTROLYTE LEAKAGE, PROLINE ACCUMULATION, PHOTOSYNTHETIC ABILITIES AND GROWTH CHARACTERS OF OIL PALM SEEDLINGS IN RESPONSE TO SALT STRESS

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Abstract

Oil palm seedlings were photo-autotrophically grown in MS medium and subsequently exposed to 0 (control), 25, 50, 100 or 200 mM NaCl. Sodium ions, proline content and the percentage of relative electrolyte leakage in seedlings subjected to salt stress increased, depending on the degree of salt concentrations. Sodium ion accumulation in oil palm seedlings grown under 200 mM NaCl was enriched and positively related to membrane injury or relative electrolyte leakage subsequently correlated with total chlorophyll degradation. Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (TC), total carotenoids (C_{x+c}), maximum quantum yield of PSII (F_v/F_m), photon yield of PSII (Φ_{PSII}) and quantum efficiency of PSII (qP) in the seedlings under salt stress dropped significantly in comparison to the control group, leading to a reduction in net-photosynthetic rate (P_n) and growth, especially in 200 mM NaCl. A positive correlation between physiological and growth parameters, including sodium ion, relative electrolyte leakage, photosynthetic pigments and water oxidation in photosystem II, P_n and plant dry weight was found. These data may further be applied to establish criteria for salt tolerance screening in oil palm breeding programs.

Introduction

Salt-affected soil problem is a member of abiotic stresses, which are a major problem world wide. It is a soil composing of various salts, which are quickly dissolved in water to produce toxic ions, especially sodium ion (Na^+). Sodium (Na^+) is a small molecule, which is frequently absorbed by root tissues and transferred to the overall plant organs through the xylem-uploading vascular tissues. Sodium ion (Na^+) is well established for negatively affecting the plant growth and development prior to plant cell death (Tester & Davenport, 2003; Davenport *et al*., 2005; Munns *et al*., 2006). In halophyte or salt tolerant species, there are a large number of plant defense mechanisms including ion homeostasis, osmoregulation, antioxidant and hormonal systems helping plant to thrive well under saline conditions (Hasegawa *et al*., 2000; Ashraf, 2004, 2009; Sairam & Tyagi, 2004; Mahajan & Tuteja, 2005; Noreen & Ashraf, 2009; Nawaz & Ashraf, 2009). In contrast, glycophytic or susceptible plant species are sensitive to salt stress, because they show reduced leaf expansion, chlorophyll contents and represent the toxic damages i.e., wilting, chlorosis, necrosis, leaf burn and plant senescence (Lutts *et al*., 1999; Iqbal *et al*., 2009).

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Oil palm is one of the most important oil production crop plants in the world and is widely cultivated in the tropical zone, especially Southeast Asia (Malaysia, Indonesia and Thailand) (Yusof & Chen, 2003; Wahid *et al*., 2005), which may create the salinity soil problem. Tolerance to abiotic stresses such as salt-affected soil, water-deficit, extreme temperature, mineral deficiency, heavy metal toxicity and ultraviolet irradiation is a fruitful topic for oil palm improvement (Jalani *et al*., 1997). In the previous reports, there is a large number of information in date palm responses to salt stress (Al-Khayri, 2002; Ramoliya & Pandey, 2003; Djibril *et al*., 2005; Tripler *et al*., 2007; Youssef & Awad, 2008; Alhammadi & Edward, 2009). A lack of the basic knowledge in the salt stress responses in oil palm is a bottle-neck to be developed. The aim of this investigation was to detect the responses of the palm plant, in terms of sodium ion, proline accumulation, relative electrolyte leakage, photosynthetic abilities and growth characters, to salt stress. The basic information relating to salt responses in oil palms is a profitable issue which should be investigated further for application in salt tolerance screening.

Materials and Methods

Plant materials: Oil palm fruits were obtained from Suksomboon Palm Co Ltd. The kernel of the fruit was removed. The seeds with the seed coat were dried in a hot air oven at 45°C for 12 h and then the seed coat was broken. The embryos, along with the endosperm were surface-disinfected once in 15% Clorox® for 20 min and once in 5% Clorox® for 30 min. The embryos were then excised to germinate in MS media (Murashige & Skoog, 1962). The pH of culture media was adjusted to 5.7 before autoclaving. Oil palm seedlings were cultured *In vitro* under conditions of 25±2°C ambient temperature, 60±5% relative humidity (RH) and 60±5 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) provided by fluorescent lamps with 16 hd^{-1} photoperiod. After two months, the seedlings were transferred aseptically to MS-liquid sugar-free media. The uncovered vessels containing photoautotrophic seedlings were transferred aseptically to culture box chambers (Carry Box Model P-850, size 26×36×19 cm, Japan) with RH controlled at 65±5% by 1.5 L saturated NaCl solution. The number of air exchanges in the culture box chambers was increased to 5.1±0.3 h^{-1} by punching the side of the plastic chambers with 32 holes and placing gas-permeable microporous polypropylene film (0.22 µm pore size) over the holes (Cha-um *et al*., 2003). Oil palm seedlings were acclimated for 14 days by placing the chambers in a Plant Growth Incubator under a temperature shift of 28±2°C/25±2°C (light/dark), 500±100 µmol CO_2 mol^{-1} concentration, 60±5% RH, 120±5 µmol m^{-2} s^{-1} PPFD provided by fluorescent lamps with 16 hd^{-1} photoperiod. Sodium chloride (NaCl) in the culture media was adjusted to 0 (control), 25, 50, 100 or 200 mM for 14 days. Ion contents, relative electrolyte leakage, proline content, photosynthetic pigments, chlorophyll fluorescence, net-photosynthetic rate (P_n) and growth characters were measured.

Data measurements: Sodium and potassium ions were assayed according to Dionisio-Sese & Tobita (1998). One-hundred milligrams of leaf tissues were ground in liquid nitrogen. Sodium and potassium ions in crude leaves were extracted by acidic method (HNO_3 and $HClO_4$) and measured using an Atomic Absorption Spectrophotometer (AA, Model M6, Thermo Elemental, MA, USA).

Relative electrolyte leakage (%) was determined according to the Dionisio-Sese & Tobita (1998) method. Proline in the root and leaf tissues was extracted and analyzed according to the method of Bates *et al*., (1973). Chlorophyll a (Chl_a), chlorophyll b (Chl_b) and total chlorophyll (TC) were analyzed following the methods of Shabala *et al*., (1998) and total carotenoid (C_{x+c}) concentrations were assayed according to Lichtenthaler (1987). Chlorophyll fluorescence emission from the adaxial surface on the leaf was measured using a fluorescence monitoring system in the pulse amplitude modulation mode, as previously described by Loggini *et al*., (1999) and Maxwell & Johnson (2000). Net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (E) was measured using a portable photosynthesis system (Infra-red Gas Analyser IRGA; Model LI 6400, LI-COR® Inc, Lincoln, Nebraska, USA) according to Cha-um *et al*., (2007).

Shoot height (SH), root length (RL), fresh weight (FW) and dry weight (DW) of oil palm seedlings were measured. Oil palm seedlings were dried at 80°C in a hot-air oven for 2 days, and then incubated in desiccators before the measurement of dry weight.

Experiment design: The experiment was arranged as Completely Randomized Design (CRD) with eight replicates (n=8). The mean values obtained were compared by Least Significant Difference (LSD) and analyzed using SPSS software. The correlations between biochemical, physiological and growth parameters were evaluated using Pearson's correlation coefficients.

Results

Sodium ion (Na^+) in the oil palm seedlings grown under salt stress was increased, relating to Sodium chloride salts in the culture medium whereas potassium ion (K^+) was significantly reduced (Fig. 1). The ratio of Na^+ / K^+ in salt stressed seedlings was gradually enlarged. An increasing Na^+ in the salt stressed seedlings was positively related to relative electrolyte leakage (REL) (Fig. 2A). The REL percentage in the leaf tissues was significantly enhanced, depending on the degree of salt concentrations in the culture medium (Table 1), leading to total chlorophyll (TC) degradation (Fig. 2B). Chlorophyll a (Chl_a) and chlorophyll b (Chl_b) contents in salt stressed leaves (≥50 m M NaCl) were significantly dropped while TC and total carotenoids (C_{x+c}) were rapidly damaged when exposed to 25 mM NaCl (Table 1). The degradation of TC and C_{x+c} in the leaf tissues was sensitive to salt stress, especially at 200 mM NaCl (degraded for 31.9 and 61.7% of control). The reduction of Chl_a and TC contents was positively correlated with maximum quantum yield of PSII (F_v/F_m) and photon yield of PSII (Φ_{PSII}) (Fig. 3). The F_v/F_m, Φ_{PSII} and quantum efficiency of PSII (qP) in salt stressed leaves (≥50 m M NaCl) were significantly diminished, in contrast the non photochemical quenching (NPQ) was enriched (Table 2). The diminishing of Φ_{PSII} in salt stressed leaves was progressively related to net photosynthetic rate (P_n) reduction (Fig. 4A), causing to low plant dry weight (Fig. 4B). In 200 mM NaCl, the P_n, stomatal conductance (g_s) and transpiration rate (E) in salt stressed seedlings were significantly decreased for 87.7, 88.6 and 54.8% of control, respectively (Table 3). The P_n and g_s parameters were statistically dropped when exposed to 25 mM salt stress. In contrast, proline contents in oil palm seedling grown under 200 mM NaCl were increasingly accumulated for 3.87 folds when compared to control (0 mM NaCl) (Table 3). In addition, growth characters including shoot height (SH), root length (RL), fresh weight (FW) and dry weight (DW) of oil palm were similarly reduced when exposed to salt stress (Table 4). In extreme salt stress (200 mM NaCl), the growth parameters were inhibited for 35.4, 63.1, 50.8 and 50.0% of control, respectively.

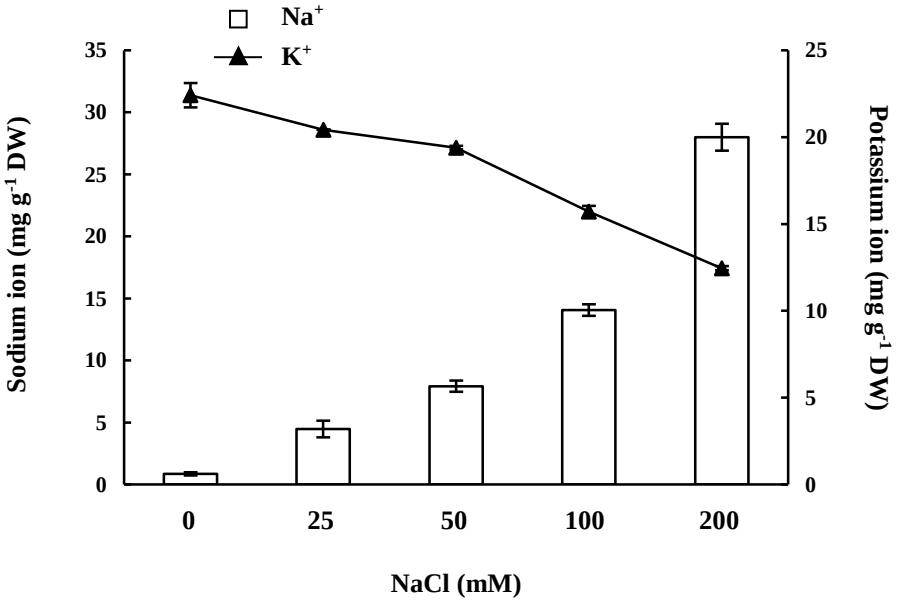


Fig. 1. Sodium and potassium ions of oil palm grown under salt stress for 14 days. Error bars represented by ±SE.

Table 1. Relative electrolyte leakage (REL), chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (TC) and total carotenoids (C_{x+c}) contents of oil palm grown under salt stress for 14 days.

NaCl (mM)	REL (%)	Chl_a (μg g^{-1}FW)	Chl_b (μg g^{-1}FW)	TC (μg g^{-1}FW)	C_{x+c} (μg g^{-1}FW)
0 (Control)	24.3e	102.8a	62.2a	165.0a	4.7a
25	30.3d	99.5ab	59.8ab	159.3b	3.3b
50	36.6c	96.0b	58.2bc	154.2c	3.0bc
100	41.2b	91.1c	56.1c	147.2d	2.6c
200	65.7a	66.2d	46.2d	112.4e	1.8d
Significant level	**	**	**	**	**

Different letters in each column show significant difference at $p \leq 0.01$ (**) by Least Significant Difference (LSD).

Table 2. Maximum quantum yield of PSII (F_v/F_m), photon yield of PSII (Φ_{PSII}), quantum efficiency of PSII (qP) and non-photochemical quenching (NPQ) of oil palm grown under salt stress for 14 days.

NaCl (mM)	F_v/F_m	Φ_{PSII}	qP	NPQ
0 (Control)	0.861a	0.515a	0.617a	0.027d
25	0.853ab	0.479ab	0.571ab	0.061c
50	0.847b	0.440b	0.528b	0.080bc
100	0.733c	0.338c	0.379c	0.098b
200	0.625d	0.167d	0.248d	0.275a
Significant level	**	**	**	**

Different letters in each column show significant difference at $p \leq 0.01$ (**) by Least Significant Difference (LSD).

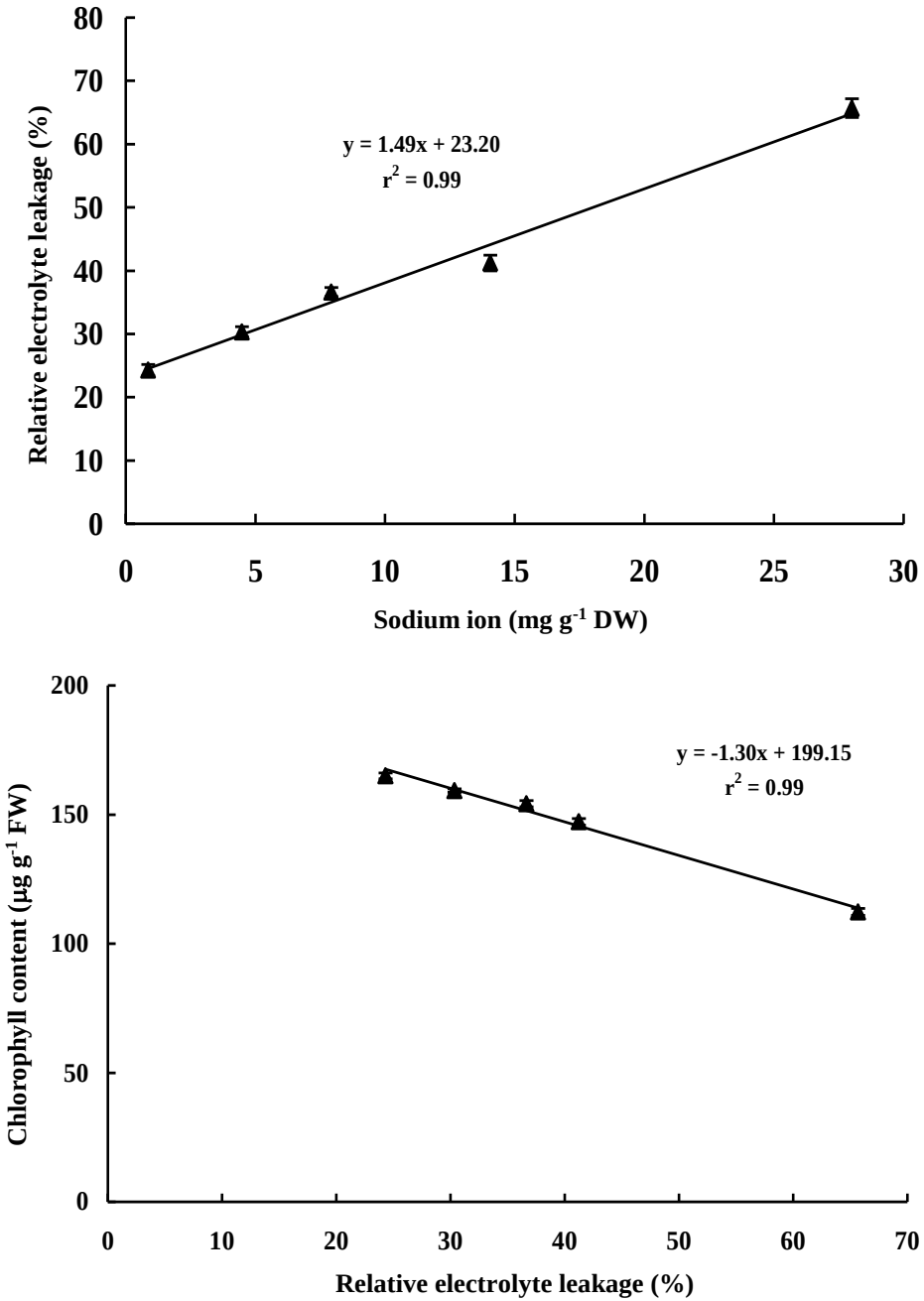
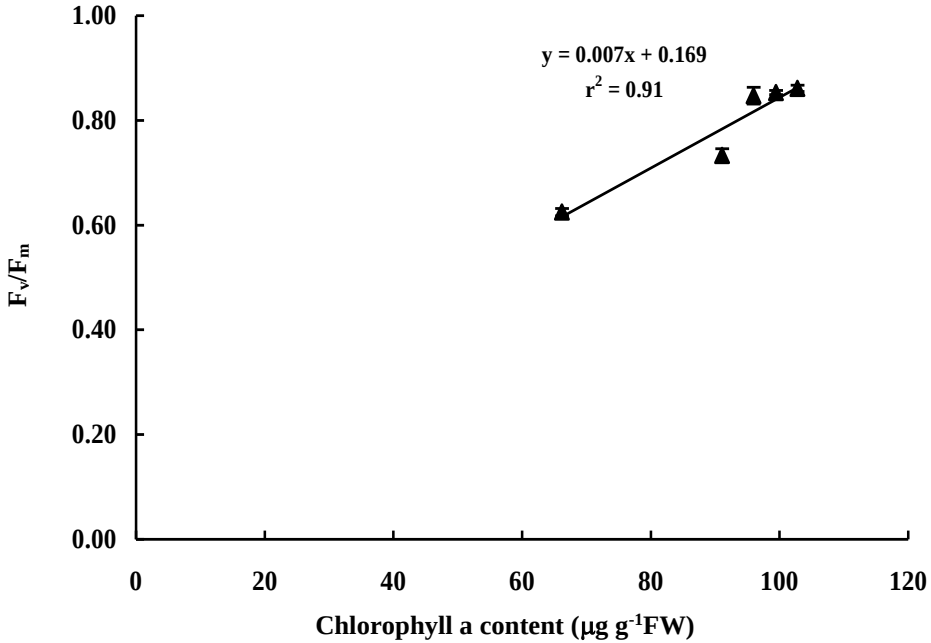


Fig. 2. Relationship between sodium ion and relative electrolyte leakage (A) and relative electrolyte leakage and total chlorophyll content (B) of oil palm grown under salt stress for 14 days. Error bars represented by ±SE.



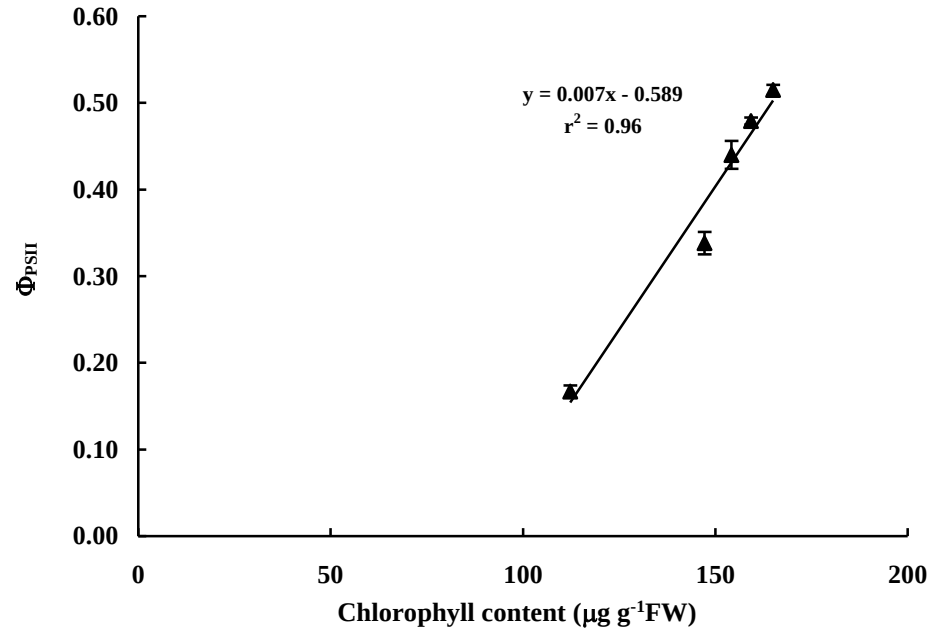


Fig. 3. Relationship between chlorophyll a content and maximum quantum yield of PSII (F_v/F_m) (A) and total chlorophyll content and photon yield of PSII (Φ_{PSII}) (B) of oil palm grown under salt stress for 14 days. Error bars represented by ± SE.

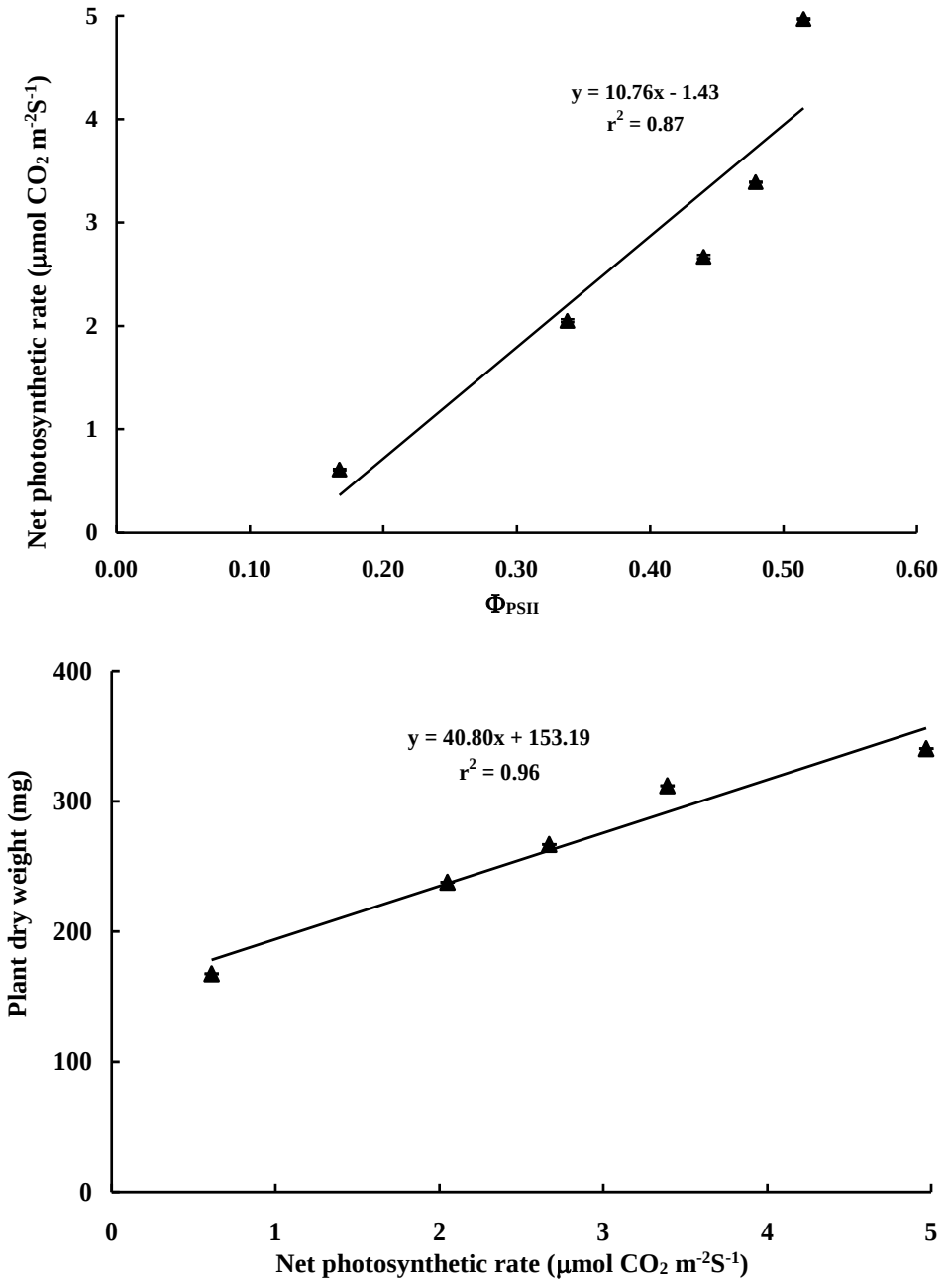


Fig. 4. Relationship between photon yield of PSII (Φ_{PSII}) and net photosynthetic rate (P_n) (A) and P_n and plant dry weight (B) of oil palm grown under salt stress for 14 days. Error bars represented by ± SE.

Table 3. Net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E) and proline contents of oil palm grown under salt stress for 14 days.

NaCl (mM)	P_n (μmol CO_2 $m^{-2}s^{-1}$)	g_s (μmol $m^{-2}s^{-1}$)	E (mmol H_2O $m^{-2}s^{-1}$)	Proline (μmol g^{-1} FW)
0 (Control)	4.97a	14.1a	0.31a	0.75c
25	3.39b	9.6b	0.29a	1.23c
50	2.67c	8.6b	0.27a	1.58b
100	2.05c	3.9c	0.23a	1.74b
200	0.61d	1.6c	0.14b	2.91a
Significant level	**	**	**	**

Different letters in each column show significant difference at $p \leq 0.01$ (**) by Least Significant Difference (LSD).

Table 4. Growth characters, shoot height (SH), root length (RL), fresh weight (FW) and dry weight (DW) of oil palm grown under salt stress for 14 days.

NaCl (mM)	SH (cm)	RL (cm)	FW (g $plant^{-1}$)	DW (g $plant^{-1}$)
0 (Control)	21.2a	18.7a	1.81a	0.34a
25	19.9ab	14.7b	1.53b	0.31a
50	19.5ab	11.8c	1.41bc	0.27b
100	18.5b	9.8c	1.25c	0.24b
200	13.7c	6.9d	0.89d	0.17c
Significant level	**	**	**	**

Different letters in each column show significant difference at $p \leq 0.01$ (**) by Least Significant Difference (LSD).

Discussion

Sodium (Na^+) in salt stress treatment of date palm was accumulated depending the degree of salt treatments in terms of EC (dS m^{-1}) (Ramoliya & Pandey, 2003; Tripler *et al*., 2007), millimolar (mM) (Al-Khayri, 2002) and parts per million (ppm) (Alhammadi & Edward 2009). Root is the major organ to accumulate the Na^+ better than shoot and leaf organs (Tripler *et al*., 2007; Alhammadi & Edward, 2009). It should be possible that the root organ is the first barrier of plant to be intact to soil salinity, which is enriched Na^+. The soft tissues as callus culture are easy to absorb the Na^+ from the NaCl-treated culture medium (Al-Khayri, 2002). K^+ is well known as ion exchanger in the membrane system of Na^+ uptake into plant cells (Akram *et al*., 2009). In the present study, the Na^+ was enriched while K^+ was dropped, leading to enhanced Na^+/K^+ ratio. It is quite similar to previous reports in date palm (Al-Khayri, 2002; Alhammadi & Edward, 2009). Enriched Na^+ in the salt-stressed seedlings causes a membrane injury, which is represented by relative electrolyte leakage (REL) percentage (Ghoulam *et al*., 2002; Youssef & Awad, 2008). In this study, an increasing REL directly injured the photosynthetic pigments including Chl_a, Chl_b, TC and C_{x+c}. The Chl_a and TC in the salt-stressed date palm seedlings (30 mS cm^{-1}) were significantly degraded for 36.2 and 23.6% of control (0 mS cm^{-1}) (Youssef & Awad, 2008). Reduction of photosynthetic abilities including pigments, chlorophyll fluorescence and CO_2 assimilation rate in the salt stressed leaves was a major cause in P_n decline. Similarly results, P_n and g_s reduction in date palm seedlings grown under 15 and 30 mS cm^{-1} EC was demonstrated (Youssef & Awad, 2008). In contrast, proline, an organic osmolyte compound, was enriched (2.91 μmol g^{-1} FW), especially in the extreme salt stress (200 mM NaCl). It is similar to salt stressed callus (255 mM NaCl) of date palm to enrich the proline for 2.8 μmol g^{-1} FW. In

date palm seedlings, proline was accumulated relating to a degree of salt stress (0.4-1.6% NaCl in the culture medium (Djibril *et al*., 2005). Proline is an important osmolyte involved in the control of osmotic pressure in the cells (Ashraf & Foolad, 2007) and one of the most sensitive indicators of salt tolerance in date palm (Djibril *et al*., 2005). Moreover, the overall growth performances are the sensitive case when exposed the oil palm plant to salt stress. In date palm, the plant height, number of leaves, relative growth rate, dry biomass weight and productivity were significantly retarded in the salt stress treatment (Ramoliya & Pandey, 2003; Tripler *et al*., 2007; Alhammadi & Edward, 2009).

In conclusion, sodium ion and relative electrolyte leakage in oil palm seedlings in response to salt stress increased, leading to damage to photosynthetic pigments, diminished the photosynthetic abilities and reduced the growth performances. The physiological and growth characters of oil palm seedlings decreased significantly, depending on the degree of salt stress. The data derived from this investigation provide the basis for the establishment for multivariate criteria for salt tolerance screening in oil palm breeding programs.

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