Remediation of salt-affected soil by the addition of organic matter - an investigation into improving glutinous rice productivity

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ABSTRACT: Soil salinity may limit plant growth and development, and cause yield loss in crop species. This study aimed at remediating saline soil using organic matter (OM) treatment, before the cultivation of RD6 rice (*Oryza sativa* L. spp. *indica*). Physiological and morphological characters of rice plants, as well as crop yield, were evaluated from salt-affected soil with varying levels of salinity. The chlorophyll *a* and total chlorophyll pigments of rice plants grown in salt-affected soil (2% salt level) with the application of OM were maintained better than in plants grown without OM treatment. The degree of reduced photosynthetic pigments in rice plants was dependent on the level of salt contamination. Pigment content was positively related to maximum quantum yield of PSII (F_v/F_m) and quantum efficiency of PSII (Φ_{PSII}), leading to reduced net photosynthetic rate (P_n) and reduced total grain weight (TGW). Photosynthetic abilities, including chlorophyll *a* and total chlorophyll pigments and Φ_{PSII}, in rice plants grown with OM treatment were greater than in those cultivated in soil without the OM treatment, especially in high salt levels (1-2% salt). The remediation of salt-affected soil in paddy fields using OM should be applied further, as an effective way of enhancing food crop productivity.
Keywords: chlorophyll *a* fluorescence, inland salinity, pigment stabilization, net photosynthetic rate, yield

Introduction

Soil salinity (electrical conductivity - ECe > 4 dS m^{-1}) is a major abiotic stress which limits plant growth and development, causing yield loss in crop species, which are defined as glycophyte or salt susceptible (Hasegawa et al., 2000; Qadir et al., 2007). Salt-affected soils are identified by excessive levels of water-soluble salts, especially sodium chloride (NaCl) (Tanji, 2002). NaCl, a major salt contaminant in soil, is a small molecule which when ionized by water, produces sodium (Na^+) and chloride (Cl^-) ions. These toxic ions cause ionic and osmotic stress at the cellular level in higher plants, especially in susceptible or glycophyte species (Mansour and Salama, 2000; Chinnusamy et al., 2005). There are many effective ways for improving salt-affected land, such as water leaching, chemical remediation and phytoremediation (Ahmad and Chang, 2002; Sharma and Minhas, 2005; Qadir et al., 2007). The remediation of salt-affected soil using chemical agents, including gypsum ($CaSO_4.2H_2O$), calcite ($CaCO_3$), calcium chloride ($CaCl_2.2H_2O$), and organic matter (farmyard manure, green manure, organic amendment and municipal solid waste), is a successful approach that has been implemented worldwide, being effective, low cost, and simple (Mitchell et al., 2000; Hanay et al., 2004; Sharma and Minhas, 2005; Tejada et al., 2006). The physical, chemical and biological properties of soil in salt-affected areas are improved by the application of OM, leading to enhanced plant growth and development. Therefore, the application of OM for soil remediation is important for sustainable land use and crop productivity (Choudhary et al., 2004; Wong et al., 2009).

Rice (*Oryza sativa* L. spp. *indica*) is one of the five main carbohydrate crops responsible for feeding the world's population, and is especially important in Asian countries. It plays a major role as a staple food, supporting more than 3 billion people and comprising 50-80% their daily calorie intake (Khush, 2005). Rice has previously been reported as being salt susceptible in both the seedling and reproductive stages (Zeng et al., 2001; Moradi and Ismail, 2007), leading to a reduction in yield of more than 50% in crops exposed to 6.65 dS m^{-1} ECe (Zeng and Shannon, 2000). RD6 is a mutant line derived from jasmine rice (non-glutinous cv. KDML105) irradiated with gamma radiation and is the dominant rice cultivar in the rain-fed lowland area of Thailand. It is a premium quality glutinous rice, in terms of aroma, cooking quality, sticky texture and long grains (Keeratipibul et al., 2008) and is widely cultivated in the Northeast region of Thailand, which is reported as being affected by soil salinity (Wongpokhom et al., 2008). The aim of the present study was to remediate saline soil using organic matter (OM) before the cultivation of RD6 rice, prior to grain harvesting.

Materials and Methods

For the nursery stock to be raised, seeds of rice cultivar RD6 were allowed to germinate and then transplanted to pots containing clay soil in 50% shading (acclimatization) light intensity for one month. Pots were placed on plastic trays (0.30 × 0.45 m), with 72 pots per tray. Irrigation was applied as water spray. One hundred acclimatized plants were transplanted directly to saline paddy fields (4 m^2 with 0.20 × 0.20 m plant to plant and row to row distance) at four sites (0.0, 0.3, 1.0 and 2.0% salt) in Sakolnakhon (17°51'4" N; 103°34'12" E), Northeast of Thailand (Table 1). Green manure (GM) and farmyard manure (FYM), in a ratio 1:1 (w/

Table 1 – Salinity level, electrical conductivity (EC_e), soil pH, nitrogen (N), phosphorus (P) and potassium (K) in the studied sites, in the Northern region of Thailand.

Plot site	Salt level	EC_e	pH	Available form of nutrients		
	%	dS m^{-1}		N (g kg^{-1})	P (mg kg^{-1})	K (g kg^{-1})
I	0	1.0	5.49	0.61	8.17	6.47
II	0.3	2.6	4.73	0.38	2.08	2.00
III	1.0	8.5	4.58	0.27	1.72	2.33
IV	2.0	20.4	4.79	0.52	12.95	4.00

w), were mixed together and then applied to the soil as organic matter (OM), at 12.5 kg m^{-2}, compared to the control (without OM). Chemical fertilizer (16:16:16; Nitrogen: Phosphorus: Potassium) was applied three times, in mid-August, September and October at 0.0156 kg m^{-2}, prior to the flowering stage. Photosynthetic pigments, chlorophyll *a* fluorescence and net photosynthetic rate (P_n) in the flag leaf were assayed. In addition, data on plant height, number of leaves, number of tillers, leaf area, spike length, spike weight, one-hundred seed weight, and total grain weight per clump were recorded in the harvesting period.

Chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b) and total chlorophyll (TC) concentrations were determined following the method of Shabala et al. (1998) and the total carotenoids (C_{x+c}) concentration was measured according to Lichtenthaler (1987). One hundred milligrams of leaf material was collected, placed in a 25 mL glass vial along with 10 mL of 95.5% acetone, and blended using a homogenizer. Chl_a, Chl_b, and C_{x+c} concentrations were measured using an UV-visible spectrophotometer. A solution of 95.5% acetone was used as a blank.

Chlorophyll *a* fluorescence emission from the adaxial surface of leaf was monitored with a Fluorescence Monitoring System (FMS 2; Hansatech Instruments Ltd., Norfolk, UK) in the pulse amplitude modulation mode, as previously described by Loggini et al. (1999) and Maxwell and Johnson (2000). Net photosynthetic rate (P_n) was measured using a Portable Photosynthesis System with an Infra-red Gas Analyser (IRGA; Model LI 6400, LI-COR® Inc, Lincoln, Nebraska, USA) according to Cha-um et al. (2007).

Plant height, number of leaves, number of tillers, leaf area, spike length, spike weight, one-hundred seed weight, and total grain weight per clump were measured. Leaf area was determined using a Leaf Area Meter DT-scan (Delta-Scan Version 2.03, Delta-T Devices, Ltd., Burwell, Cambridge, UK).

The experiment was arranged as 4 × 2 factorial in Completely Randomized Design (CRD) with ten replicates (n = 10). Analysis of variance (*ANOVA*) in each parameter was analyzed. The mean values were compared using Tukey-B test and analyzed with SPSS software (SPSS for Windows, SPSS Inc., Chicago, USA). The correlations between physiological and morphological parameters were evaluated by Pearson's correlation coefficients.

Results and Discussion

The chlorophyll *a* (Chl_a) content in the leaf tissue of RD6 rice grown in salt affected soil decreased, by 5.4, 19.6 and 26.4%, when exposed to 0.3, 1.0 and 2.0% salt levels, respectively. Chlorophyll *b* (Chl_b), total chlorophyll (TC) and total carotenoids (C_{x+c}) content had a similar trend to that of Chl_a. The Chl_a content in salt stressed leaves of RD6 rice was stabilized in the saline soil treated by OM, especially in the 1-2 % salt levels (Table 2). TC pigments in rice plants grown with OM treatment in 2% salt levels were maintained better than in the control (without OM) (Table 2). Photosynthetic pigment contents in the leaves of salt-stressed rice plants were decreased, depending on increasing salt levels in the soil. The Chl_a content in the salt stressed leaves was positively related to maximum quantum yield of PSII (F_v/F_m) (r^2 = 0.80) (Figure 1A). F_v/F_m dropped, relating to salt concentration in the soil (Table 3). Quantum efficiency of PSII (Φ_{PSII}) in the salt-stressed leaves declined by 1.5, 5.3 and 12.7% when exposed to 0.3, 1.0 and 2.0% salt levels, respectively. The Φ_{PSII} in salt stressed leaves of RD6 rice was improved in the saline soil treated by organic matter (OM), especially in the 2.0% salt levels (Table 3). A positive relationship between F_v/F_m and Φ_{PSII} was displayed (r^2 = 0.59) (Figure 1B) which subsequently affected the net photosynthetic rate (P_n) (r^2 = 0.46) (Figure 2A). P_n, a sensitive parameter, reduced drastically, by 26.2, 26.7 and 33.3% when subjected to 0.3, 1.0 and 2.0% salt levels, respectively (Table 3). In con-

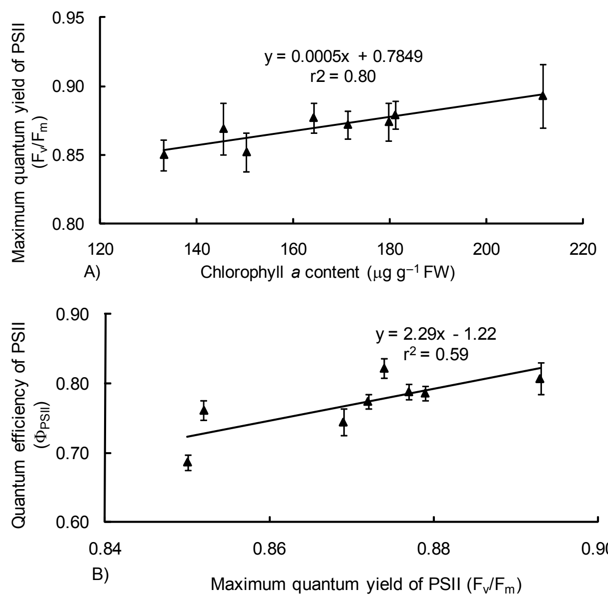

Figure 1 – Relationships between chlorophyll *a* (Chl_a) content and maximum quantum yield of PSII (F_v/F_m) (A) and F_v/F_m and quantum efficiency of PSII (Φ_{PSII}) (B) in leaf tissues of glutinous rice (cv. RD6) cultivated in field trials with salinity and remediation procedures.

trast, P_n in OM treated plants was sustained to a greater degree than in plants grown without OM (19.0, 22.3 and 22.8%), leading to high productivity as indicated by total grain weight per clump (r^2 = 0.45) (Figure 2B). Overall growth performance, demonstrated by plant height, number of leaves and leaf area, was evidently retarded in plants grown in 1.0 - 2.0% salt levels, especially in those without OM (control) treatment (Table 4). In contrast, the growth

Table 2 – Chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b), total chlorophyll (TC) and total carotenoids (C_{x+c}) in the leaf tissues of glutinous rice (cv. RD6) cultivated in field trials with different salinity and remediation procedures.

Salt level	Remediation	Chl_a	Chl_b	TC	C_{x+c}
%		$\mu g\ g^{-1}$ FW			
0	Control	181.1 b	48.2 b	229.3 b	62.4 ab
	OM	211.7 a	71.7 a	283.4 a	68.3 a
0.3	Control	171.3 bc	44.2 bc	215.5 bc	60.5 bc
	OM	179.8 b	45.7 bc	225.5 b	60.8 bc
1.0	Control	145.5 de	39.0 cd	184.5 de	50.8 de
	OM	164.2 bc	43.6 bc	207.8 cd	53.4 cd
2.0	Control	133.2 e	32.9 d	166.1 e	45.6 e
	OM	150.3 cd	38.1 cd	188.4 cd	52.7 de
ANOVA		**	**	**	**

Significant at $p \le 0.01$ is represented by **Means followed by the different letters are different (Tukey-B test, $p \le 0.01$).

Table 3 – Maximum quantum efficiency of PSII (F_v/F_m), quantum efficiency of PSII (Φ_{PSII}) and net photosynthetic rate (P_n) in leaf tissues of glutinous rice (cv. RD6) cultivated in field trials with salinity and remediation procedures.

Salt level	Remediation	F_v/F_m	Φ_{SII}	P_n
%				$\mu mol\ m^{-2}\ s^{-1}$
0	Control	0.879 ab	0.786 bc	17.1 a
	OM	0.893 a	0.807 ab	17.5 a
0.3	Control	0.872 ab	0.774 bc	12.7 b
	OM	0.874 ab	0.822 a	14.2 ab
1.0	Control	0.869 ab	0.744 c	12.6 b
	OM	0.877 ab	0.788 bc	13.6 b
2.0	Control	0.850 b	0.686 d	11.4 b
	OM	0.852 ab	0.761 bc	13.5 b
ANOVA		**	**	**

Significant at $p \le 0.01$ and significant at $p \le 0.05$ are represented by ** and *, respectively. Means followed by the different letters are different at $p \le 0.05$ and $p \le 0.01$ by Tukey-B test.

Table 4 – Plant height, number of leaves and leaf area of glutinous rice (cv. RD6) cultivated in field trials with salinity and remediation procedures.

Salt level	Remediation	Plant height	Number of leaves	Leaf are
%		cm		cm^2
0	Control	136.0 a	4.7 a	191.2 b
	OM	139.3 a	4.8 a	273.4 a
0.3	Control	118.2 bc	4.0 ab	180.1 b
	OM	128.6 ab	4.5 ab	217.5 ab
1.0	Control	104.4 cd	3.9 ab	161.4 b
	OM	127.8 ab	4.1 ab	209.8 b
2.0	Control	84.9 e	3.6 b	89.3 c
	OM	99.3 de	3.9 ab	99.4 c
ANOVA		**	**	**

Significant at $p \le 0.01$ is represented by **. Means followed by the different letters are different at $p \le 0.01$ by Tukey-B test.

Table 5 – Number of tillers, spike length, spiklet weight and 100-seed weight of glutinous rice (cv. RD6) cultivated in field trials with salinity and remediation procedures.

Salt level	Remediation	Number of tillers	Spike length	Spike weight	100-seed weight
%			cm	------------------------- g	-------------------------
0	Control	11.8 bc	25.2 b	3.1 bc	2.55 bcd
	OM	13.6 ab	25.9 b	3.7 ab	2.71 ab
0.3	Control	9.3 cd	25.2 b	3.0 bc	2.80 a
	OM	15.4 a	27.3 a	4.4 a	2.79 a
1.0	Control	7.9 d	23.5 c	2.5 c	2.70 ab
	OM	10.0 c	26.4 ab	4.4 a	2.76 ab
2.0	Control	6.6 d	23.9 c	2.6 c	2.45 d
	OM	9.1 cd	24.7 bc	2.7 c	2.52 cd
ANOVA		**	**	**	**

Significant at $p \leq 0.01$ is represented by **. Means followed by the different letters are different at $p \leq 0.01$ by Tukey-B test.

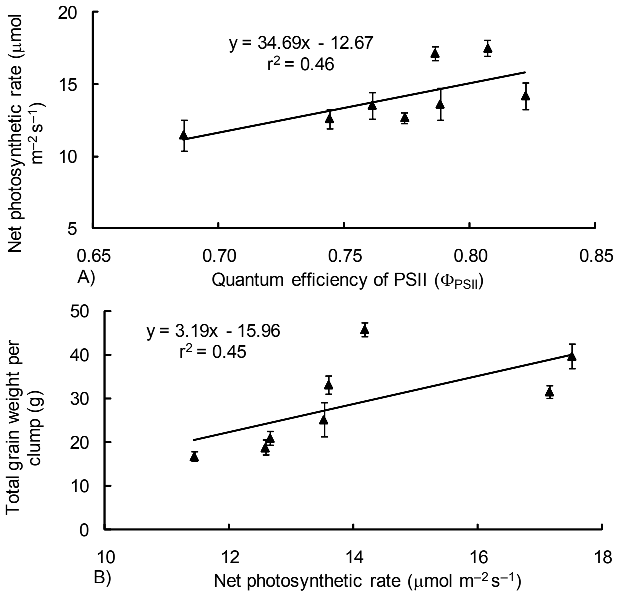

Figure 2 – Relationships between quantum efficiency of PSII (Φ_{PSII}) and net photosynthetic rate (P_n) (A) and P_n and total grain weight (B) of glutinous rice (cv. RD6) cultivated in field trials with salinity and remediation procedures.

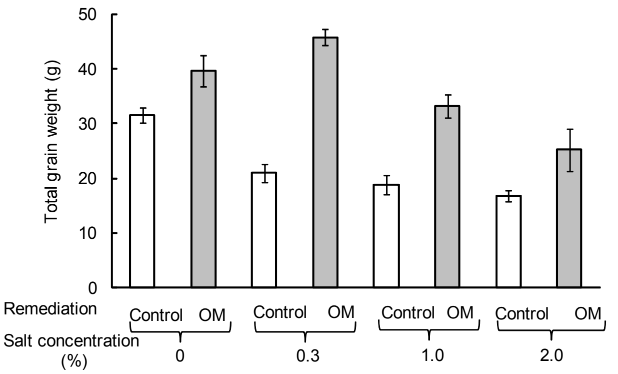

Figure 3 – Total grain weight per clump of glutinous rice (cv. RD6) cultivated in field trials with salinity and remediation procedures.

characters of OM treated plants were an improvement, except in the case of extreme salt contamination (2% salt level). Overall, salt stress adversely affected yield traits, including number of tillers, spike length, spike weight and 100-seed weight (Table 5), as well as total grain weight (TGW) (Figure 3).

Levels of Chl_a and TC pigments in rice grown in saline soil (2% salt level) treated with OM were maintained better than in the control (without OM treatment), leading to a high water oxidation rate in photosystem II and high P_n, and subsequently high productivity. In *Hordeum maritimum*, the photosynthetic pigments Chl_a, Chl_b and TC in plants cultivated in saline water treatment (4 g L^{-1} NaCl) with municipal solid waste (MSW) application (40 t ha^{-1}) were stabilized better than in the control (without MSW) by 48, 49 and 48%, respectively (Lakhdar et al., 2008). Organic matter (OM), including farmyard manure (FYM) and green manure (GM), may function as salt ion binding agents which detoxify the toxic ions, especially Na^+ and Cl^-, as indicated by low EC_e in soil treated with OM (Zaka et al., 2003; Hanay et al., 2004; Tejada et al., 2006; Zahid and Niazi, 2006). In the case of rice, remediation of saline paddy fields using OM improved productivity in the reproductive stage (Qadir et al., 2001; Zaka et al., 2003; Amanullah, 2008; Ghafoor et al., 2008; Murtaza et al., 2009). The most serious cases of yield reduction in crops grown in saline paddy fields that have not undergone remediation, are a subsequent effect of toxic ion accumulation in the plant cells, which causes pigment reduction, diminishes chlorophyll *a* fluorescence and lowers P_n. There are many reports on rice cultivation which clearly demonstrate the relationship between physiological and morphological changes in reproductive development during seed set, when plants are exposed to salt-affected soil without remediation (Zeng and Shannon, 2000; Zeng et al., 2003; Ali et al., 2004). OM application to saline paddy soil is an effective remediation procedure, in terms of the physical, chemical and biological properties of the soil (Mitchell et al., 2000; Hanay et al., 2004; Tejada et al., 2006; Wong et al., 2009), which can be used to enhance the growth and development of rice crops prior to grain harvesting (Ghafoor et al., 2008; Murtaza et al., 2009).

In conclusion, the level of chlorophyll *a* and total chlorophyll pigments in RD6 rice grown in saline soil (2% salt level) treated with OM was higher than in the control (without OM), resulting in improved photosynthetic abilities. Net-photosynthetic rate (P_n) in the salt-stressed leaves was strongly related to total grain weight, so, OM treatment in paddy fields could effectively alleviate the problem of soil salinity, resulting in yield improvement.

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