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Remediation of salt-affected soil by gypsum and farmyard manure – Importance for the production of Jasmine rice

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Abstract

The aim of this investigation was to remediate saline soil using gypsum and/or FYM before the cultivation of rice. Subsequently, the physiological and morphological characters of the rice plants, as well as crop yield, were evaluated. In this study, rice grown in soil with the application of gypsum and FYM had 79.6% spikelet fertility, while rice grown in soil without the gypsum and FYM treatment had only 46.4%. Sodium ion accumulation in both root and shoot organs of jasmine rice cultivated in the soil treated with gypsum and FYM treatments was lower than in rice grown in the soil without gypsum and FYM, while the potassium ion level was enriched. In addition, the low sodium ion accumulation in gypsum and FYM treated rice was positively related to water use efficiency and pigment stabilization, leading to high efficiency of photosystem II (Φ_{PSII}), and net-photosynthetic rate (P_n). The sugar content in the flag leaf of rice cultivated with gypsum and FYM treatment was enriched, leading to high productivity. The exogenous application of gypsum and FYM in saline fields may be used as effective remediation, which will lessen plant defects caused by the contaminating salts in saline soil.

Keywords: pigment stabilization, potassium ion, net photosynthetic rate, photosystem II, sodium ion, sugar content.
Abbreviations: Chl_a_chlorophyll a; Chl_b_chlorophyll b; EC_electrical conductivity; Fruc_fructose; F_v/F_m_maximum quantum yield of PSII; FYA_Farmyard manure; Gluc_ glucose; g_s_stomatal conductance; LA_leaf area; K_potassium; Na_sodium; NPC_number of panicle per culms; OSW_one-hundred seed weight; P_n_net-photosynthetic rate; Φ_{PSII}_photon yield of PSII; SF_spikelet fertility; Suc_sucrose; E_ transpiration rate; C_{x+c}_total carotenoids; TC_total chlorophyll; TGW_total grain weight per culms; TSS_total soluble sugar; WUE_water use efficiency.

Introduction

Saline (ECe >4 dS m^{-1}), or salt affected soil, is a major environmental issue, as it limits plant growth and development, causing loss of productivity, especially in crop species, which are defined as glycophyte (Hasegawa et al., 2000; Qadir et al., 2008). Salt affected soils are characterized by excessively high levels of water-soluble salts, including sodium chloride (NaCl), sodium sulfate (Na_2SO_4), calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$), among others. (Tanji, 2002). In the case of inland salinity, NaCl is a major salt contaminant in the soil. It has a small molecule size and when oxidized by water, producing sodium ions (Na^+) and chloride ions (Cl^-), which are easily absorbed by the root cells of higher plants and transferred to the whole plant using xylem uploading channels (Maathuis and Amtmann, 1999; Tester and Davenport, 2003; Rodriguez-Navarro and Rubio, 2006). Those toxic ions cause ionic and osmotic stresses at the cellular level of higher plants, especially in susceptible species (Mansour and Salama, 2004; Chinnusamy et al., 2005). There are many procedures that can be used to improve salt affected land, such as, water leaching, chemical remediation and phytoremediation (Ahmad and Chang, 2002; Sharma and Minhas, 2005; Qadir et al., 2007; Feizi et al., 2010). The remediation of saline soil using chelating agents such as gypsum ($CaSO_4$ $2H_2O$), calcite ($CaCO_3$), calcium chloride ($CaCl_2$) and organic matter (farmyard manure, green manure, organic amendment and municipal solid waste), is a fruitful topic of investigation and can be applied worldwide, being low cost, effective and simple to implement (Mitchell et al., 2000; Hanay et al., 2004; Sharma and Minhas, 2005; Tejada et al., 2006, Makoi and Verplancke, 2010). The physical, chemical and biological properties of salt affected soil are improved by the application of gypsum and/or FYM as remediation for sustainable land usage and crop productivity, leading to enhanced plant growth and development (Ghafoor et al., 2001; Choudhary et al., 2004; Wong et al., 2009). Rice (*Oryza sativa* L. spp. *indica*) is one of the top five major carbohydrate crops for the world's population, especially in Asia. It is a major staple food, supporting more than 3 billion people, comprising 50-80% of their daily calorie intake (Khush, 2005). Rice has previously been reported as being salt-sensitive in the vegetative and reproductive stages (Zeng et al., 2001; Moradi and Ismail, 2007), leading to a reduction in crop yield of more than 50% when exposed to 6.65 dS m^{-1}

Table 1. Sodium and potassium ions (mg g^{-1}FW) in the root and flag leaf tissues of Thai jasmine rice cultivated in the saline field treated with gypsum and/or farmyard manure (FYM).

Gypsum (g m^{-2})	FYM (g m^{-2})	Root		Leaf	
		Sodium	Potassium	Sodium	Potassium
0	0	16.04a	3.74b	30.28a	6.11b
	500	12.50ab	3.88ab	22.30b	11.47a
62.5	0	8.59b	5.02ab	20.54b	12.33a
	500	3.29c	5.11a	16.87b	12.40a
Significant level		**	**	**	**

Different letters in each column show significant difference at $p \leq 0.01$ (**) by Duncan's New Multiple Range Test (DMRT).

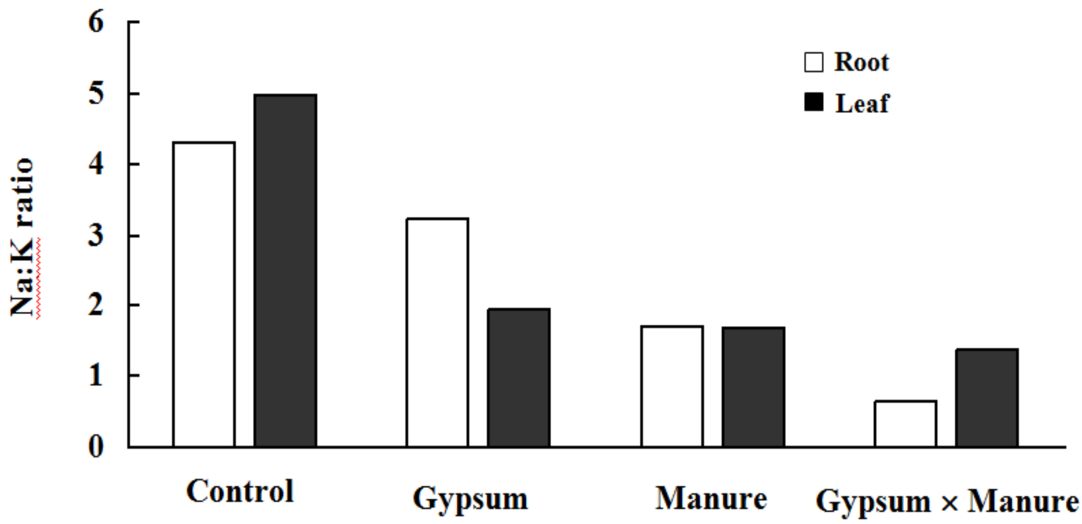

Fig 1. Sodium and potassium ratio (Na:K) in the root and leaf tissues of Thai jasmine rice cultivated in the saline field treated with gypsum and/or farmyard manure.

electrical conductivity (EC) salinity (Zeng and Shannon, 2000). In Thailand, jasmine rice is a premium quality grain in terms of aroma, cooking quality, soft texture and long grains, and is the most popular rice in the world (Ariyaphanphitak et al., 2005; Laohakunjit and Kerdchoechuen, 2007). It is widely cultivated in the northeastern region of Thailand, which is reported as being affected by salinity (Wongpokhom et al., 2008). The aim of the present study is to investigate the physiological adaptation and productivity of jasmine rice cultivated in saline soil remediated using gypsum and/or FYM.

Materials and methods

Plant materials and experimental site

Seeds of Thai jasmine rice cultivars (cv. KDML105), previously reported as salt susceptible (Cha-um et al., 2009), provided by Pathumthani Rice Research Center, (Rice Research Institute, Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand) were germinated and transplanted to pots containing clay soil in 50% shading (acclimatization) light intensity for 1 month. The pots were placed on plastic trays (30 × 45 cm) with 72 pots per tray. Water irrigation was applied using moisture spray. One-hundred acclimatized plants were transferred directly to a paddy field (4 m^2 with 20 × 20 cm in between rows) at the saline field site of Pimai Salt Co. Ltd., Pimai, Nakhon Ratchasima. Soil conditions were; clay soil texture (pH = 5.16, EC_e =12.5 dS m^{-1} (1.25% total salt contamination), total nitrogen = 0.38 g kg^{-1}, available phosphorus = 2.08 mg kg^{-1} and available potassium = 2.00 mg kg^{-1}). Gypsum ($CaSO_4$ $2H_2O$) and/or farmyard manure (FYM) were applied to the soil at 62.5 g m^{-2} and/or 500 g m^{-2}, respectively, compared to the control (without gypsum and FYM). Chemical fertilizer (16:16:16; Nitrogen: Phosphorus: Potassium) was applied 3 times, in mid-August, September and October 2009 at 0.0156 kg m^{-2}, prior to the flowering stage. Sodium and potassium ions, photosynthetic pigments, chlorophyll a fluorescence, net photosynthetic rate and soluble sugars in the flag leaf were assayed. In addition, seed set percentage, one-hundred seed weight, number of spikes per culm, total grain weight per culm and flag leaf area measurements were collected in the harvesting period.

Data collection

One hundred milligrams of root and flag leaf tissues were ground in liquid nitrogen. Sodium and potassium ions were extracted by the acidic method (HNO_3 and $HClO_4$) and assayed according to Dionisio-Sese and Tobita (1998) using an Atomic Absorption Spectrophotometer (AA, Model M16, Thermo Elemental, MA, USA). Chlorophyll a (Chl_a), chlorophyll b (Chl_b) and total chlorophyll (TC) concentrations were determined following the method of Shabala et al. (1998) and total carotenoids (C_{x+c}) concentration was measured according to Lichtenthaler (1987). One hundred milligrams of leaf material was collect-

Table 2. Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (TC) and total carotenoids (C_{x+c}) in the flag leaf of Thai jasmine rice cultivated in the saline field treated with gypsum and/or farmyard manure (FYM).

Gypsum ($g\ m^{-2}$)	FYM ($g\ m^{-2}$)	Chl_a ($\mu g\ g^{-1}$)	Chl_b ($\mu g\ g^{-1}$)	TC ($\mu g\ g^{-1}$)	C_{x+c} ($\mu g\ g^{-1}$)
0	0	249.32	158.57b	407.89b	75.55
	500	250.88	166.59b	417.47ab	81.84
62.5	0	252.26	169.64b	421.90ab	82.26
	500	254.13	203.81a	457.94a	83.37
Significant level		NS	**	**	NS

Different letters in each column show significant difference at $p \leq 0.01$ (**) by Duncan's New Multiple Range Test (DMRT). Non significant level in statistical analysis represent by ^{NS}.

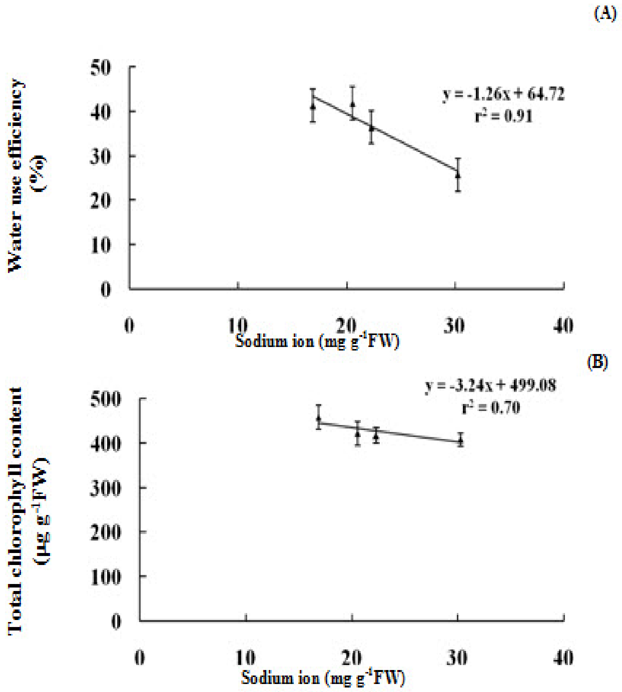

Fig 2. Relationship between sodium ion and water use efficiency (A) and sodium ion and total chlorophyll contents (B) in the flag leaf of Thai jasmine rice cultivated in the saline field treated with gypsum and/or farmyard manure. Error bars represent ±SE.

ed, placed in a 25 mL glass vial, along with 10 mL 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; $\mu mol\ m^{-2}s^{-1}$), transpiration rate (E; $mmol\ m^{-2}\ s^{-1}$) and stomatal conductance (g_s; $\mu mol\ H_2O\ m^{-2}\ s^{-1}$) were measured using a Portable Photosynthesis System with an Infra-red Gas Analyser (IRGA; Model LI 6400, LI-COR® Inc, Lincoln, Nebraska, USA). The E and g_s were measured by continuously monitoring the H_2O content of the air entering and existing in the IRGA headspace chamber. Water use efficiency (WUE) of acclimatized plantlets was calculated by the ratio of P_n to E (Cha-um et al., 2007a).

$$WUE = \frac{P_n}{E} \times 100$$

Soluble sugars; sucrose, glucose and fructose, in flag leaf tissues were analyzed according to the modified Karkacier method (2003). One hundred-milligrams fresh weight tissue was ground with liquid nitrogen in a pre-cooled mortar, extracted with 1 mL nanopure water, vigorously shaken for 15 s, sonicated for 15 min, and then centrifuged at 12,000 rpm for 15 min. The supernatant was filtered through a 0.45 µm membrane filter and stored at –20°C prior to the measurement of sugar content (sucrose, glucose and fructose) using high performance liquid chromatography (HPLC). Fifty-microlitters of crude extracts were automatically injected into the HPLC system with a Waters 600 pump. On-line detection was done using a Waters 410 differential refractrometer detector and the data was analyzed by Empower software. The analytical column was a MetaCarb 87C equipped with a guard column. Deionized water was used as the mobile phase with a 0.5 $mL\ min^{-1}$ flow rate. Glucose, fructose and sucrose were used as standards.Spikelet fertility percentage, one-hundred seed weight, number of panicles per culm, total grain weight per culm and flag leaf area were measured. Flag leaf area was

Table 3. Maximum quantum yield of PSII (F_v/F_m), photon yield of PSII (Φ_{PSII}), net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (E) in the flag leaf of Thai jasmine rice cultivated in the saline field treated with gypsum and/or farmyard manure (FYM).

Gypsum ($g\ m^{-2}$)	FYM ($g\ m^{-2}$)	F_v/F_m	Φ_{PSII}	P_n ($\mu mol\ m^{-2}s^{-1}$)	g_s ($\mu mol\ m^{-2}s^{-1}$)	E ($mmol\ m^{-2}s^{-1}$)
0	0	0.843b	0.802c	1.45b	11.2b	5.08b
	500	0.849b	0.807bc	1.85ab	12.1b	5.60b
62.5	0	0.857ab	0.820ab	2.62a	14.7a	6.25a
	500	0.871a	0.824a	2.63a	15.1a	6.35a
Significant level		**	**	**	**	**

Different letters in each column show significant difference at $p \leq 0.01$ (**) by Duncan's New Multiple Range Test (DMRT).

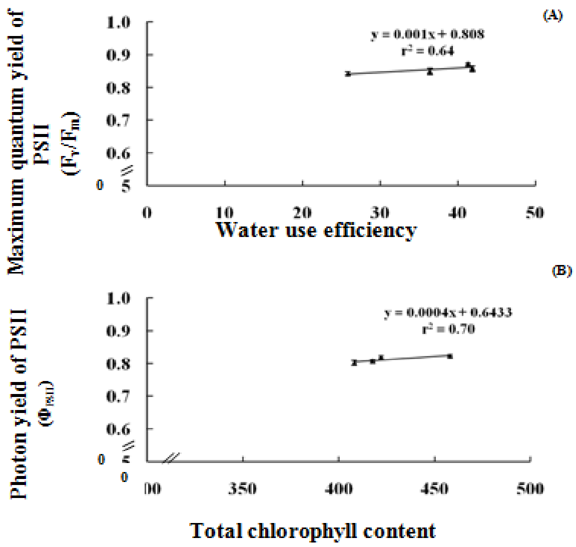

Fig 3. Relationship between water use efficiency and maximum quantum yield of PSII (A) and total chlorophyll contents and photon yield of PSII (B) in flag leaf of Thai jasmine rice cultivated in the saline field treated with gypsum and/or farmyard manure. Error bars represent ±SE.

determined using a Leaf Area Meter DT-scan (Delta-Scan Version 2.03, Delta-T Devices, Ltd., Burwell, Cambridge, UK).

Experiment design

The experiment was arranged as 2×2 factorials in Completely Randomized Design (CRD) with ten replicates (n=10). Analysis of variance (*ANOVA*) was assayed. The mean values were compared using Duncan's New Multiple Range Test (DMRT) and analyzed by SPSS software (SPSS for Windows, SPSS Inc., Chicago, USA). The correlations between physiological and biochemical parameters were evaluated by Pearson's correlation coefficients.

Results and Discussion

The levels of accumulated sodium ions in the root and flag leaf tissues of jasmine rice grown under saline field trial conditions, treated with gypsum and farmyard manure (FYM) were lower than those grown in untreated soil by 4.88 and 1.79 times, respectively, whereas potassium ions were enriched (Table 1). The sodium to potassium ratio (Na:K) increased in the root and flag leaf tissues in plants grown in soil without treatment by gypsum and FYM (control) (Fig. 1). The level of sodium ions in both root and flag leaf tissues decreased significantly, while that of potassium ions increased, the level depending on whether the soil treatment was by gypsum, FYM or a combination of the two (Table 1). Na^+ and Cl^- are the major ions which contaminate saline soil. They are absorbed rapidly by the root system of rice and accumulate in the whole plant, which will then display symptoms of toxicity in both vegetative and reproductive stages (Zeng et al., 2003; Ali et al., 2004; Cha-um et al., 2007b). In the present study, Na^+ in jasmine rice grown in saline soil treated with gypsum and FYM was very low, whereas K^+ was enriched when compared to the control (without gypsum and FYM). The properties of organic matter (Tejada et al. 2006) and gypsum (Chaudhry, 2001; Ghafoor et al., 2001) in treating saline soil have been reported for the purposes of saline soil remediation (Hanay et al., 2004; Wong et al., 2009; Makoi and Verplancke, 2010), for the cultivation of crops such as rice, wheat (Qadir et al., 2001), sugarcane (Choudhary et al., 2004), cotton and tomatoes (Mitchell et al.,

Table 4. Sucrose (Suc), glucose (Gluc), fructose (Fruc) and total soluble sugar (TSS) in the flag leaf of Thai jasmine rice cultivated in the saline field treated with gypsum and/or farmyard manure (FYM).

Gypsum ($g\ m^{-2}$)	FYM ($g\ m^{-2}$)	Suc ($\mu mol\ g^{-1}$)	Gluc ($\mu mol\ g^{-1}$)	Fruc ($\mu mol\ g^{-1}$)	TSS ($\mu mol\ g^{-1}$)
0	0	7.19b	7.19c	4.73c	19.11b
	500	8.75b	7.23c	4.58c	20.56b
62.5	0	15.81a	10.79b	9.06b	35.66a
	500	19.86a	14.25a	14.13a	48.24a
Significant level		**	**	**	**

Different letters in each column show significant difference at $p \leq 0.01$ (**) by Duncan's New Multiple Range Test (DMRT).

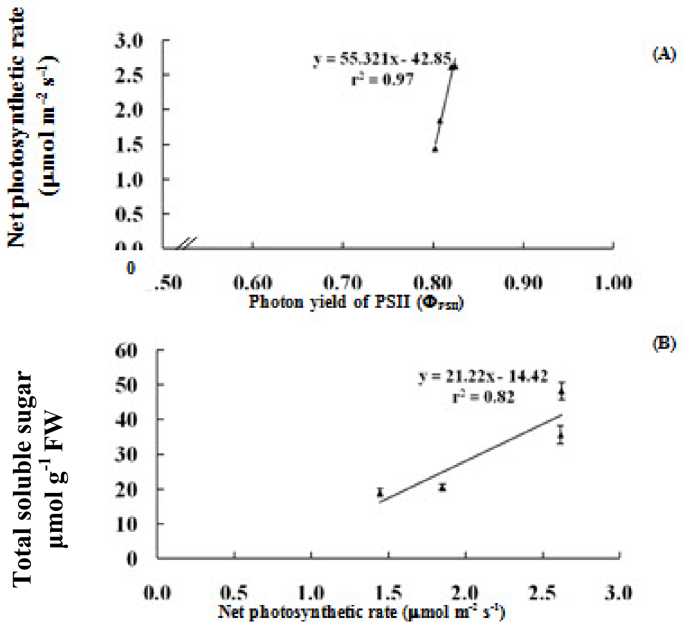

Fig 4. Relationship between photon yield of PSII and net photosynthetic rate (A) and net photosynthetic rate and total soluble sugar contents (B) in flag leaf of Thai jasmine rice cultivated in the saline field treated with gypsum and/or farmyard manure. Error bars represent ±SE.

2000). Organic matter, including FYM and green manure and gypsum may function as salt-ion chelating agents which detoxify the toxic ions, especially Na^+ and Cl^-, as indicated by low EC_e in soil treated with both FYM and gypsum (Zaka et al., 2003; Hanay et al., 2004; Tejada et al., 2006; Zahid and Niazi, 2006). Water use efficiency (WUE) (r^2 = 0.91) and total chlorophyll content (r^2 = 0.70) in flag leaf tissues were negatively related to the increase in sodium ions (Fig. 2). Chlorophyll b (Chl_b) and total chlorophyll (TC) content of the flag leaves of rice grown in soil treated with both gypsum and FYM were maintained better than in the control. This was also the case if the treatment was solely gypsum or FYM, except for chlorophyll a (Chl_a) and total carotenoids (C_{x+c}) (Table 2). The WUE and TC in the flag leaf tissues were positively correlated with maximum quantum yield of PSII (F_v/F_m) (r^2 = 0.64) and photon yield of PSII (Φ_{PSII}) (r^2 = 0.70), respectively (Fig. 3). Chlorophyll a fluorescence parameters, including F_v/F_m and $\Phi_{PSII,}$ in the flag leaf of rice grown in soil treated with gypsum were significantly better than the control and those grown in soil treated solely with gypsum or FYM (Table 3). The Φ_{PSII} in the flag leaf was positively related to net photosynthetic rate (P_n) (r^2 = 0.97) (Fig. 4A), leading to soluble sugar accumulation (r^2 = 0.82) (Fig. 4B). The P_n, stomatal conductance (g_s) and transpiration rate (E) in rice cultivated in soil treated with gypsum increased to a significantly greater degree than those of the control by 1.81, 1.35 and 1.25 times, respectively (Table 3). Sucrose (Suc), glucose (Gluc), fructose (Fruc) and total soluble sugar (TSS) in the flag leaf tissue of rice cultivated in soil treated with gypsum and FYM accumulated to a higher level than those in the control by 2.76, 1.98, 2.99 and 2.52 times, respectively (Table 4). The accumulation of soluble sugar in the flag leaf was directly related to the total grain yield of jasmine rice (r^2 = 0.91) (Fig. 5). Spikelet fertility (SF), one-hundred seed weight (OSW), number of panicles per culm (NPC), total grain weight per culm (TGW) and flag leaf area (LA) parameters at the harvesting stage increased significantly (Table 5), and were regulated by gypsum, FYM and their interactions, except in the case of TGW and LA of plants grown in soil treated with gypsum. In this study, water use efficiency and levels of photosynthetic pigments in rice grown in saline soil treated with gypsum and

Table 5. Spikelet fertility (SF), 100-seed weight (OSW), number of panicle per culms (NPC), total grain weight per culms (TGW) and flag leaf area (LA) of Thai jasmine rice cultivated in the saline field treated with gypsum and/or farmyard manure (FYM).

Gypsum ($g\ m^{-2}$)	FYM ($g\ m^{-2}$)	SF (%)	OSW (g)	NPC	TGW (g)	LA (cm^2)
0	0	46.44b	1.55c	9.35c	15.87b	23.27c
	500	71.42a	1.94b	9.55c	17.92b	24.77bc
62.5	0	73.33a	2.32a	16.30b	53.37a	27.64ab
	500	79.59a	2.50a	24.50a	58.04a	29.83a
Significant level		**	**	**	**	**

Different letters in each column show significant difference at $p \leq 0.01$ (**) by Duncan's New Multiple Range Test (DMRT).

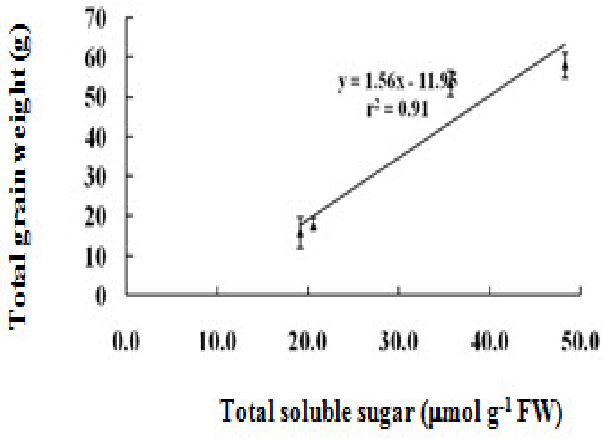

Fig 5. Relationship between total soluble sugar contents in flag leaf and total grain weight of Thai jasmine rice cultivated in the saline field treated with gypsum and/or farmyard manure. Error bars represent ±SE.

FYM were maintained better than those in the control (without treatment with gypsum and FYM), leading to a high water oxidation rate in photosystem II, high P_n and sugar content, as well as high productivity. The most serious cases of yield reduction from 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 low water use efficiency, pigment degradation, diminished water oxidation in PSII, low P_n and low sugar content (Qadir et al., 2001; Chaudhry et al., 2001; Zaka et al., 2003; Sharma and Minhas, 2005; Zahid and Niazi, 2006; Amanullah, 2008; Thammam et al., 2008; Hasaneen et al., 2009). The relationship between physiological and morphological changes in reproductive development during seed set in salt stressed rice without remediation have been demonstrated (Zeng and Shannon, 2000; Zeng et al., 2003; Ali et al., 2004). Gypsum and FYM applications to paddy saline 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 (Zaka et al., 2003; Sharma and Minhas, 2005). Individual applications of gypsum or FYM are ineffective in remediating saline soil (Qadir et al., 2001; Amanullah, 2008). In conclusion, the level of sodium ions in jasmine rice grown in saline soil treated with gypsum and FYM was lower than in the control, as well as being reduced in the Na:K ratio, resulting in higher water use efficiency and chlorophyll stabilization. Water oxidation and chlorophyll enrichment in the leaf tissues were strongly related to F_v/F_m and Φ_{PSII}, leading to high P_n and high soluble sugar accumulation as well as high total grain weight. Gypsum and FYM treatment should effectively remedy the saline soil problem, resulting in yield improvement from paddy fields.

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