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THESIS

IN VITRO RICE (*Oryza sativa* L. subsp. *indica*) RESPONSES TO SALT STRESS UNDER ISO-OSMOTIC CONDITION

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A Thesis Submitted in Partial Fulfillment of
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Salt stress affects the plant physiological responses by disturbing metabolic processes which result in the reduction of plant growth and development. However, previous studies did not explain clearly about ionic effects on plant physiological responses under salt stress. This study aims to investigate the ionic effect of NaCl on osmotic potential, photosynthetic pigment concentrations, photosynthetic performances and growth in salt-tolerant (Homjan; HJ) and salt-sensitive (Pathumthani1; PT1) rice varieties under iso-osmotic condition. Without the osmotic control, the osmotic potential, photosynthetic pigment concentrations, photosynthetic performances and growth in salt-sensitive PT1 seedlings were severely reduced more than those in salt-tolerant HJ seedlings with the increasing of NaCl concentration and salt exposure time. Under the iso-osmotic condition, sodium ion (Na^+), Na^+:K^+ ratio, root electrolyte leakage (EL_{root}), glucose, fructose, sucrose, raffinose and stachyose in PT1 salt-stressed seedlings were higher than those in HJ salt-stressed seedlings, while potassium ion (K^+) in PT1 seedlings was lower than that in HJ seedlings. The reduction of the physiological responses in PT1 seedlings exposed to salt stress under iso-osmotic condition was similar to the non-osmotic control condition. An exogenous application of KNO_3 and sucrose in the culture medium could increase the salt tolerance ability. These results indicated that there was no difference of the physiological responses in salt-stressed seedlings between the iso-osmotic control and non-osmotic control conditions.

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Kongake Siringam

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LIST OF ABBREVIATIONS

∅	=	diameter
Ψ_w	=	water potential
Ψ_π	=	osmotic potential
ρ_w	=	density of water
%	=	percentage
Φ_{PSII}	=	quantum efficiency of PSII
a	=	osmolarity
ABA	=	abscisic acid
AKT1	=	K^+ uptake channel
ANOVA	=	analysis of variance
APX	=	ascorbate peroxidase
ATP	=	adenosine triphosphate
°C	=	degree Celcius
C_3	=	C_3 carbon fixation
CAM	=	crassulacean acid metabolism
CAT	=	catalase
Ca^{2+}	=	calcium ion
Chl *a*	=	chlorophyll *a*
Chl *b*	=	chlorophyll *b*
CI	=	chlorophyll index
Cl^-	=	chloride ion
CLC	=	voltage-dependent chloride channel
CO_2	=	carbon dioxide
C_{x+c}	=	total carotenoids
CRD	=	completely randomized design
Cu/Zn-SOD	=	copper/zinc superoxide dismutase
d	=	day
DHAR	=	dehydroascorbate reductase
D_i	=	optical density at the wavelength i

LIST OF ABBREVIATIONS (Continued)

DM	=	dry matter
DMRT	=	Duncan's multiple range tests
DNA	=	deoxyribonucleic acid
dS m^{-1}	=	Deci Siemens per meter
DW	=	dry weight
EC_e	=	electrical conductivity
EL	=	electrolyte leakage
ESP	=	exchangeable sodium percentage
FBP	=	fructose-1,6-bisphosphatase
FK	=	fructokinase
F_v/F_m	=	maximum quantum yield of PSII
FW	=	fresh weight
g	=	gram
GAS	=	ground water associated salinity
Gols	=	galactinol synthase
GPx	=	glutathione peroxidase
GR	=	glutathione reductase
h	=	hour
ha	=	hectare
HAK	=	high-affinity K^+ uptake
H^+-ATPase	=	electrogenic proton pump
h d^{-1}	=	hour per day
HJ	=	Homjun rice cultivar
HKT	=	K^+-Na^+ co-transporter
H_2O_2	=	hydrogen peroxide
HPLC	=	High Performance Liquid Chromatography
IAS	=	irrigation associated salinity
IRRI	=	International Rice Research Institute
K^+	=	potassium ion
KDML 105	=	Khao Dawk Mali 105 rice cultivar

LIST OF ABBREVIATIONS (Continued)

KDML 19669	=	Khao Dawk Mali 19669 rice cultivar
KH_2PO_4	=	potassium phosphate
KNO_3	=	potassium nitrate
K_2SO_4	=	potassium sulfate
LPT123	=	Leung Pra Tiew 123 rice cultivar
LOP	=	leaf osmotic potential
m	=	meter
mg	=	milligram
min	=	minute
mL	=	milliliter
mm		millimeter
mM	=	millimolar
mol	=	mole
mL min^{-1}	=	milliliter per minute
mol kg^{-1}	=	mole per kilogram
MDHAR	=	monodehydroascorbate reductase
Mg^{2+}	=	magnesium ion
Mn-SOD	=	manganese superoxide dismutase
MPa	=	Mega Pascal
MS	=	Murashige and Skoog medium
μ	=	micro
μg	=	microgram
μL	=	microliter
μm	=	micrometer
μmol	=	micromolar
μg g^{-1} FW	=	microgram per gram fresh weight
μmol CO_2 h^{-1}	=	micromole carbon dioxide per hour
μmol g^{-1} FW	=	micromole per gram fresh weight
μmol m^{-2} s^{-1}	=	micromole per square meter per second

LIST OF ABBREVIATIONS (Continued)

nm	=	nanometer
Na^+	=	sodium ion
NaCl	=	sodium chloride
$Na^+ : K^+$	=	sodium potassium ratio
NAS	=	non-groundwater-associated salinity
NHX	=	Na^+/H^+ antiporter
NPQ	=	non-photochemical quenching
NSC	=	non-selective channel
O_2	=	oxygen
O_2^-	=	superoxide
$^1O_2$	=	singlet oxygen
$OH^\bullet$	=	hydroxyl radical
P	=	significant level
PM-ATPase	=	plasma membrane H^+-ATPase
p5cs	=	Δ^1-pyrroline-5-carboxylate synthetase
POD	=	peroxidase
PPF	=	photosynthetic photon flux
PSII	=	photosystem II
PT1	=	Pathumthani 1 rice cultivar
qP	=	photochemical quenching
r	=	relation
rpm	=	round per minute
RFS	=	raffinose synthase
RH	=	relative humidity
ROSs	=	reactive oxygen species
s	=	second
subsp.	=	subspecies
SAR	=	sodium absorption ratio
SDW	=	shoot dry weight

LIST OF ABBREVIATIONS (Continued)

S.E.	=	standard error
SFW	=	shoot fresh weight
SKOR	=	stelar K^+ outward rectifier
SO_4^{2-}	=	sulfate ion
SOD	=	superoxide dismutase
STS	=	stachyose synthase
t	=	temperature
TC	=	total chlorophyll
USSL	=	United State Salinity Laboratory
v	=	volume
v/v	=	volume by volume
V-ATPase	=	vacuolar H^+-ATPase
VHA	=	vacuolar Na^+/H^+ antiporter
V-PPase	=	vacuolar pyrophosphatase
w	=	weight
w/v	=	weight by volume

IN VITRO RICE (*Oryza sativa* L. subsp. *indica*) RESPONSES TO SALT STRESS UNDER ISO-OSMOTIC CONDITION

INTRODUCTION

Rice (*Oryza sativa* L. subsp. *indica*) is an important crop which is a carbohydrate source for three billions of the world population, particularly in Asia. Thailand is a number one of rice exporter in the world market, peculiarly aromatic rice. However, the rice growth and productivity are limited by environmental problem, especially salinity which obstacles the crop productivity. In Thailand, the rice cultivation area in northeastern and nearby seashore is affected by salinity. Many researches demonstrated that the increasing of salt stress induced osmotic and ionic effects. Osmotic effect lowered soil water potential which decreases water availability that affects plant water uptake. In addition, the ionic effect is generated by excess salt ion accumulations such as sodium ion (Na^+) and chloride ion (Cl^-). The ionic effect induces nutrient deficiencies and ion imbalance (Na^+:K^+ ratio) which results in the electrolyte leakage induction of the plasma membrane. However, most research works explained the ionic effect on plant physiological responses without the osmotic control.

Generally, plants adapt to the negative effects of salt stress by operating salt defensive mechanisms including; ion homeostasis and compartmentalization, osmoregulation, antioxidant system and hormonal regulation. Under non-osmotic control, the physiological responses to salt stress could not indicate the salt defensive mechanism which functions in salt-stressed rice seedlings. The ion homeostasis and osmoregulation are major salt defensive mechanisms. Potassium (K) is an important inorganic cation which plays a role on ion homeostasis. Many researches showed the lowering Na^+:K^+ ratio increasing salt tolerance ability during salt stress. Recently, the improvement of the salt tolerance ability by potassium fertilizer applications shows the reduction of Na^+ accumulation while K^+ is increased. It leads to maintain the lowering Na^+:K^+ ratio and reduces plant damage.

In addition, the osmoregulation plays key role on preventing and detoxifying from salinity. Soluble sugars are the major carbohydrate that plays a role on plant tolerance to abiotic stress such as water deficit, chilling and salinity. The soluble sugars perform the osmotic adjustment by reducing osmotic potential which results in turgor pressure maintenance and continuing water influx into the cells under salinity. Moreover, the soluble sugars play an important role on the reserve energy and membrane stabilization by retaining the integrity of membranes during stress conditions. Previous researches show that the sugar accumulation in many species is regulated by salt stress.

Therefore, this study was to elucidate influences of NaCl on physiological responses i.e. ion contents, osmotic potential, electrolyte leakage, photosynthetic pigment concentrations, chlorophyll *a* fluorescence parameters, growth and sugar content, in the salt-tolerant and salt-sensitive rice seedlings under iso-osmotic condition. The research clarifies the improvement of salt tolerance ability in salt-sensitive rice variety by exogenous potassium fertilizer and sugar applications.

OBJECTIVES

Aim of this research was to understand roles of NaCl on physiological responses for increasing salt tolerance capability of rice grown under salt stress. Current research was performed as three specific approaches as following;

1. To study the physiological responses of salt-tolerant and salt-sensitive rice varieties to different NaCl concentrations and salt exposure times

2. To study the physiological responses of salt-tolerant and salt-sensitive rice varieties to salt stress under iso-osmotic condition

3. To study the salt tolerance ability improvement by exogenous potassium nitrate (KNO_3) and sucrose application

LITERATURE REVIEW

1. Rice

1.1. Botanical characteristics

Rice, a monocotyledon plant, belongs to family *Gramineae* (*Poaceae*). It is an annual cereal crop which produces inflorescence (panicle) composing spikelets and flowers that produce seed or grain (Figure 1). Rice was domesticated in India around 3000 B.C. and distributed to China, Indonesia and Japan. The cultivated species are diploid (2n = 24) such as *Oryza sativa* L. (Asian rice) and *Oryza glaberrima* Steud. (West Africa rice) while wild species may be tetraploid. The most cultivated species is *Oryza sativa* L. which is categorized into three subspecies: *indica, japonica* and *javanica*. An *indica* rice is grown in the tropical climate. It is a popular subspecies since this species has higher yield (high productivity) and better adaptation to various growing conditions than other subspecies. Seed characteristics of *indica* rice include medium to long grain, higher amylose content which results in dry and fluffy cooked rice.

Mostly, *indica* rice is cultivated in the Asia and Americas continents (Christou, 1994). Rice is a carbohydrate source for the necessary daily consumption for more than a half of the world's population (International Rice Research Institute, 1990; Dawe, 1999; Khush, 2005). Rice is an exporting crop in many countries such as Thailand, China, India and Vietnam. In the present, the demand of rice grain depends on the increasing of the world population. The rice consumption of the world population is expected to reach 780 million tons in 2020 (Shabbir *et al.*, 2001). However, growth and productivity of rice were decreased by environmental stress conditions such as drought, extreme temperature, nutrient deficiencies and salinity (Lutts *et al.*, 1995; Toenniessen, 1995; Shannon *et al.*, 1998; Zeng and Shannon, 2000; Zeng *et al.*, 2001; Yokoi *et al.*, 2002; Zeng *et al.*, 2003).

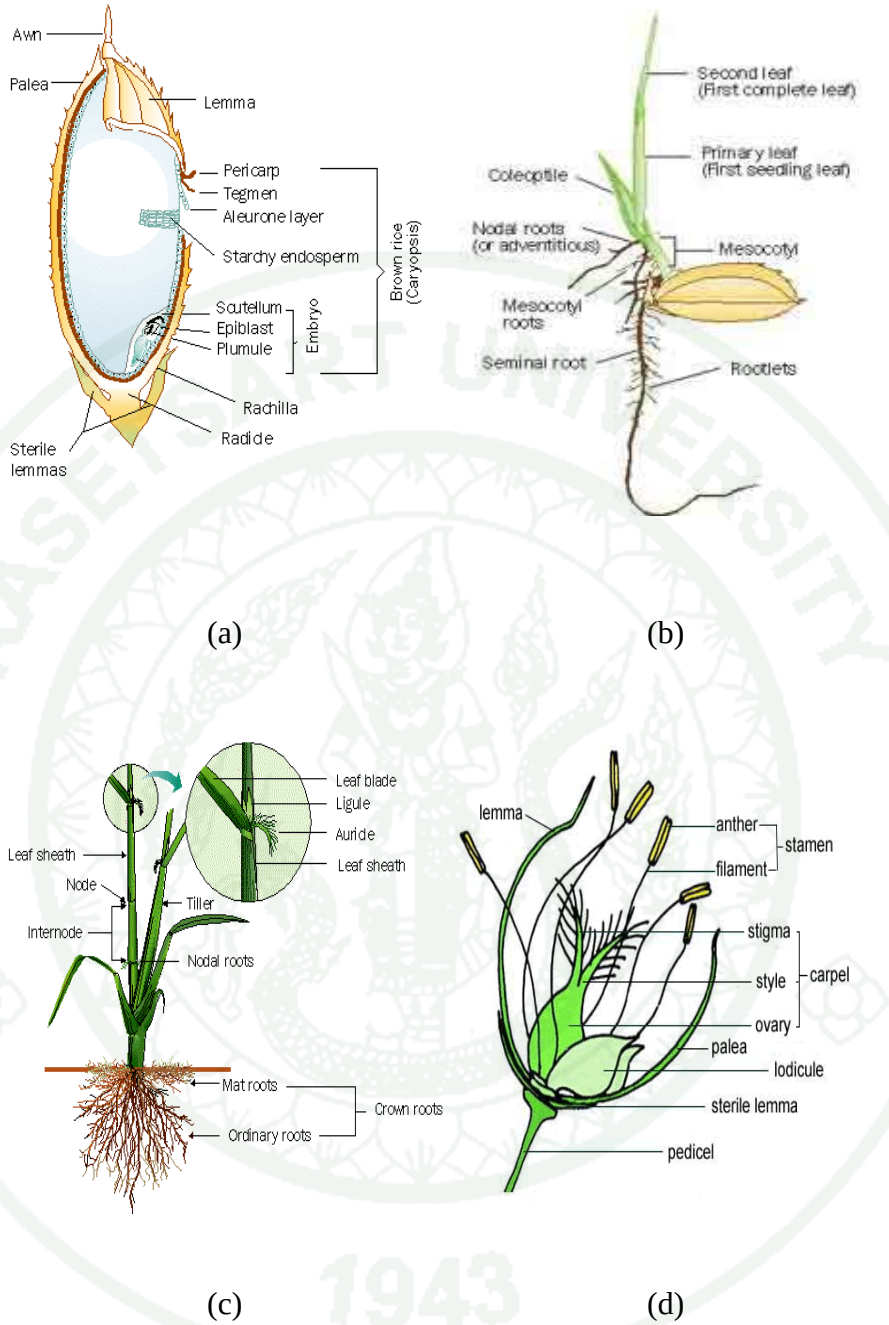

Figure 1 Morphology of rice seed (a), seedling (b), tillering (c) and flowering (d).

Source: Anonymous (2000)

1.2. Variation of salt tolerance ability in rice

In the salt-affected soil, the growth and yield of rice are reduced since rice is a salt-sensitive crop (Shannon *et al.*, 1998). Survival rate, seedling growth and yield component of M-202 rice cultivar subjected to salt stress (EC = 11.5 mS cm^{-1}) were reduced more than 50% in comparing to the control (EC = 0.9 mS cm^{-1}) (Zeng and Shannon, 2000).

Salt tolerance ability in twelve rice genotypes exposing to salt stress (EC = 4.5-8.3 mS cm^{-1}) was identified by physiological characters; ion uptakes, ion selectivity and leaf area index. The Na^+ and Cl^- in salt-sensitive M-103 rice were higher than those in salt-tolerant IR63731-1-1-4-3-2 rice. This resulted in the reduction of the leaf area index and grain yield in M-103 rice more than that in IR63731-1-1-4-3-2 rice (Zeng *et al.*, 2003).

In addition, the reproductive growth (seed yield, seed weight per panicle, spikelet number per panicle and tiller number per plant) in twelve rice genotypes were reduced by the increasing of salt stress (EC = 8.3 mS cm^{-1}). However, the reduction of the reproductive growth in salt-tolerant genotypes (IR63731-1-1-4-3-2, Agami and GZ1368-5-4) was lower than that in salt-sensitive genotypes (M-103 and IR71657-5R-B-12PB) (Zeng *et al.*, 2002).

Classical salt-tolerant rice cultivar included Agami, Cherveruppu, Daeyabyeo, Nona Bokra, Kalarata, Pokkali and SR26B. Salt-sensitive rice cultivar compromised IR28, IR29 and Sakha101 (Quijano-Guerta and Kirk, 2002; Zeng *et al.*, 2004; Zeng, 2005). Lee *et al.* (2003) demonstrated that the salt tolerance ability (growth reduction percentage and ion accumulation) in *indica* subspecies (Pokkali, IR45427-2B-2-2B-1-1 and TCCP 266) was lower than that in *japonica* subspecies (Agami M1 and Namyang 7).

Salt tolerancece ability in rice was developed by using breeding program that produced breeding lines such as IR63731-1-1-4-3-2, GZ5291-7-1-2, IR50184-3B-18-2B-1 and AC26. These rice lines expressed high growth and grain yield under high salinity (EC = 8.3 dS m^{-1}) (Zeng *et al.*, 2002). Senadhira *et al.* (2002) developed salt-tolerant rice line (IR51500-AC11-1 as PSBRc50) from anther culture which the rice line showed high salt tolerance ability and high-yielding ability.

1.3. Salt tolerance classification in Thai rice

Thai aromatic rice is an economic crop which is exported to the world market. However, salinity is a seriously problem with the reduction of growth and rice yield. Previously, salt tolerance ability in Thai aromatic rice is classified by physiological and biochemical responses (Wanichananan *et al.*, 2003; Cha-um *et al.*, 2009c). Salt stress induced proline accumulation in Thai aromatic rice lines. The highest proline content was found in Leuang Tang Mo rice while the lowest content of proline was found in Khao Dawk Mali19669 (KDML19669) and other KDML rice lines. In addition, the proline content in Khao Dawk Mali105 (KDML105) and Pathumthani1 (PT1) were classified in the middle and low level, respectively. The chlorophyll index (CI) in Hawm Naipon, Hawm Durian, Hawm Paepalo, Hawm Phrae, Hawm Thong, Hawm Tang, Hawm Jampa, Hawm Nang Nuan, Hawm Sadung, Hawm and Hawm Maejan was higher than that in KDML105 when exposed to 513 mM NaCl for 8 days. This result demonstrated that the chlorophyll index (CI) indicated the salt tolerance ability in Thai aromatic rice (Wanichananan *et al.*, 2003).

Cha-um *et al.* (2009c) showed that salt stress increased sodium ion (Na^+) and Na^+:K^+ ratio which led to the enhancement of the relative electrolyte leakage in Khao Dawk Mali105 (KDML105) more than that in Hawm Jan (HJ). Photosynthetic pigments, photosynthetic performances and water use efficiency in HJ rice were higher than those in KDML105 and resulted in higher growth performance during salt stress. In addition, an increase in salt stress (85-427 mM NaCl) induced Na^+ content while K^+ decreased in Pathumthani1 (PT1), Khao Dawk Mali105 (KDML105) and Hawm Jan (HJ). Moreover, the increase in osmolarity was positively related to the

photosynthetic pigment degradations which resulted in the growth inhibition. The photosynthetic pigment degradations indicated that the HJ was salt-tolerant rice variety whereas PT1 and KDML105 were salt-sensitive rice varieties (Cha-um *et al.*, 2007b).

Recently, there were many researches which compared physiological and biochemical characteristics between Thai aromatic rice, classical salt-tolerant and salt-sensitive rice varieties. Pongprayoon *et al.* (2008) compared the proline content in eleven Thai aromatic rice cultivars including; Hawm Paepalo (HPL), Hawm Sadung (HSD), Hawm Jan (HJ), Hawm Nang Nuan (HNN), Hawm Tang (HT), Hawm Maejan (HMJ), Hawm Phrae (HP), Hawm Thong (HT), Hawm Jampa (HJP), Hawm (Hom), Hawm Durian (HDR) to the classical salt-tolerant rice (Pokkali; Pok) and classical salt-sensitive rice (IR29). According to the proline content, Thai rice cultivars were classified into three classes, high (HTH, HPL and HJP), moderate (HMJ, HNN, HT, Pok, HJ and HSD) and low (HDR, IR29, HP and Hom) when exposed to 513 mM NaCl for 4 days. Moreover, this study found that the green leaf area was reduced when NaCl concentration and proline content were increased.

Cha-um *et al.* (2009a) indicated that the photosynthetic pigments and photosynthetic performances in term of chlorophyll *a* fluorescence in Thai aromatic (Hawm Jan; HJ) rice variety were higher than those in classical salt-tolerant rice variety (Pokkali), classical salt-sensitive rice variety (IR29) and Pathumthani1 (PT1) when exposed to salt stress (342 mM NaCl) for 4 days. These physiological characteristics can be used to identify the salt tolerance ability in Thai rice. Cha-um *et al.*, (2010a) reported that the Kumuangluang (KML), Khao Dawk Mali105 (KDML105), Pokkali (POK), Homjan (HJ), Dokpayom (DPY), Chewmaejan1 (CMJ1), Chewmaejan2 (CMJ2), upland rice1 (UR1), upland rice2 (UR2) and Chowho (CH) were classified into the salt-tolerant rice variety. In addition, the R258, Pathumthani1 (PT1), IR29 and upland rice2 (UR2) were salt-sensitive rice varieties.

From the previous mentions, the salt tolerance ability in term of physiological and biochemical expressions in Hawm Jan (HJ) rice variety was closely related to the classical salt-tolerant rice variety (Pokkali) during salt stress. The HJ rice variety is a local rice variety (salt-tolerant rice) that grows well in the salt-affected paddy fields nearby coastal area in the southern region of Thailand. It is a short-day photoperiod sensitivity rice, low tillering, short grain and environmental stress tolerant. Pathumthani1 (PT1) rice variety is similar to the classical salt-sensitive rice variety (IR29). The PT1 rice variety is originated from conventional breeding of BKNA6-18-3-2 × PTT85061-86-3-2-1 and is identified as a salt-sensitive rice, photoperiod insensitivity, high tillering, high amylose content and high grain yield (Cha-um *et al*., 2007b). The salt-tolerant HJ and salt-sensitive PT1 rice varieties were selected for this research.

2. Saline soil

Saline soil is a serious environmental problem which is initiated by accumulation of water soluble salts in the soil that influences on the agricultural production, environmental health and economic welfare (Rengasamy *et al.,* 2006). The salinity induces osmotic and ionic effects which lead to the the water uptake limitation and plant nutrient deficiencies. These cause the reduction of plant growth and development. The saline soil is typically dominated by sodium ion (Na^+), magnesium ion (Mg^{2+}), chloride ion (Cl^-) and sulfate ion (SO_4^{2-}) (Läuchli and Lüttge, 2002), especially Na^+ which can be used to classify the type of saline soil.

2.1. Classification of saline soil

Abrol *et al.* (1988) classified the saline soil and effect on crop plants into five groups by following the differences of the electrical conductivity (EC); non-saline, slightly saline, moderately saline, strongly saline and very strongly saline soils (Table 1). The saline soil generally appeared in more than one hundred countries in the world (Szabolcs, 1989) (Table 2). It is a major abiotic stress in agricultural land in worldwide. It degrades the soil structure which results in the reduction of the soil

productivity. About 900 million ha of agricultural land was saline soil and it tended to the dispersing in the cultivated area (Flowers and Yeo, 1995; Ghassemi *et al.*, 1995; Szabolcs, 1999). The salinity disturbed plant metabolisms that affected the decreasing of plant growth and development such as germination, seedling growth, maturation, flowering and grain yield (Shabbir *et al.*, 2001; Khan and Abdullah, 2003; Zafar *et al.*, 2004).

Table 1 Classification of saline soil and crop growth.

Soil salinity class	EC (mS cm^{-1})	Effect on Crop Plants
Non saline	0 - 2	Salinity effects negligible
Slightly saline	2 - 4	Yields of sensitive crops may be restricted
Moderately saline	4 - 8	Yields of many crops are restricted
Strongly saline	8 - 16	Only tolerant crops yield satisfactorily
Very strongly saline	> 16	Only a few very tolerant crops yield satisfactorily

EC = electrical conductivity of extract of saturated soil paste

Source: Abrol *et al.* (1988)

2.2. Origin of saline soil

Origin of saline soil includes natural and anthropogenic or human-induced. The salinity is generated by salt in the natural area such as rainfall, wind transportation and seawater intrusion in the landscape. The saline soil is induced by human practice on the environment, especially poor irrigation management which leads to the salt accumulation in irrigated soils (Abrol, 1986; Szabolcs, 1999; Läuchli and Lüttge, 2002).

Rengasamy *et al.* (2006) defined the type of salinity by following soil and ground water associations. Firstly, GAS, in discharge areas of the landscape, the water exits from groundwater and brings the dissolved salts to the soil surface. High salt accumulation was found when the water table was less than 1.5 m below the soil surface. However, this threshold depth may vary depending on soil hydraulic properties and climatic conditions.

Secondly, NAS, the water table was depth and drainage was poor in landscapes. The salts, introduced by rain, weathering, and aeolian depositions, were stored within the soil. In dry climatic zones, the salts were usually found in the deeper soil layers. However, poor hydraulic properties of shallow soil layers led to the accumulation of salts in the topsoil and subsoil layers that affected the agricultural productivity.

Finally, IAS, the salts were introduced and stored in the root zone by irrigation because of insufficient leaching. The poor quality of irrigation water, low hydraulic conductivity of soil layers (heavy clay soils and sodic soils) and high evaporative conditions induced salinity. Highly saline effluent water and improper drainage, and soil management increased the risk of salinity in irrigated soils. In many irrigation regions, rising saline groundwater interacting with the soils in the root zone can induce the salinity problem (Figure 2).

Table 2 Global distribution of saline soil.

Continent	Saline soil (million hectares)
North America	6.2
Central America	2.0
South America	69.4
Africa	53.5
South Asia	83.3
North and Central Asia	91.6
Southeast Asia	20.0
Europe	7.8
Australasia	17.4
Total	351.5

Source: Szabolcs (1989)

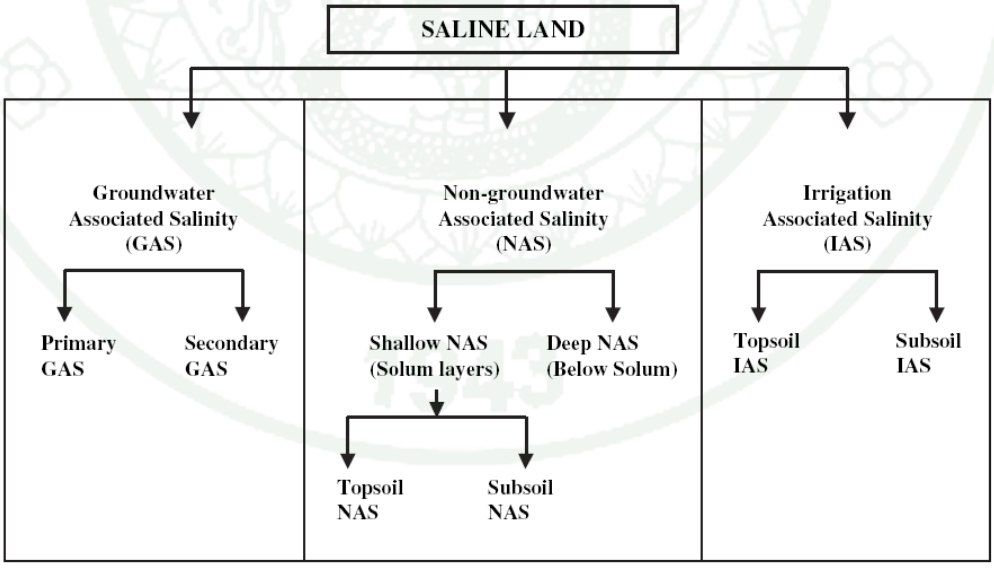

Figure 2 Major types of salinity in world soils based on salinization processes.

Source: Rengasamy *et al.* (2006)

3. Physiological characteristic responses in plants to salinity stress

Salinity stress generated the excess salts, especially sodium ion (Na^+) which induced osmotic (water deficit) and ionic (ion imbalance and ion toxicity) effects. Change of osmotic and ionic effects led to the reduction of plant metabolisms which resulted in the decreasing of plant growth and development (Flowers and Hajibagheri, 2001; Munns, 2002; Yokoi *et al.*, 2002; Tester and Davenport, 2003; Parida and Das, 2005). The physiological responses of plants to salinity stress were divided into four types;

Firstly, the salt accumulations reduced the osmotic potential that caused the water deficit condition, leading to the reduction of the plant water absorption (Allakhverdiev *et al.*, 2000; Aziz and Khan, 2001; Munns, 2002; Parida and Das, 2005; Diédhiou and Golldack, 2006; Yasar *et al.*, 2006; Hu *et al.*, 2007). Many researches reported that tomato (Romero-Aranda *et al.*, 2001), maize (Çiçek and Cakirlar, 2002), sorghum (Netondo *et al.*, 2004) and rice (Cha-um *et al.*, 2007b) adjusted the osmotic potential for maintaining the turgor pressure and water uptake ability. Moreover, the decreasing of water absorption reduced the plant water relation such as stomatal conductance, transpiration rate and photosynthetic rate that affected the survival rate and growth inhibition in many species such as pea (Hernández *et al.*, 1995), tomato (Mohammad *et al.*, 1998), cotton (Meloni *et al.*, 2001), wild rice (Nakamura *et al.*, 2002), sorghum (de Lacerda *et al.*, 2005), barley (Chen *et al.*, 2007), *Eucalyptus* (Nasim *et al.*, 2008) and cactus pear (Silva-Ortega *et al.*, 2008). The root cap tissues proliferation and basal part of the root tip were observed in salt-tolerant rice (Pokkali and Nona Bokra) and salt-sensitive rice (IR24 and Nipponbare) after exposed to the salinity. The root cap length in salt-sensitive IR24 and Nipponbare rice was reduced by 50 mM NaCl due to peeling off (Ferdose *et al.*, 2009).

Secondly, salt stress increased sodium ion (Na^+) accumulation while potassium ion (K^+) was decreased. This resulted in the Na^+ toxicity and nutrient uptake disturbances (McKersie and Leshem, 1994; Aziz and Khan, 2001) in sorghum

(de Lacerda *et al.*, 2005), rice (Cha-um *et al.*, 2007b), tomato (Khelil *et al.*, 2007) and wheat (El-Hendawy *et al.*, 2009; Mahmood, 2009). In addition, an increase of Na^+ was positively related to induction of Na^+:K^+ ratio in cotton (Meloni *et al.*, 2001), olive (Ben Ahmed *et al.*, 2008) and rice (Singh *et al.*, 2007). Luo *et al.* (2005) suggested that Na^+ and Na^+:K^+ ratio in salt-sensitive soybean (N23232 and Zhongzi huangdou-yi) seedlings were higher than those in salt-tolerant soybean (BB52 and Nannong 1138-2) seedlings after exposed to 150 mM NaCl for 6 days. In rice plant, the salt-sensitive I Kong Pao seedlings showed higher Na^+ and Na^+:K^+ ratio than those the salt-tolerant Pokkali seedlings (Lefèvre *et al.*, 2001). The Na^+:K^+ can be used to identify the salt tolerance ability in wheat (Ahmad and Jabeen, 2005), sorghum (de Lacerda *et al.*, 2005) and rice (Singh *et al.*, 2007). The excess of Na^+ resulted in the decreasing of K^+ uptake by the expression of K^+ uptake channel (*AKT1*) in roots and shoots (Fuchs *et al.*, 2005) while the expression of high-affinity K^+ uptake (*HAK5*) in *Arabidopsis* roots was induced by K^+ starvation (Rodríguez-Navarro and Rubio, 2006). Moreover, the increase of Na^+ reduced Ca^{2+} content which resulted in the initiation of membrane injury (Parida *et al.*, 2004).

Thirdly, the lipids were an effective source of storage energy and structural constituents of the cellular membranes (Singh *et al.*, 2002). The stress conditions such as salinity, drought, waterlogging, temperature extremes and high light intensity, etc. induced reactive oxygen species (ROSs) production such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\bullet$) and singlet oxygen ($^1O_2$) (Ashraf, 1994; Mittova *et al.*, 2000; Mittler, 2002; Munns, 2002). The electron transportation in mitochondria and chloroplasts were disturbed by ROSs which caused protein degradation and DNA mutation (Smirnoff, 1993; Alscher *et al.*, 1997; McCord, 2000). In rice plant, the lipid peroxidation in salt-sensitive *japonica* Hitomebore and salt-sensitive *indica* (IR28) was higher than that in salt-tolerant *indica* (Pokkali) (Dionisio-Sese and Tobita, 1998). The lipid peroxidation can be used to indicate the salt tolerance ability in rice (Dionisio-Sese and Tobita, 1998), wheat (Sairam and Srivastava, 2002), marigold (Chaparzadeh *et al.*, 2004), corn (Hajlaoui *et al.*, 2009) and alfalfa (Wang *et al.*, 2009).

Finally, the salinity reduced the photosynthesis in term of water oxidation and gas exchange (Shennan *et al.*, 1987; Tyree *et al.*, 1994; Hwang and Chen, 1995; Delfine *et al.*, 1998; Parida *et al.*, 2004; Choat *et al.*, 2005; Hacke *et al.*, 2006; Çavuşoğlu *et al.*, 2007; Çavuşoğlu *et al.*, 2008). The light-dependent reactions occur in the thylakoid membranes of the chloroplast and use light energy to synthesize ATP and NADPH (Figure 3). The light-dependent reactions can be evaluated by measurement the chlorophyll *a* fluorescence. In light-dependent reactions, the light energy is absorbed by chlorophyll molecules in a leaf and undergoes one of three fates: it can be used to drive photosynthesis (photochemistry), excess energy can be dissipated as heat or it can be re-emitted as light-chlorophyll fluorescence.

The chlorophyll *a* fluorescence informs the changes of the efficiency of photochemistry and heat dissipation can be gained. The chlorophyll *a* fluorescence parameters compose of;

i) Maximum quantum yield of PSII (F_v/F_m). The F_v/F_m estimates PSII maximum efficiency in the dark-adapted state. For healthy plant, the F_v/F_m value is closely to 0.8. In contrast, lower F_v/F_m indicates that the PSII reaction centers are damaged by stress conditions.

ii) Quantum efficiency of PSII (Φ_{PSII}). The Φ_{PSII} measures the efficiency of photosystem II. The Φ_{PSII} demonstrates the proportion of light absorbed by PSII and can give a measure of the electron transport. High Φ_{PSII} value indicates high efficiency of the PSII.

iii) Photochemical quenching (qP). The yield of chlorophyll *a* fluorescence from PSII can be decreased (quenched) by photochemistry (photochemical quenching) or by one or more processes that are not directly linked to photochemistry (non-photochemical quenching). There is an inverse, non-linear relationship between the fraction of PSII centres in the open state and the yield of chlorophyll *a* fluorescence.

iv) Non-photochemical quenching (NPQ). The NPQ shows protective roles on non-photochemical mechanisms which quench singlet-excited chlorophylls (Chl) and dissipate excess excitation energy as heat relating to energization of the thylakoid membrane due to lumen acidification. The NPQ helps to regulate and protect photosynthesis in stress environments in which light energy absorption exceeds the capacity for light utilization (Maxwell and Johnson, 2000).

Moreover, salinity increased an epidermal and mesophyll thickness, palisade cell length, palisade and spongy cells diameter in leaves of bean, cotton, and *Atriplex* (Longstreth and Nobel, 1979). The leaf area and stomatal density in tomato were reduced by salinity (Romero-Aranda *et al.,* 2001). In general, the photosynthetic pigments such as chlorophyll and carotenoid contents are reduced by salt stress. The contents of total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotene in tomato leaves were also decreased by salt stress (Khavari-Nejad and Mostofi, 1998). Reduction of photosynthetic pigments was directly related to photosynthetic apparatus particularly chloroplasts which became disorganization by exposing with salt stress (Hernández *et al.,* 1995). The result led to the decreasing of the water oxidation in PSII. Disturbance in water oxidation in photosystem II resulted in the reduction of photosynthesis and plant growth. Cha-um and Kirdmanee (2008) showed that the reduction of water oxidation in PSII in *Eucalyptus,* Rain tree and Thai neem was gradually reduced with the increasing of salinity which resulted in the photosynthetic rate and plant growth reductions.

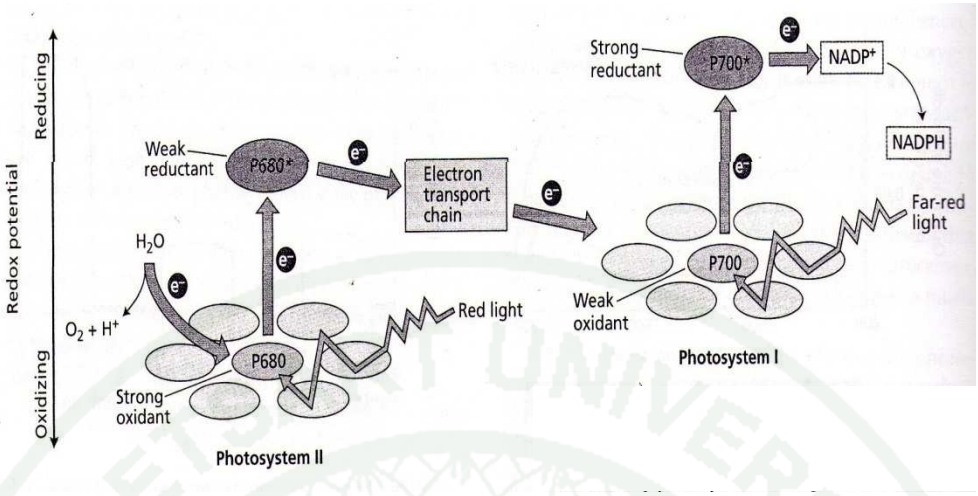

Figure 3 Light-dependent reactions of photosynthesis.

Source: Taiz and Zeiger (2002)

4. Salt defensive mechanisms

The defensive mechanisms to salinity are the complex organization and compose of many processes such as ion regulation and compartmentalization, osmoregulation, antioxidative system and hormonal regulation system (Parida and Das, 2005). Each kind of defensive mechanism was discussed as follows:

4.1. Ion regulation and compartmentalization

The ion homeostasis restricted to the excess toxic salts, especially Na^+ which disturbed ion homeostasis when Na^+ moved into the cell. The Na^+ interacted with other cations, especially K^+ (Aziz and Khan, 2001; Hu *et al.*, 2007). Since Na^+ competed with K^+ for intracellular influx which increased the Na^+:K^+ selectivity (Parida and Das, 2005). The size of Na^+ (molecular weight = 23.0; electron shell = 2, 8, 1) is smaller than K^+ (molecular weight = 39.1; electron shell = 2, 8, 8, 1) (Masterton and Hurley, 2009). In general, size of the molecule plays a role on the movement into the cell. Small molecule can pass into the cell better than the large molecule.

Cha-um *et al.* (2009c) showed that the ion selectivity in salt-sensitive KDML105 rice was lower than that in salt-tolerant Homjan rice. However, the increasing of Na^+:K^+ ratio and electrolyte leakage in salt-tolerant Pokkali rice were less than those in salt-sensitive IR29 rice, resulting in maintaining survival rate (Theerakulpisut *et al.*, 2005). Plants cannot tolerate to large amounts of Na^+ in the cytoplasm. Therefore, the restriction of Na^+ by compartmentalization in vacuole was necessary to plant survival under salinity (Reddy *et al.*, 1992; Maathuis and Amtmann, 1999; Zhu, 2003).

Removing of Na^+ between cytoplasm and vacuole depended on ion transporters (Na^+/H^+ antiporter; *NHX1*), ion channel (non-selective channel; *NSC*) and electrogenic H^+ pumps (plasma membrane H^+-ATPase; PM-ATPase, vacuolar type H^+-ATPase; V-ATPase and vacuolar pyrophosphatase; V-PPase) (Zhu *et al.*, 1993). Kader *et al.* (2006) demonstrated that the expression of *OsHKT2* (K^+-Na^+ co-transporter) and *OsVHA* (energizer for tonoplast Na^+/H^+ antiporter) were induced immediately by salinity in the salt-tolerant Pokkali rice while their expressions were delayed and lowered in the salt-sensitive BRRI Dhan29 rice. Activity of ion transporters and electrogenic H^+ pumps might confer salt defensive response in rice by maintaining a low cytosolic Na^+ level and a correct ratio of cytosolic Na^+:K^+. The *OsCLC1* (voltage-dependent Cl^- channel) and vacuolar H^+-ATPase (*OsVHA-B*) transcript were up-regulated in salt-tolerant Pokkali rice while they were down-regulated in salt-sensitive IR29 rice (Diédhiou and Golldack, 2006).

Other salt regulation mechanisms included i) salt secretion occured through development of unique cellular structures called salt glands that functioned to secrete salt (especially NaCl) from leaves to maintain internal ion concentration (Hogarth, 1999), ii) Salt exclusion occured through roots to regulate the salt content of their leaves in many halophytes (Levitt, 1980), and iii) ion or solute selective accumulations adjusted osmotic pressure which resulted in the increasing of water retention and sodium exclusion.

4.2. Osmoregulation

Osmoregulation is a plant defensive mechanism which accumulates compatible solutes that are low-molecular-mass compounds and do not interfere with normal biochemical reactions (Yancey *et al.*, 1982; Ford, 1984; Ashihara *et al.*, 1997; Hasegawa *et al.*, 2000; Zhifang and Loescher, 2003). Compatible solutes control osmotic potential and water potential adjustments in plant cells for continuing water influx (or reduced efflux). The osmotic adjustment facilitates water retention in the cytoplasm. The compatible solutes (sugars, sugar alcohols, complex sugars, quaternary nitrogen compounds, protein and amino acid derivatives) play important role on the osmoregulation system (Hasegawa *et al.*, 2000).

The compatible solutes prevented cellular structures by interacting with the membranes, protein complexes or enzymes. The compatible solutes protected macromolecules from the adverse effects of the increasing ionic strength in the surrounding media (Crowe *et al.*, 1992). Glycinebetaine content was induced by salt stress in a number of plants (Saneoka *et al.*, 1999; Khan *et al.*, 2000; Muthukumarasamy *et al.*, 2000; Wang and Nil, 2000). The increasing of salinity induced compatible solute accumulations such as proline, soluble sugars and polyamine in *indica* rice (Lin and Kao, 1995; Lefèvre *et al.*, 2001; Ahmad *et al.*, 2007). Cha-um *et al.* (2007a) showed that the glycinebetaine accumulation in salt-tolerant rice line (GS No. 4371) was greater than that in salt-sensitive rice line (GS No. 7032) under salinity. Accumulation of glycinebetaine helped pigment stabilizations. The glycinebetaine content increased in shoots, but was not significant in roots of *Haloxylon recurvum* under salt stress (Khan *et al.*, 2000).

Many plants accumulated proline as a non-toxic and protective osmolyte under salinity (Lee and Liu, 1999; Khatkar and Kuhad, 2000; Muthukumarasamy *et al.*, 2000; Singh *et al.*, 2000; Jain *et al.*, 2001; Cha-um *et al.*, 2010b). The proline accumulation in Thai aromatic rice maintained photosynthetic pigments. Accumulation of proline conserved the photosynthetic efficiency which resulted in the increasing of survival percentage under salinity (Wanichananan *et al.*, 2003;

Pongprayoon *et al.*, 2008). In mulberry, free amino acids were increased at low salinity, but decreased at high salinity, in addition, the glycinebetaine was accumulated more than the proline (Agastian *et al.*, 2000). Carbohydrates, especially sugar and starch accumulations, were major osmoprotection, osmotic adjustment, carbon storage and radical scavenging under salinity (Hasegawa *et al.*, 2000; Zhu, 2001; Parida *et al.*, 2002; Borsani *et al.*, 2003; Minorsky, 2003; Ashraf and Harris, 2004; Bartels and Sunkar, 2005; Ashraf and Foolad, 2007).

Salinity increased reducing sugars (glucose, fructose), sucrose and fructans in a number of plants while starch content was decreased (Kerepesi and Galiba, 2000; Khatkar and Kuhad, 2000; Singh *et al.*, 2000; Parida *et al.*, 2002). Under salinity, sugar content was unchanged in some genotypes of rice while it was decreased in some genotypes. Starch content in rice roots was declined, but remained constant in shoots (Alamgir and Ali, 1999). Udomchalothorn *et al.* (2009) suggested that sucrose accumulation and sucrose/starch ratio in leaves were increased while starch was decreased in salt-sensitive LPT123 rice and salt-tolerant LPT123-TC171 rice seedlings when exposed to 85 mM NaCl for 9 days. Moreover, sucrose accumulation was remarkably increased in the salt-tolerant LPT123-TC171 rice cultivar. Silva-Ortega *et al.* (2008) demonstrated that the *Osp5cs* (Δ^1-pyrroline-5-carboxylate synthetase) was gradually up-regulated with the increasing of salinity in cactus pear.

4.3. Antioxidative systems

Antioxidative enzyme activities, lipid peroxidation and salt responsive gene expressions responded to salinity (Hasegawa *et al.*, 2000). Reactive oxygen species (ROSs) composed of O_2^-, $OH^\bullet$, H_2O_2 and $^1O_2$ which were induced by salinity. The ROSs induced oxidative damage to lipids, protein and nucleic acids (Fridovich, 1986; Wise and Naylor, 1987; Imlay and Linn, 1988). Dionisio-Sese and Tobita (1998) reported that the Na^+ accumulation, lipid peroxidation and electrolyte leakage in salt-sensitive IR28 rice were rapidly increased more than those in salt-tolerant Pokkali rice under extreme salinity (EC = 12 mS cm^{-1}). The ROSs production and

activity of antioxidative enzymes scavenging can be used to indicate the plant survival ability under salinity (Spychalla and Desborough, 1990). Plants responded to the ROSs by the increasing of antioxidative enzymes such as catalase (CAT), peroxidase (POD), glutathione reductase (GR) and superoxide dismutase (SOD) resulted in the increasing of the scavenging reactive oxygen species (Parida and Das, 2005). The activity of SOD, CAT, POD, APX and GR increased under salt stress in plants and a correlation between these enzyme levels and salt tolerance exists (Gossett *et al.*, 1994; Hernández *et al.*, 1995; Sehmer *et al.*, 1995; Kennedy and de Fillippis, 1999; Benavides *et al.*, 2000; Hernández *et al.*, 2000; Sreenivasulu *et al.*, 2000; Lee *et al.*, 2001; Mittova *et al.*, 2002). Hernandez *et al.* (2000) reported that the activities of antioxidative enzymes such as APX, GR, monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and Mn-SOD increased while Cu/Zn-SOD remained constant and total ascorbate and glutathione content decreased in wheat under salinity.

Under salinity, the activity of APX, CAT and GR were decreased, the SOD and reduced glutathione were increased, and malondialdehyde and total protein remained constant in root nodules of soybean (Comba *et al.*, 1998). In rice plant, Vaidyanathan *et al.* (2003) reported that the activity of CAT, APX, GR in salt-tolerant Pokkali was higher than those in salt-sensitive Pusa Basmati1 rice, resulting in lowering membrane damage. Activity of CAT indicated the efficiency to scavenging of ROSs which might identify salt defensive response in rice by lowering of hydrogen peroxide. The activities of APX, MDHAR, DHAR, and GR increased in the shoots and decreased in the roots (Meneguzzo *et al.*, 1999). Excess ROSs generated lipid peroxidation which produced highly reactive species that modified proteins and DNA (Singh *et al.*, 2002). Wu *et al.* (1998) reported that the salinity reduced sterols and phospholipids in root plasma membrane of salt marsh grass (*Spartina patens*). In rice plant, the lipid peroxidation in salt-sensitive IR28 rice and salt-tolerant Pokkali rice were enhanced by the increasing of salinity (Dionisio-Sese and Tobita, 1998). Under salinity, the superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) activities decreased in both salt-tolerant Lunishree and salt-sensitive Begunbitchi rice while peroxidase (POX) was increased. In addition, ascorbate and

glutathione contents increased in salt-tolerant Lunishree rice whereas they were decreased in salt-sensitive Begunbitchi rice (Khan and Panda, 2008). Menezes-Benavente *et al.* (2004) reported that the expression of *APx*, *CatB*, *GR*, *SodCc1* and *SodCc2* genes in eleven-day-old seedlings of *indica* rice (EMBRAPA-7 Taim) were up-regulated while *CatA*, *CatC*, and *GPx* were unchanged when exposed to 250 mM NaCl.

4.4. Plant hormones

Abscisic acid (ABA) plays an important role on salt tolerance ability. Gómez-Cadenas *et al.* (1998) suggested that ABA, aminocyclopropane-1-carboxylic acid and ethylene production were enhanced by salinity. Moreover, the ABA promoted stomatal closure by changing ion fluxes in guard cells and led to the alleviation of the inhibitory effect of NaCl on photosynthesis, growth and translocation of assimilates (Popova *et al.*, 1995). Moreover, high salinity triggered an increase in levels of cytokinins (Thomas *et al.*, 1992; Aldesuquy, 1998; Vaidyanathan *et al.*, 1999). ABA was responsible for the alteration of salt stress-induced genes (de Bruxelles *et al.*, 1996). Protein phosphorylation, modification of cytosolic calcium levels and pH acted as intermediate by ABA signal transduction (Leung and Giraudat, 1998). The ABA also promoted a switching of photosynthesis from C_3 to crassulacean acid metabolism (CAM) in *Mesembryanthemum crystallinum* under salinity (Thomas *et al.*, 1992). Rising of the ABA enhanced Ca^{2+} uptake which contributed to membrane integrity maintenance (Chen *et al.*, 2001). Chen and Plant (1999) showed that the ABA level in root of *Ailsa Craig* (AC) and ABA-deficient mutant (*flc*) were enhanced by salinity. Gupta *et al.* (1998) showed that the ABA-inducible genes played an important role on the salt tolerance mechanism in rice.

5. Role of potassium and sugars in plants respond to salinity stress

5.1. Potassium ion (K^+)

Potassium is an abundant inorganic cation in the cytoplasm of organisms. It plays an important role on osmoregulation, turgor maintenance and cell expansion (Mengel and Kirkby, 1982). The potassium ion (K^+) transportation is inhibited by sodium ion (Na^+). Under salt stress, detoxification of by Na^+ compartmentalization into the vacuole reduced biochemical machinery damage in the cytosol (Tester and Davenport, 2003; Apse and Blumwald, 2007). Salt-tolerant species maintained a high cytosolic K^+:Na^+ homeostasis during salt stress (Flowers and Hajibagheri, 2001; Carden *et al.*, 2003; Cuin *et al.*, 2003; Golldack *et al.*, 2003; Peng *et al.*, 2004). K^+ uptake into the roots depended on K^+-selective channels characterized on the plasma membrane such as *AKT1* (Hirsch *et al.*, 1998; Kim *et al.*, 1998; Broadley *et al.*, 2001; Gierth *et al.*, 2005). Spalding *et al.* (1999) reported that low K^+ concentration disrupted *AKT1* and finally resulted in growth inhibition.

In addition, *HAK5* gene transporter contributed the high-affinity potassium uptake in *Arabidopsis* roots (Gierth *et al.*, 2005). Moreover, K^+ transportation at long distance was regulated by *SKOR* (Gaymard *et al.*, 1998) and *AKT2* (Lacombe *et al.*, 2000). In addition, Na^+ was regulated by Na^+/H^+ antiporter (*NHX1)* which mediated Na^+ and K^+ coupling transport in vacuoles (Zhang and Blumwald, 2001) and its over-expression improved plant salt tolerance (Apse *et al.*, 1999; Zhang and Blumwald, 2001; Zhang *et al.*, 2001). Another Na^+/H^+ antiporter (*NHX5*) had been identified in salt tolerance by controlling the accumulation of Na^+ and K^+ in shoots. Recently, application of potassium fertilizers such as potassium phosphate (KH_2PO_4) (Kaya *et al.*, 2003), potassium nitrate (KNO_3) (Akinci and Simsek, 2004) and potassium sulfate (K_2SO_4) (Akram *et al.*, 2009) enhanced salt tolerance ability. Satti and Lopez (1994) demonstrated that the addition of 4-8 mM KNO_3 to saline solution containing 50 mM NaCl improved growth and fruit set in tomato.

5.2. Sugars

Sugars (simple sugars and complex sugars) are compatible solutes which play an important role on osmoregulation. The compatible solutes can accumulate to high level without disturbing intracellular biochemistry. Higher level of sugar accumulation leads to the movement of water into plant, resulting in turgor pressure maintenance (Bohnert and Jensen, 1996; Mahajan and Tuteja, 2005). Moreover, sugars preserved the activity of enzymes and scavenged the ROSs under salinity (Cushman, 2001; Yokoi *et al.*, 2002). Ashraf and Tufail (1995) showed that the sugar contents in sunflower were increased under salinity, moreover, the soluble sugar contents in salt-tolerant line were generally higher than those in the salt-sensitive line. Garcia *et al.* (1997) showed that the increasing of osmoprotectants, especially sucrose, glucose and fructose in rice roots and leaves were generally regulated by salinity.

Moreover, sucrose accumulation inhibited fusion and leakage in liposomes from phospholipids in membrane (Crowe *et al.*, 1987; Koster and Leopold, 1988; Hoekstra *et al.*, 1991). Khelil *et al*. (2007) found that the sucrose content in tomato was rapidly increased when exposed to 100 and 200 mM NaCl in young and old leaves, but slightly increased at 100 mM NaCl when compared to the control. In the parallel way, the hexose content in tomato young and old leaves at 200 mM NaCl was enhanced in comparing to the control. Moreover, the accumulation of sucrose depended on the sucrose synthesis (SuSy) activity. The SuSy activity in old and young tomato leaves was increased when exposed to 100 mM NaCl. The sucrose was cleavaged into glucose and fructose by acid invertase activity in old and young leaves. This result showed the decreasing during early salt stress period while it increased at late salt stress period. An increase in SuSy and invertase activity correlated with the increasing of the sucrose and hexose contents.

In rice plant, the total soluble sugar and sucrose contents were accumulated in salt-sensitive KDML105 rice but they were not increased in salt-tolerant Luang Anan and Pokkali rice. Starch content in salt-tolerant Pokkali rice was increased whereas it was decreased in salt-tolerant Luang Anan and salt-sensitive

KDML105 rice during salt stress. The activity of the sucrose phosphate synthase in salt-sensitive KDML105 rice was reduced while it was induced in salt-tolerant Luang Anan and Pokkali rice when exposed to 50-150 mM NaCl. The acid invertase activity in salt-tolerant Luang Anan and Pokkali rice tended to increase while decreased in KDML105 rice (Pattanagul and Thitisaksakul, 2008).

The activity of enzymes and gene expressions involved with sugar biosynthesis that induced the sugar accumulations. Cha-um *et al.* (2009b) showed that the activity and gene expression of fructose-1,6-bisphosphatase (FBP; EC 3.1.3.11) and fructokinase (FK; EC 2.7.1.4) involved with gluconeogenesis and sucrose synthesis in salt-tolerant HJ rice. The enzymes activities and gene expressions in salt-tolerant HJ rice were gradually better than those in salt-sensitive PT1 rice. This result showed the high sucrose, glucose and fructose accumulations in salt-tolerant HJ rice.

The complex sugars include stachyose and raffinose which are members of raffinose family oligosaccharides. These sugars are α-galactosyl derivatives of sucrose which are carbohydrate reserves and play a role in stress tolerance (Minorsky, 2003). Bentsink *et al.* (2000) suggested that the oligosaccharides may protect membranes, proteins, and nucleic acids against the damage which occurred during and upon the withdrawal of water. This protective role of oligosaccharides has been explained mainly by their capacity to retain the integrity of membranes through their interaction with the phospholipid headgroups, thus replacing water during dehydration. Numerous reports demonstrated that raffinose and stachyose functioned as a stabilization of membranes under chilling stress and salt stress (Lineberger and Steponkus 1980; Castonguay *et al.*, 1995; Bohnert and Jensen, 1996; Bentsink *et al.*, 2000; Zuther *et al.*, 2004; Morsy *et al.*, 2007).

However, the oligosaccharides content depended on galactinol synthase (*GolS*), raffinose synthase (*RFS*) and stachyose synthase (*STS*) which involved in raffinose family oligosaccharides biosynthesis (Figure 4). Pukacka and Wojkiewicz (2002) reported that an increase in the activity of *GolS* enhanced raffinose and stachyose contents in Norway maple and Sycamore seeds under desiccation condition.

The galactinol and raffinose contents were enhanced by overexpression of *GolS* and *RFS* in transgenic potato plants (Hannah *et al.*, 2006). Taji *et al.* (2002) showed that the *ATGolS1* and *ATGolS2* were induced by drought and salinity while *ATGolS3* was induced by cold stress. In addition, the overexpression of *ATGolS2* in transgenic *Arabidopsis* induced galactinol and raffinose contents and reduced transpiration. Furthermore, alteration of raffinose and stachyose was closely related to the change of disaccharide (sucrose) and monosaccharide (glucose and fructose) under drought and salt stress conditions (Santarius and Milde, 1977; Norwood *et al.*, 2003; Heldt, 2005; Morsy *et al.*, 2007).

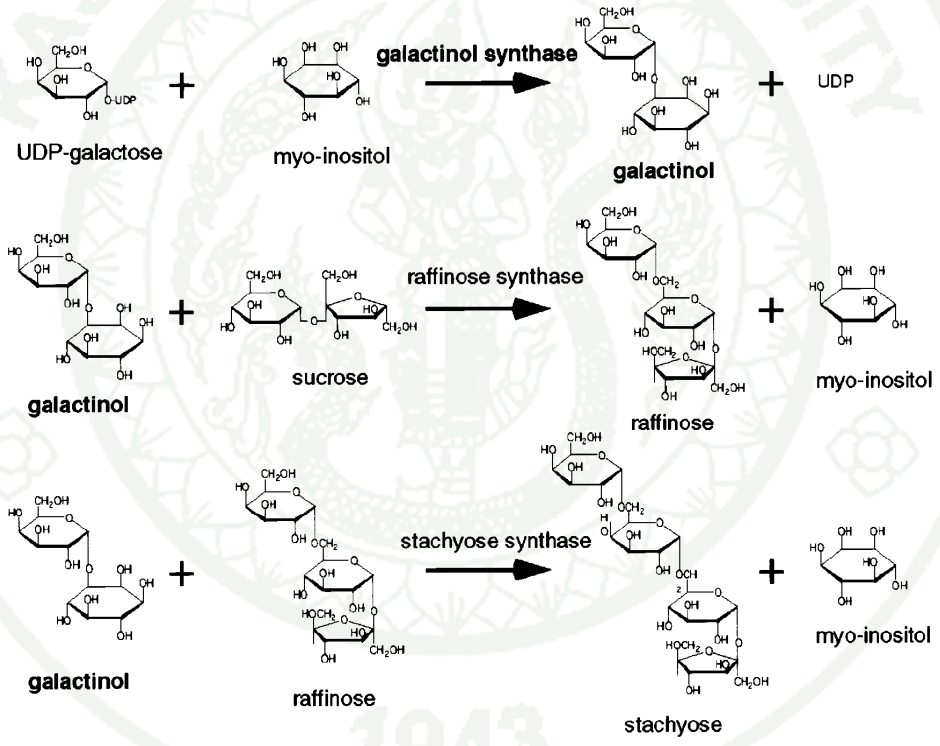

Figure 4 Metabolic pathway of galactinol and raffinose family oligosaccharides (RFOs) in plants.

Source: Taji *et al.* (2002)

MATERIALS AND METHODS

Experiment 1. Physiological responses of salt-tolerant and salt-sensitive rice varieties to different NaCl concentrations and salt exposure times

1.1. Plant material and growth condition

Seeds of salt-tolerant Homjan (HJ) and salt-sensitive 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 (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose and adjusted to pH 5.7 (Appendix Figure 1A). 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.

1.2. 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 (Appendix Figure 1B). 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 (∅ 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.

Experiment 2. Physiological responses of salt-tolerant and salt-sensitive rice varieties to salt stress under iso-osmotic condition

2.1. Iso-osmotic condition adjustment

To investigate the ionic effect of NaCl on physiological responses, the culture medium was adjusted to salt stress under iso-osmotic condition (Table 1). An osmotic potential of the culture medium was adjusted approximately -1.75 ± 0.20 MPa. Physiological responses and sugar contents in HJ and PT1 seedlings to salt stress under iso-osmotic condition were measured.

2.2. Salt stress treatments under iso-osmotic condition

The HJ and PT1 seedlings were prepared as described in 1.1. Fourteen-day-old HJ and PT1 HJ and PT1 seedlings were aseptically transferred to MS sugar-free liquid medium as described in 1.2. Consequently, the culture medium of HJ and PT1 seedlings was adjusted to salt stress under iso-osmotic. After 4 days of salt treatments, ion contents, Na^+:K^+ ratio, electrolyte leakage of roots, osmotic potential of roots and leaves, photosynthetic pigment concentrations, Chl *a* fluorescence parameters, growth and sugar contents were determined.

Table 3 Osmotic potential in the MS medium was reduced by adding NaCl and mannitol.

Medium	NaCl (mM)	Mannitol (mM)	Osmotic potential
MS	0.0	0.0	-0.45
		109.8	-0.65
		219.6	-0.76
		329.4	-1.25
		439.2	-1.49
		548.9	**-1.75**
	85.5	0.0	-0.69
		109.8	-1.33
		219.6	-1.44
		329.4	**-1.93**
		439.2	-2.17
		548.9	-2.43
	171.0	0.0	-1.01
		109.8	-1.65
		219.6	**-1.76**
		329.4	-2.24
		439.2	-2.48
		548.9	-2.75
	256.5	0.0	-1.28
		109.8	**-1.92**
		219.6	-2.03
		329.4	-2.51
		439.2	-2.75
		548.9	-3.01
	342.0	0.0	**-1.73**
		109.8	-2.34
		219.6	-2.48
		329.4	-2.96
		439.2	-3.20
		548.9	-3.46

Experiment 3. Salt tolerance ability improvement by exogenous potassium nitrate (KNO_3) and sucrose application

3.1. Effect of exogenous potassium nitrate (KNO_3) on salt tolerance ability in salt-sensitive rice variety

The HJ and PT1 seedlings were prepared as described in 1.1. Fourteen-day-old HJ and PT1 seedlings were aseptically transferred to 60 mL MS liquid medium supplemented with 0.0, 9.4, 11.8 and 14.1 mM KNO_3 by using vermiculite as supporting material for 14 days. The HJ and PT1 seedlings were cultured as described in 1.2. Consequently, the culture medium of HJ and PT1 seedlings was adjusted to 0 and 342 mM NaCl. After 4 days of salt treatments, osmotic potential of roots and leaves, photosynthetic pigment concentrations, Chl *a* fluorescence parameters and growth were evaluated.

3.2. Improvement of salt tolerance ability in salt-sensitive rice variety by exogenous sucrose application

The HJ and PT1 seedlings were prepared as described in 1.1. Fourteen-day-old HJ and PT1 seedlings were aseptically transferred to 60 mL MS liquid medium supplemented with 0.0, 29.2, 58.4 and 116.8 mM sucrose by using vermiculite as supporting material for 14 days. The HJ and PT1 seedlings were cultured as described in 1.2. Consequently, the culture medium of HJ and PT1 seedlings was adjusted to 0 and 342 mM NaCl. After 4 days of salt treatments, sugar contents, osmotic potential of roots and leaves, photosynthetic pigment concentrations, Chl *a* fluorescence parameters and growth were evaluated.

4. Physiological response measurements

4.1. Osmotic potential

Root and leaf osmolalities were measured according to Lanfermeijer *et al.* (1991) by using a vapor pressure osmometer (5520 Vapro®, Wescor, Inc., USA). The osmolality was converted to the osmotic potential according to Kozai *et al.* (1986) equation;

$$\text{Osmotic potential (bar)} = 4.6153 \times (273.16 + t) \times \rho_w \times \ln (55.509/(55.509 + a))$$

where, t was temperature (^oC)

ρ_w was density of water at t ^oC (g cm^{-3})

a was osmolality (mol kg^{-1})

$$\text{Osmotic potential (MPa)} = \frac{\text{Osmotic potential (bar)}}{10}$$

4.2. Sodium (Na^+) and potassium (K^+)

One hundred milligrams of root and leaf fresh weights were ground in liquid-nitrogen and extracted by acidic method (Dionisio-Sese and Tobita, 1998). Na^+ and K^+ contents were analyzed by atomic absorption spectrophotometer (Model M6 Thermo Elemental, MA, USA). The Na^+:K^+ ratio was calculated by following Lee *et al.* (2003).

4.3. Electrolyte leakage

Electrolyte leakage in roots (EL_{root}) was determined by following Dionisio-Sese and Tobita (1998). The rice roots were cut into 5.0 ± 0.2 mm in length and placed in the glass vessels (Opticlear®; KIMBLE, Vineland, New Jersey, USA) containing 10 mL deionized water. The glass vessels were cap and maintained at

room temperature (25°C) for 2 h. The initial electrical conductivity (EC_1) was measured by using an electrical conductivity meter (Model ID1010, INDEX, Kuala Lumpur, Malaysia). Then, rice roots were boiled at 100°C in the water bath for 30 min, cooled down at 25°C after that the electrical conductivity (EC_2) was measured. The EL_{root} was calculated by using the following formular**;**

$$EL_{root} = \frac{EC_1}{EC_2} \times 100$$

where, EC_1 was the initial electrical conductivity of the samples before boiling

EC_2 was the electrical conductivity of the samples after boiling

4.4. 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.* (1998) and Lichtenthaler (1987). 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 4ºC 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;

$$\text{Chl } a = 9.784D_{662} - 0.99D_{644}$$

$$\text{Chl } b = 21.42D_{644} - 4.65D_{662}$$

$$\text{TC} = \text{Chl } a + \text{Chl } b$$

$$C_{x+c} = \frac{(1000D_{470} - 1.90\text{Chl } a - 63.14\text{Chl } b)}{214}$$

where, D_i was an optical density at the wavelength i

4.5. 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 Loggini *et al.* (1999). 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 (2000).

4.6. Growth

To compare growth of seedlings among the control and the NaCl-treated samples, 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^{o}C in a hot-air oven (Memmert, Model 500, Germany) for 48 h prior to cooling down in a desiccator and measuring the DW.

4.7. Sugar contents

4.7.1. Sugar extraction

Glucose, fructose, sucrose, raffinose and stachyose in HJ and PT1 roots and leaves were extracted according to modified Karkacier *et al.* (2003) method. Fifty milligrams of fresh weights were ground to pestle in liquid-nitrogen with the pre-cooled eppendorf tube. The sample was mixed with 1 mL nanopure water, sonicated for 15 min, and then centrifuged at 12,000 rpm for 15 min. Supernatant was filtrated through 0.45 μm membrane filter (VertiCleanTM; NYLON Syringe, Vertical Chromatography Co., Ltd., Thailand) and stored at -20 ºC prior to sugar analysis.

4.7.2. Sugar analysis

Glucose, fructose and sucrose contents in HJ and PT1 roots and leaves were analyzed by High Performance Liquid Chromatography (HPLC Water, Milford, MA, USA) equipped with 4214 differential refractive index (RI) detector and a Waters 600 gradient controller pump (Water, Milford, MA, USA). Metacarb 87C column (7.8×300 mm) (Varian, USA) were used for glucose, fructose and sucrose separations. Nanopure water was used as mobile phase. The injection volume was 40 μL and the flow rate was 0.4 mL min^{-1}.

Raffinose and stachyose were separated by VertiSep PRP-NH_2 (4.6×250 mm) (LIGAND SCIENTIFIC Co., Ltd.). Acetonitrile: nanopure water (75:25; v/v) was used as mobile phase. The authentic raffinose and stachyose was added as internal standard. The injection volume was 40 μL with 1.0 mL min^{-1} flow rate.

Quantification of sugars was performed by comparing the peak areas with those of the standard solutions. The glucose, fructose, sucrose, raffinose and stachyose (Sigma, Germany) were used as standard and sugar contents were calculated using a standard curve equation (Appendix Figure 2). The total soluble sugar is the sum of detected sugars.

5. Experimental design and statistical analysis

The experiment 1 and 2 was designed as 2×3×3 and 2×5 factorials in a Completely Randomized Design (CRD), respectively. The experiment 3 was conducted in CRD. 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).

6. Place and duration

The experiments were conducted at the Plant Physio-Biochemistry Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Klong Luang, Pathumthani from June 2006 to August 2010.

RESULTS

Experiment 1. Physiological responses of salt-tolerant and salt-sensitive rice varieties to different NaCl concentrations and salt exposure times

Physiological responses in term of the osmotic potential, photosynthetic pigment concentrations, chl *a* fluorescence parameters and growth in salt-tolerant HJ and salt-sensitive 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 (Figure 5A and 5B) 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 (Figure 5A and 5B).

The leaf osmotic potential in HJ and PT1 rice was rapidly decreased with the increasing of NaCl concentration (171 and 342 mM) and salt exposure time (2, 4 and 8 days) (Figure 5C and 5D). 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 (Figure 5C and 5D).

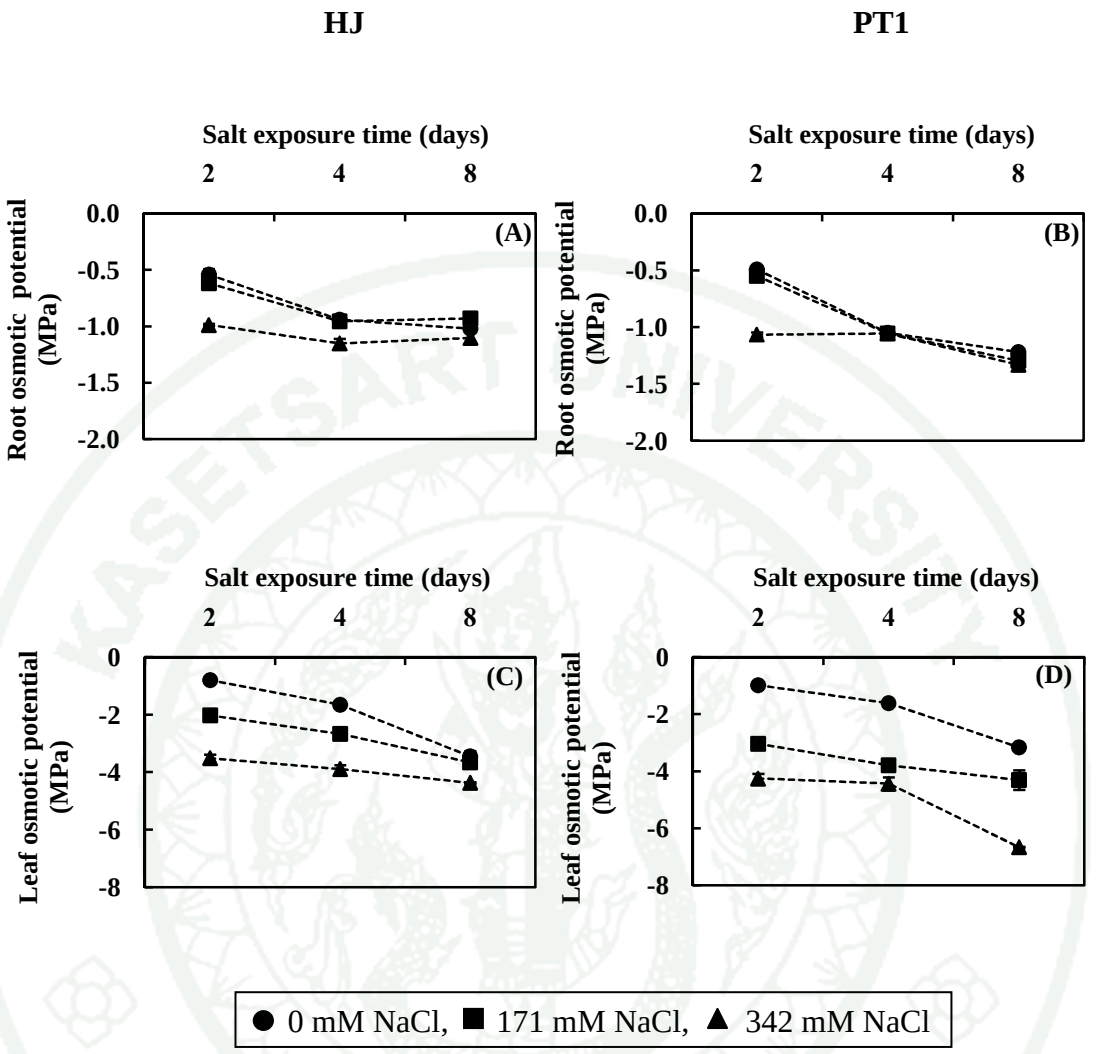

Figure 5 Change of the root and leaf osmotic potentials in HJ (A and C) and PT1 (B and D) seedlings when exposed to 0, 171 and 342 mM NaCl for 2, 4 and 8 days.

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 (Figure 6A and Figure 7A). 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 (Figure 6).

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 (Figure 7A and 7B).

After 4 and 8 days of salt stress induction, the C_{x+c} in HJ and PT1 seedlings was decreased (Figure 7C and 7D). 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.

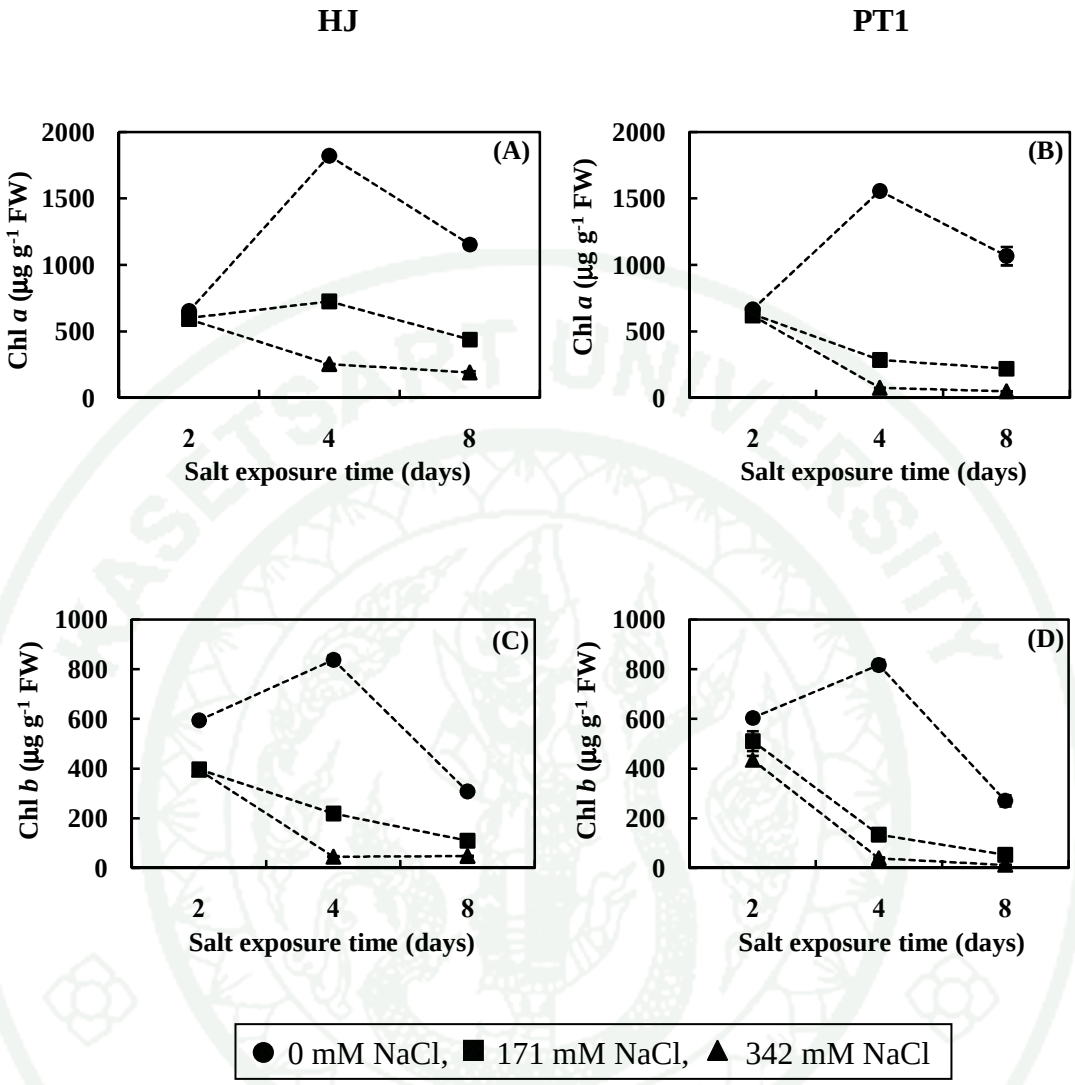

Figure 6 Change of the Chl *a* and Chl *b* in HJ (A and C) and PT1 (B and D) seedlings when exposed to 0, 171 and 342 mM NaCl for 2, 4 and 8 days.

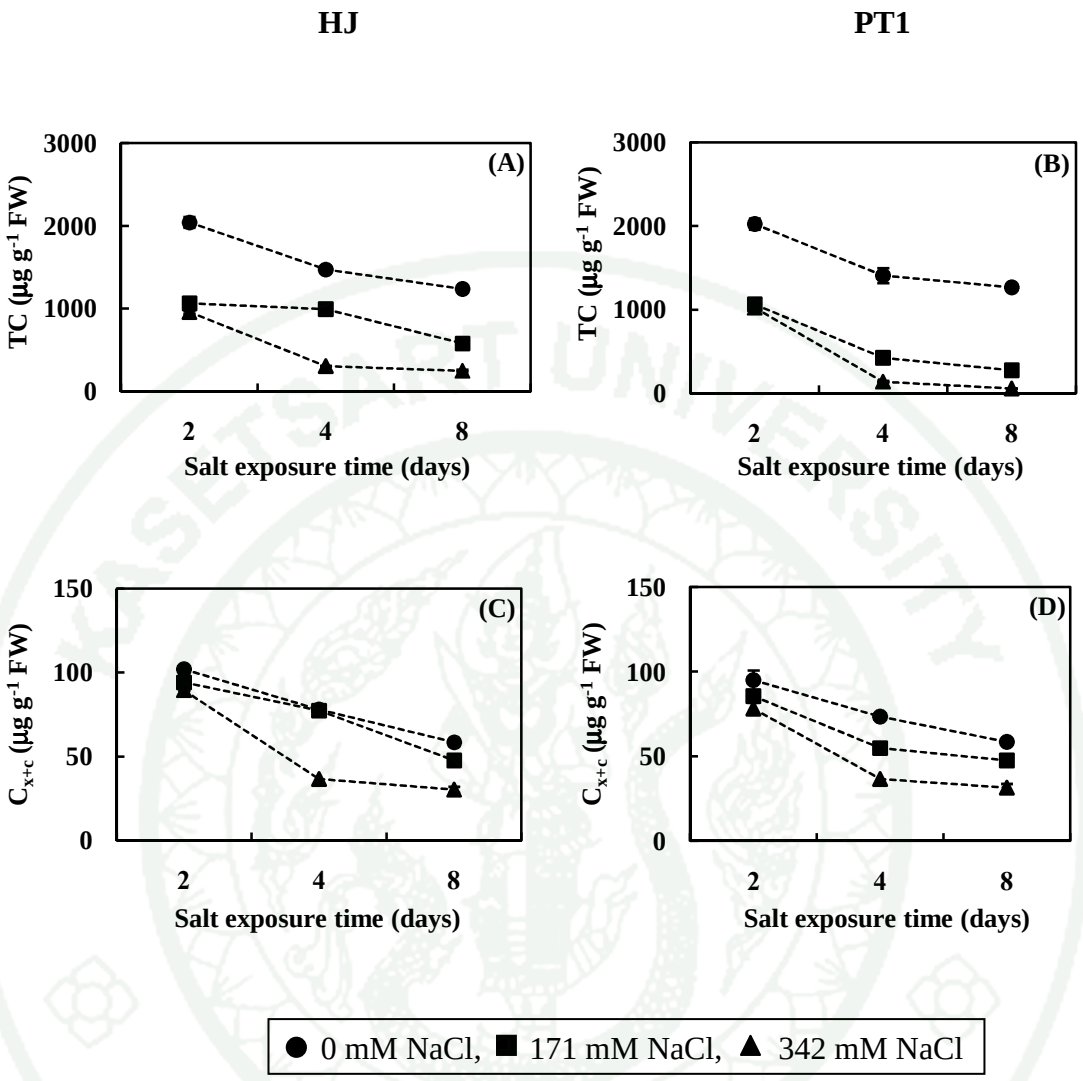

Figure 7 Change of the TC and C_{x+c} in HJ (A and C) and PT1 (B and D) seedlings when exposed to 0, 171 and 342 mM NaCl for 2, 4 and 8 days.

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 (Figure 8A and 8B). 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 (Figure 8A and 8B).

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 (Figure 8C and 8D).

The qP in HJ and PT1 salt-stressed seedlings was similar to the reduction of the Φ_{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 (Figure 9A and 9B).

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 (Figure 9C and 9D). The protective role of the anti-oxidative system in PT1 salt-stressed seedlings were significantly reduced more than those in HJ salt-stressed seedlings.

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 4 and Table 5). 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 4 and Table 5).

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. The table 6-11 showed the positively correlated coefficients between growth and physiological responses in HJ and PT1 salt-stressed seedlings at all salt exposure times.

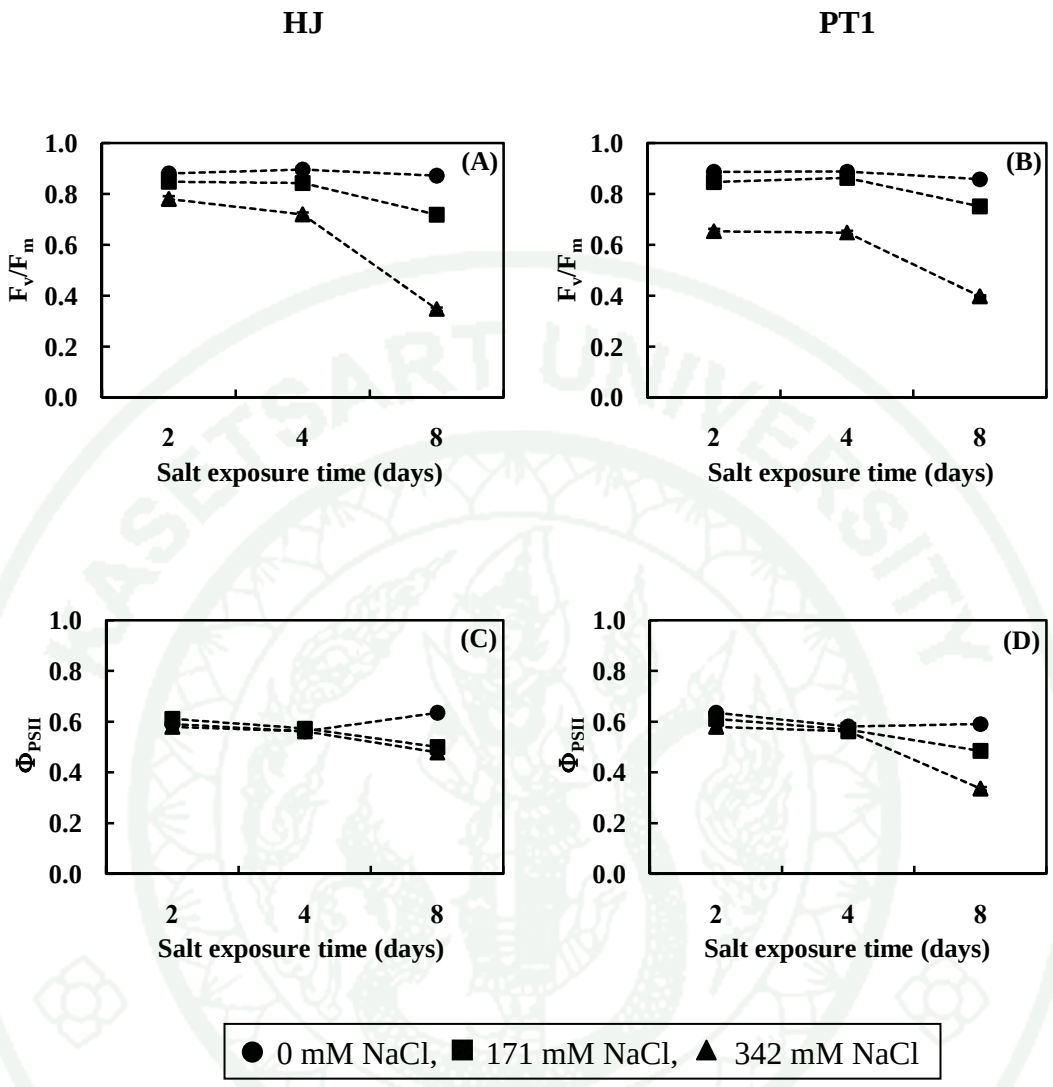

Figure 8 Change of the F_v/F_m and Φ_{PSII} in HJ (A and C) and PT1 (B and D) seedlings when exposed to 0, 171 and 342 mM NaCl for 2, 4 and 8 days.

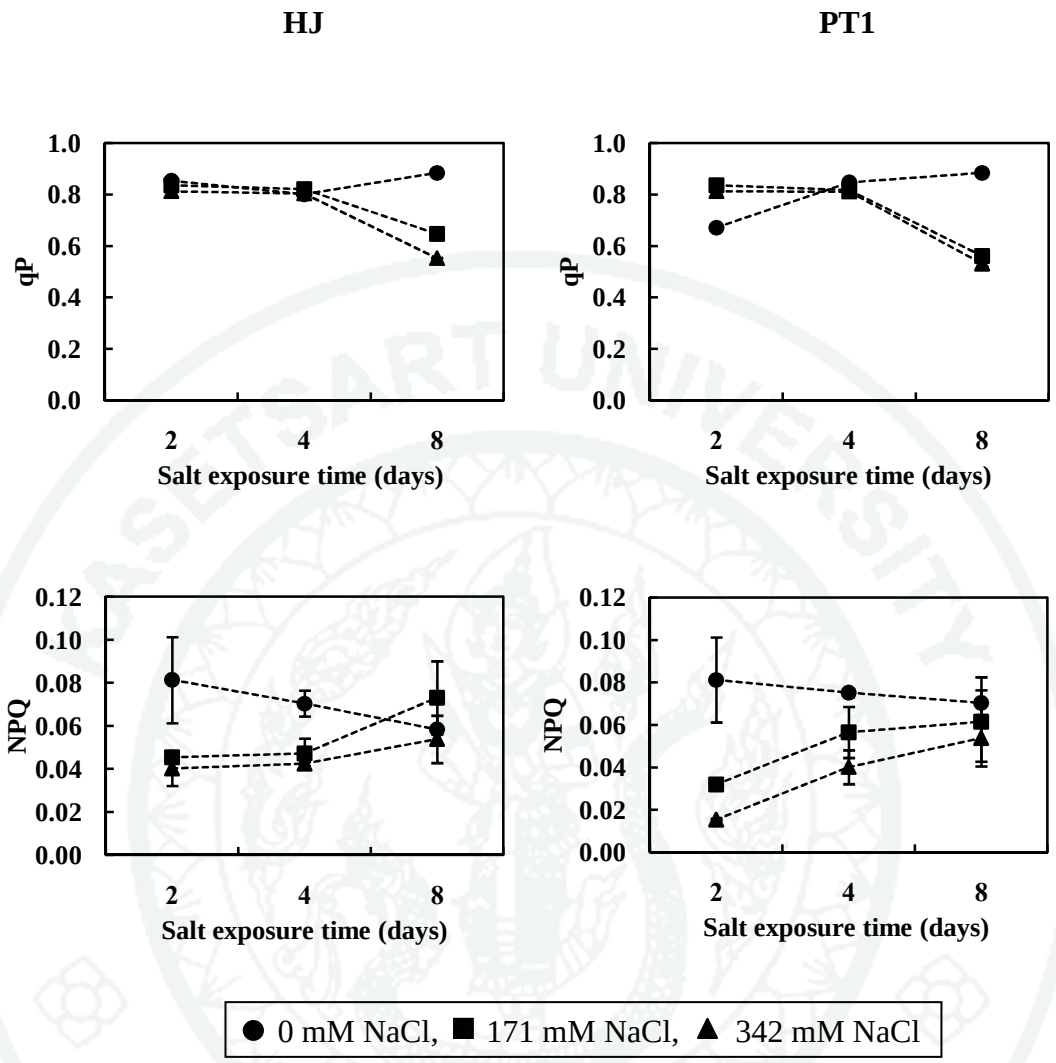

Figure 9 Change of the qP and NPQ in HJ (A and C) and PT1 (B and D) seedlings when exposed to 0, 171 and 342 mM NaCl for 2, 4 and 8 days.

Table 4 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	
			FW (mg)	DW (mg)
HJ	0	2	53.5	6.5
		4	67.2	4.6
		8	56.9	4.2
	171	2	47.5	5.5
		4	54.5	3.7
		8	41.5	2.6
	342	2	37.9	4.7
		4	34.2	3.1
		8	34.1	2.4
PT1	0	2	56.3	6.6
		4	44.2	2.4
		8	52.9	4.1
	171	2	44.8	5.8
		4	40.5	2.3
		8	41.6	3.3
	342	2	41.5	5.0
		4	39.7	2.0
		8	41.0	2.7
Significant level				
Rice			ns	*
NaCl			**	**
Salt exposure time			ns	**
Rice × NaCl			*	ns
Rice × Salt exposure time			*	**
NaCl × Salt exposure time			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).

Table 5 Fresh weight (FW) and dry weight (DW) in HJ and PT1 shoots 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)	Shoots	
			FW (mg)	DW (mg)
HJ	0	2	194.7 ab	35.6 bcd
		4	216.6 a	43.1 b
		8	149.9 cd	61.9 a
	171	2	178.7 abc	31.5 bcd
		4	205.9 a	34.6 bcd
		8	141.3 cde	27.0 cde
	342	2	178.0 abc	28.1 cde
		4	98.2 fg	24.9 de
		8	92.3 fg	25.8 cde
PT1	0	2	196.8 ab	38.2 bc
		4	101.1 fg	18.1 e
		8	129.4 def	32.8 bcd
	171	2	189.7 ab	32.4 bcd
		4	82.5 g	17.9 e
		8	129.2 def	31.9 bcd
	342	2	163.9 bcd	29.2 cde
		4	82.3 g	17.2 e
		8	106.7 efg	28.6 cde
Significant level				
Rice			**	**
NaCl			**	**
Salt exposure time			**	**
Rice × NaCl			*	**
Rice × Salt exposure time			**	**
NaCl × Salt exposure time			ns	ns
Rice × NaCl × Salt exposure time			**	*

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).

Table 6 Correlation coefficients of the physiological responses in HJ salt-stressed seedlings exposed to salt stress for 2 days.

	LOP	Chl *a*	Chl *b*	TC	C_{x+c}	F_v/F_m	Φ_{PSII}	qP	NPQ	SFW	SDW
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	-	-
SFW	0.691*	**0.886****	0.790**	0.734**	0.644*	0.467^{ns}	0.422^{ns}	0.569^{ns}	0.711**	1	-
SDW	**0.958****	**0.869****	**0.859****	**0.856****	0.674*	**0.836****	0.674*	**0.875****	0.679*	**0.857****	1

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

Table 7 Correlation coefficients of the physiological responses in PT1 salt-stressed seedlings exposed to salt stress for 2 days.

	LOP	Chl *a*	Chl *b*	TC	C_{x+c}	F_v/F_m	Φ_{PSII}	qP	NPQ	SFW	SDW
LOP	1	-	-	-	-	-	-	-	-	-	-
Chl *a*	0.502ns	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	-	-
SFW	0.575^{ns}	0.563^{ns}	0.536^{ns}	0.446^{ns}	**0.808****	0.639*	0.631*	0.626*	0.787**	1	-
SDW	0.693*	0.647*	0.659*	0.646*	**0.885****	0.612*	0.662*	0.574^{ns}	**0.852****	**0.929****	1

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

Table 8 Correlation coefficients of the physiological responses in HJ salt-stressed seedlings exposed to salt stress for 4 days.

	LOP	Chl *a*	Chl *b*	TC	C_{x+c}	F_v/F_m	Φ_{PSII}	qP	NPQ	SFW	SDW
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^{ns}	0.678*	0.705*	0.595*	0.360^{ns}	0.553^{ns}	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^{ns}	0.652*	0.513^{ns}	**0.844****	1	-	-
SFW	0.750**	0.611*	0.580*	0.757**	0.796**	0.781**	0.206^{ns}	0.564^{ns}	0.683*	1	-
SDW	0.721**	0.651*	0.651*	0.701*	0.627*	0.686*	0.313^{ns}	0.624*	0.782**	**0.923****	1

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

Table 9 Correlation coefficients of the physiological responses in PT1 salt-stressed seedlings exposed to salt stress for 4 days.

	LOP	Chl *a*	Chl *b*	TC	C_{x+c}	F_v/F_m	Φ_{PSII}	qP	NPQ	SFW	SDW
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*	0.421^{ns}	**0.808****	1	-	-
SFW	0.454^{ns}	0.387^{ns}	0.347^{ns}	0.436^{ns}	0.432^{ns}	0.215^{ns}	0.117^{ns}	0.516^{ns}	0.785**	1	-
SDW	0.139^{ns}	0.081^{ns}	0.050^{ns}	0.119^{ns}	0.182^{ns}	0.095^{ns}	-0.146^{ns}	0.228^{ns}	0.627*	**0.877****	1

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

Table 10 Correlation coefficients of the physiological responses in HJ salt-stressed seedlings exposed to salt stress for 8 days.

	LOP	Chl *a*	Chl *b*	TC	C_{x+c}	F_v/F_m	Φ_{PSII}	qP	NPQ	SFW	SDW
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^{ns}	0.412^{ns}	0.428^{ns}	0.385^{ns}	0.374^{ns}	0.315^{ns}	0.383^{ns}	0.384^{ns}	1	-	-
SFW	**0.970****	0.770**	0.770**	**0.800****	**0.927****	**0.934****	0.671*	0.765**	0.515^{ns}	1	-
SDW	0.736**	**0.803****	**0.804****	0.779**	0.650*	0.594*	0.791**	0.776**	0.532^{ns}	0.609*	1

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

Table 11 Correlation coefficients of the physiological responses in PT1 salt-stressed seedlings exposed to salt stress for 8 days.

	LOP	Chl *a*	Chl *b*	TC	C_{x+c}	F_v/F_m	Φ_{PSII}	qP	NPQ	SFW	SDW
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^{ns}	0.287^{ns}	0.295^{ns}	0.266^{ns}	0.323^{ns}	0.219^{ns}	0.284^{ns}	0.232^{ns}	1	-	-
SFW	**0.834****	0.513^{ns}	0.505^{ns}	0.539^{ns}	0.762**	**0.814****	0.759**	0.478^{ns}	0.374^{ns}	1	-
SDW	0.716**	0.567^{ns}	0.599*	0.563^{ns}	0.681*	0.691*	0.683*	0.497^{ns}	0.426^{ns}	0.622*	1

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

Experiment 2. Physiological responses of salt-tolerant and salt-sensitive rice varieties to salt stress under iso-osmotic condition

Under iso-osmotic condition, higher NaCl concentration (85.5-342.0 mM NaCl) in the culture medium significantly increased sodium ion (Na^+), Na^+:K^+ ratio and root electrolyte leakage (EL_{root}) in HJ and PT1 seedlings. The Na^+ in salt-tolerant HJ and salt-sensitive PT1 seedlings exposed to 342 mM NaCl were highly accumulated in roots and leaves when compared to the control (Table 12 and 13). In the meantime, the K^+ in HJ salt-stressed seedlings was increased while K^+ in PT1 salt-stressed seedlings was unchanged (Table 12 and 13, Figure 10 and 11).

The increase of Na^+ and K^+ in HJ salt-stressed seedlings could maintain the Na^+:K^+ ratio in roots (Figure 12) but not in leaves (Figure 13), therefore the EL_{root} in HJ rice was lower than that in the PT1 rice (Figure 14). The osmotic potential in HJ

and PT1 salt-stressed seedlings was reduced in leaf tissues (Figure 15) while osmotic potential was not affected in roots (Figure 16).

Table 12 Na^+, K^+, Na^+: K^+ ratio and electrolyte leakage (EL_{root}) in HJ and PT1 roots after cultured in liquid MS medium for 7 days and subsequently exposed to 0.0, 85.5, 171.0, 256.5 and 342.0 mM NaCl under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

Rice	NaCl (mM)	Mannitol (mM)	Na^+ (μg g^{-1} FW)	K^+ (μg g^{-1} FW)	Na^+: K^+ ratio	EL_{root} (%)
HJ	0.0	548.9	9.1 g	42.5 b	0.217 de	31.3 e
	85.5	329.4	16.6 d	178.5 a	0.093 e	39.0 de
	171.0	219.6	18.9 c	186.3 a	0.104 e	47.9 cd
	256.5	109.8	28.3 b	247.4 a	0.116 e	49.3 c
	342.0	0.0	31.0 a	262.8 a	0.130 e	51.0 c
PT1	0.0	548.9	0.7 i	13.8 b	0.058 e	45.1 cd
	85.5	329.4	3.5 h	10.0 b	0.473 cd	45.2 cd
	171.0	219.6	11.3 f	15.1 b	0.749 bc	66.3 b
	256.5	109.8	14.4 e	14.0 b	1.043 ab	71.5 ab
	342.0	0.0	13.4 e	11.8 b	1.138 a	79.2 a
Significant level						
Rice			**	**	**	**
NaCl			**	*	**	**
Rice × NaCl			**	*	**	*

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

Table 13 Na^+, K^+ and Na^+: K^+ ratio in HJ and PT1 leaves after cultured in liquid MS medium for 7 days and subsequently exposed to 0.0, 85.5, 171.0, 256.5 and 342.0 mM NaCl under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

Rice	NaCl (mM)	Mannitol (mM)	Na^+ (μg g^{-1} FW)	K^+ (μg g^{-1} FW)	Na^+: K^+ ratio
HJ	0.0	548.9	0.3 f	22.8 e	0.013
	85.5	329.4	6.9 de	64.1 bc	0.109
	171.0	219.6	20.4 c	62.0 bc	0.328
	256.5	109.8	26.5 c	74.7 b	0.355
	342.0	0.0	44.0 a	99.3 a	0.456
PT1	0.0	548.9	1.2 ef	43.0 d	0.027
	85.5	329.4	7.6 d	39.3 de	0.193
	171.0	219.6	24.7 c	48.4 cd	0.511
	256.5	109.8	22.8 c	37.4 de	0.630
	342.0	0.0	33.0 b	48.0 cd	0.696
Significant level					
Rice			ns	**	**
NaCl			**	**	**
Rice × NaCl			*	**	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).

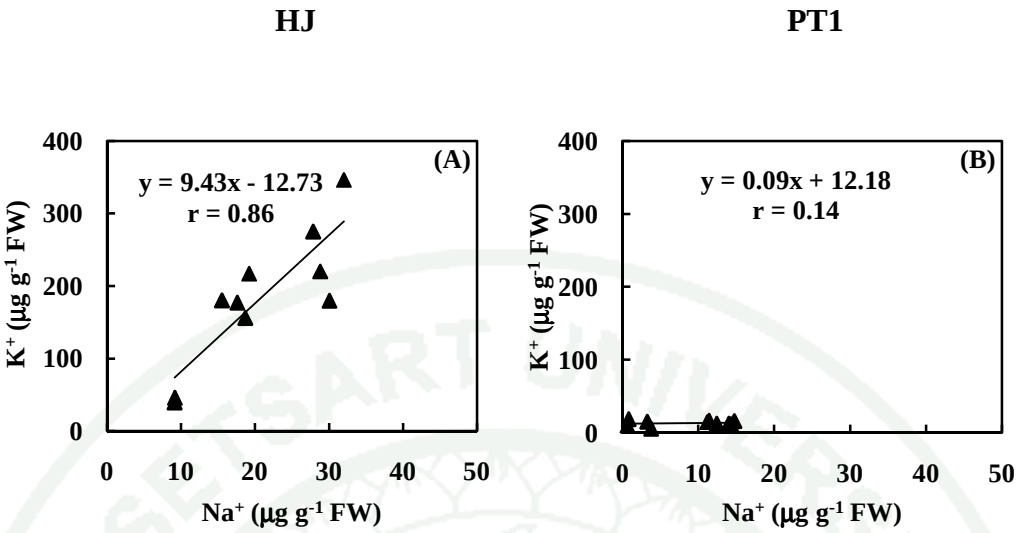

Figure 10 Correlation between Na^+ and K^+ in HJ (A) and PT1 (B) roots when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

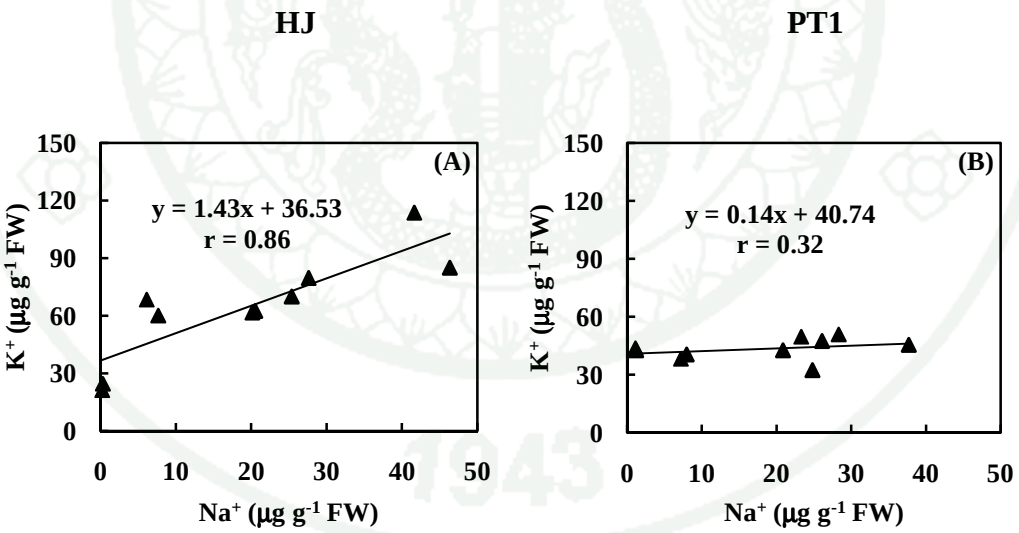

Figure 11 Correlation between Na^+ and K^+ in HJ (A) and PT1 (B) leaves when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

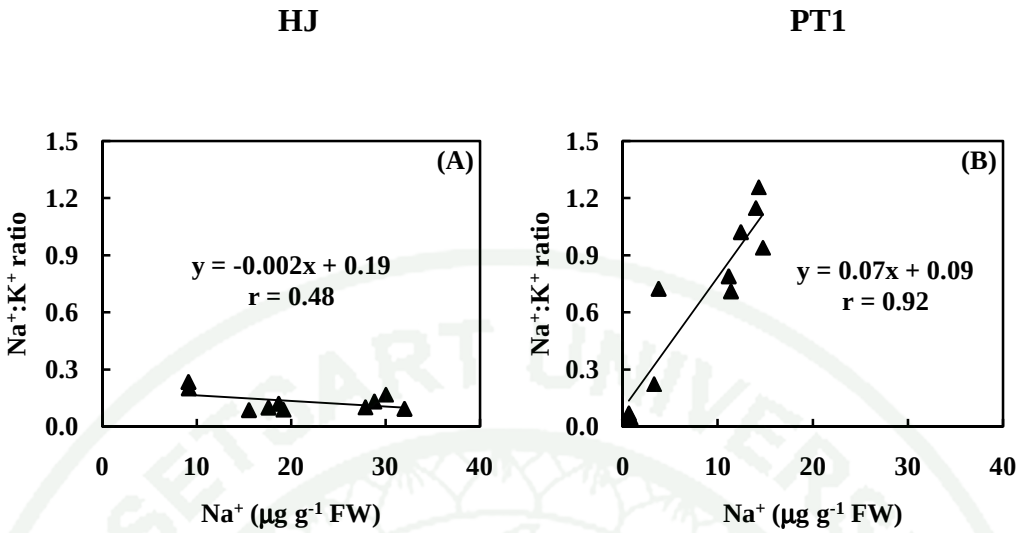

Figure 12 Correlation between Na^+ and Na^+:K^+ ratio in HJ (A) and PT1 (B) roots when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

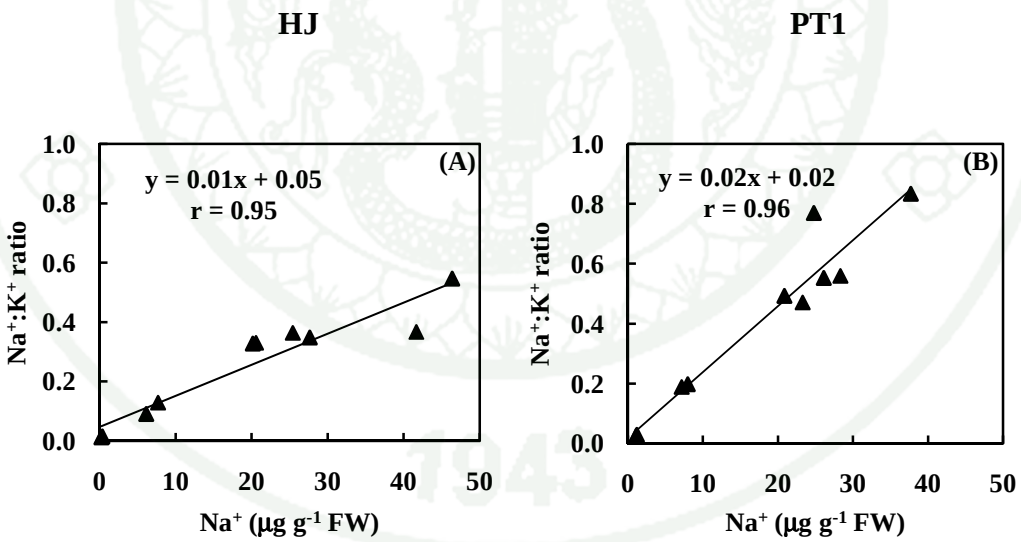

Figure 13 Correlation between Na^+ and Na^+:K^+ ratio in HJ (A) and PT1 (B) leaves when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

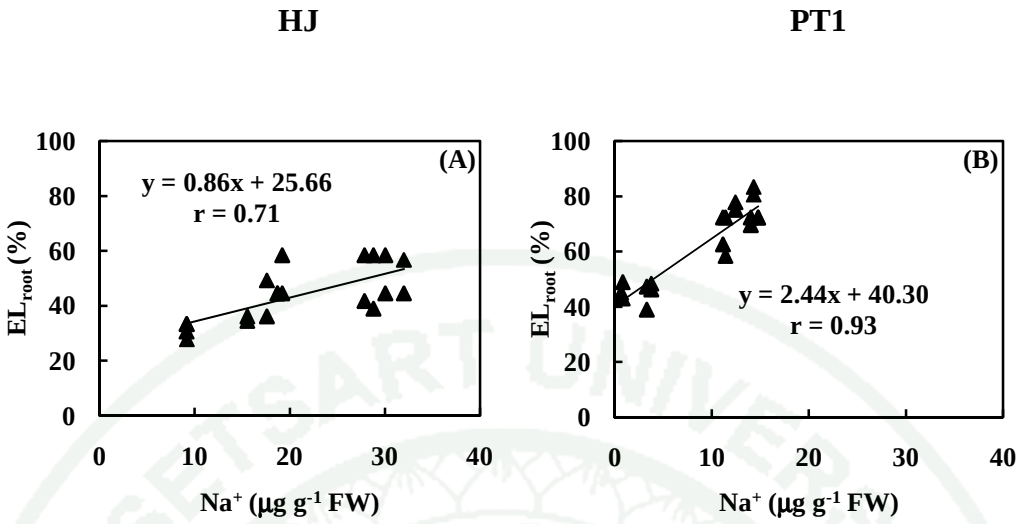

Figure 14 Correlation between Na^+ and EL_{root} in HJ (A) and PT1 (B) rice when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

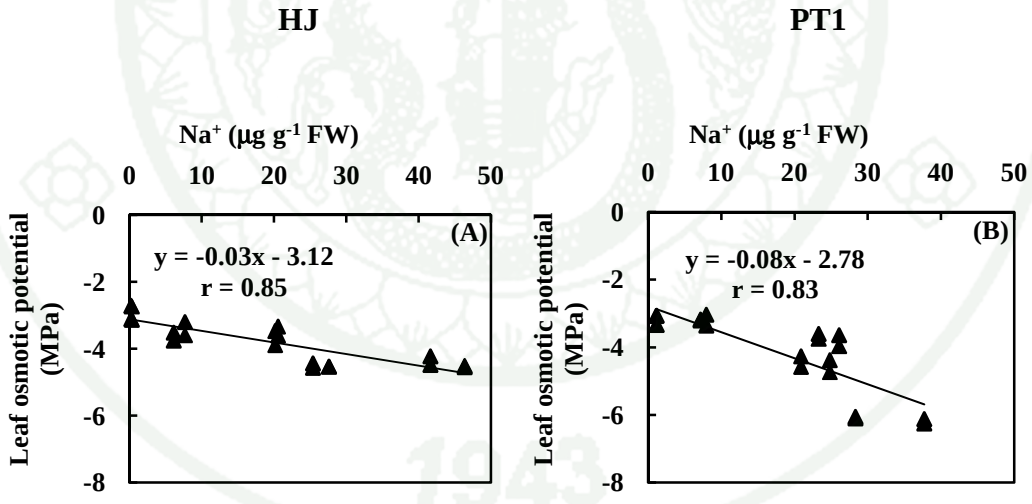

Figure 15 Correlation between Na^+ and osmotic potential in HJ (A) and PT1 (B) leaves when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

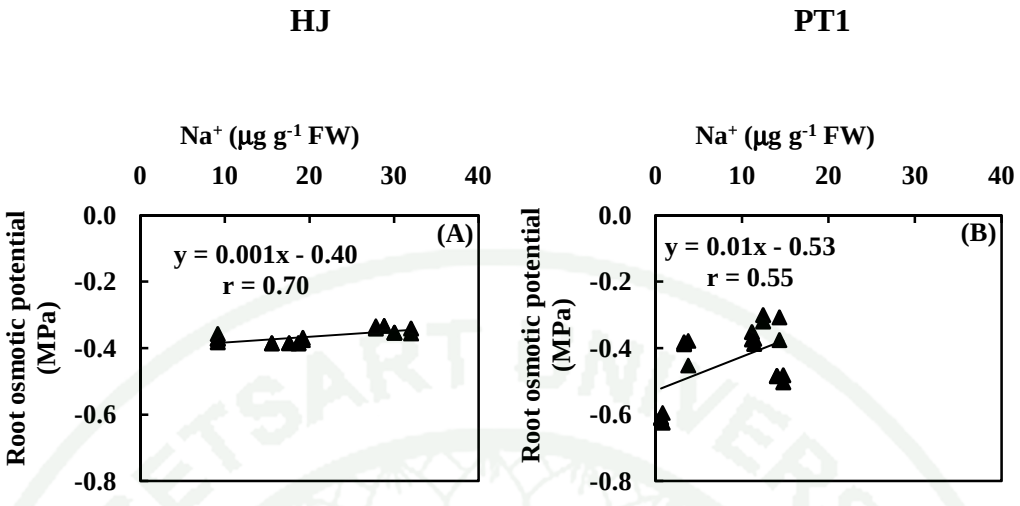

Figure 16 Correlation between Na^+ and osmotic potential in HJ (A) and PT1 (B) roots when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

Under iso-osmotic condition, photosynthetic pigments, Chl *a*, Chl *b*, TC and C_{x+c} in HJ and PT1 salt-stressed seedlings were severely reduced when the NaCl concentration was increased. Half reduction of the photosynthetic pigments in PT1 salt-stressed seedlings was found at 342 mM NaCl in comparing to the control (Table 14). This result clearly demonstrated that the increase of Na^+ under iso-osmotic condition reduced the photosynthetic pigments in both HJ and PT1 seedlings (Figure 17 and 18).

In parallel way, Chl *a* fluorescence parameters i.e. F_v/F_m, Φ_{PSII}, qP and NPQ in HJ and PT1 salt-stressed seedlings were significantly decreased when compared to the control (Table 15). This result indicated that the increasing of Na^+ inhibited the water oxidation in the PSII in HJ and PT1 seedlings (Figure 19 and 20). Moreover, increasing of the Chl *a* and TC were closely related to the increasing of F_v/F_m (Figure 21) and Φ_{PSII} (Figure 22), respectively. The increase of C_{x+c} was positively related to the increment of NPQ (Figure 23) in both HJ and PT1 seedlings. Furthermore, the relationship between Chl *a* fluorescence parameters i.e. increasing of F_v/F_m induced Φ_{PSII} which resulted in the increase of the photosynthesis (Figure 24).

The increase of NaCl concentration reduced photosynthesis i.e. photosynthetic pigment concentrations and Chl *a* fluorescence parameters which caused the growth reduction (FW and DW) in HJ and PT1 salt-stressed seedlings under iso-osmotic condition. Growth of seedlings was significantly different among the HJ and PT1 varieties and NaCl concentration (Table 16). Moreover, the growth of PT1 salt-stressed seedlings was more severely reduced than that of HJ salt-stressed seedlings (Table 16).

Table 14 Photosynthetic pigment concentrations in HJ and PT1 seedlings after cultured in liquid MS medium for 7 days and subsequently exposed to 0.0, 85.5, 171.0, 256.5 and 342.0 mM NaCl under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

Rice	NaCl (mM)	Mannitol (mM)	Pigment concentrations (μg g^{-1} FW)			
			Chl *a*	Chl *b*	TC	C_{x+c}
HJ	0.0	548.9	319.5 b	661.0 b	904.9 b	40.1 a
	85.5	329.4	259.7 d	529.2 c	802.3 c	36.4 b
	171.0	219.6	238.9 e	450.9 e	702.3 d	29.7 d
	256.5	109.8	231.7 e	440.4 ef	702.9 d	29.2 d
	342.0	0.0	213.2 f	412.9 f	632.4 e	26.5 e
PT1	0.0	548.9	375.3 a	697.9 a	966.6 a	37.2 b
	85.5	329.4	288.6 c	492.5 d	779.3 c	31.4 c
	171.0	219.6	192.0 g	466.7 de	629.3 e	23.2 f
	256.5	109.8	178.4 g	411.9 f	558.2 f	21.6 g
	342.0	0.0	154.4 h	169.6 g	425.4 g	21.4 g
Significant level						
Rice			**	**	**	**
NaCl			**	**	**	**
Rice × NaCl			**	**	**	**

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

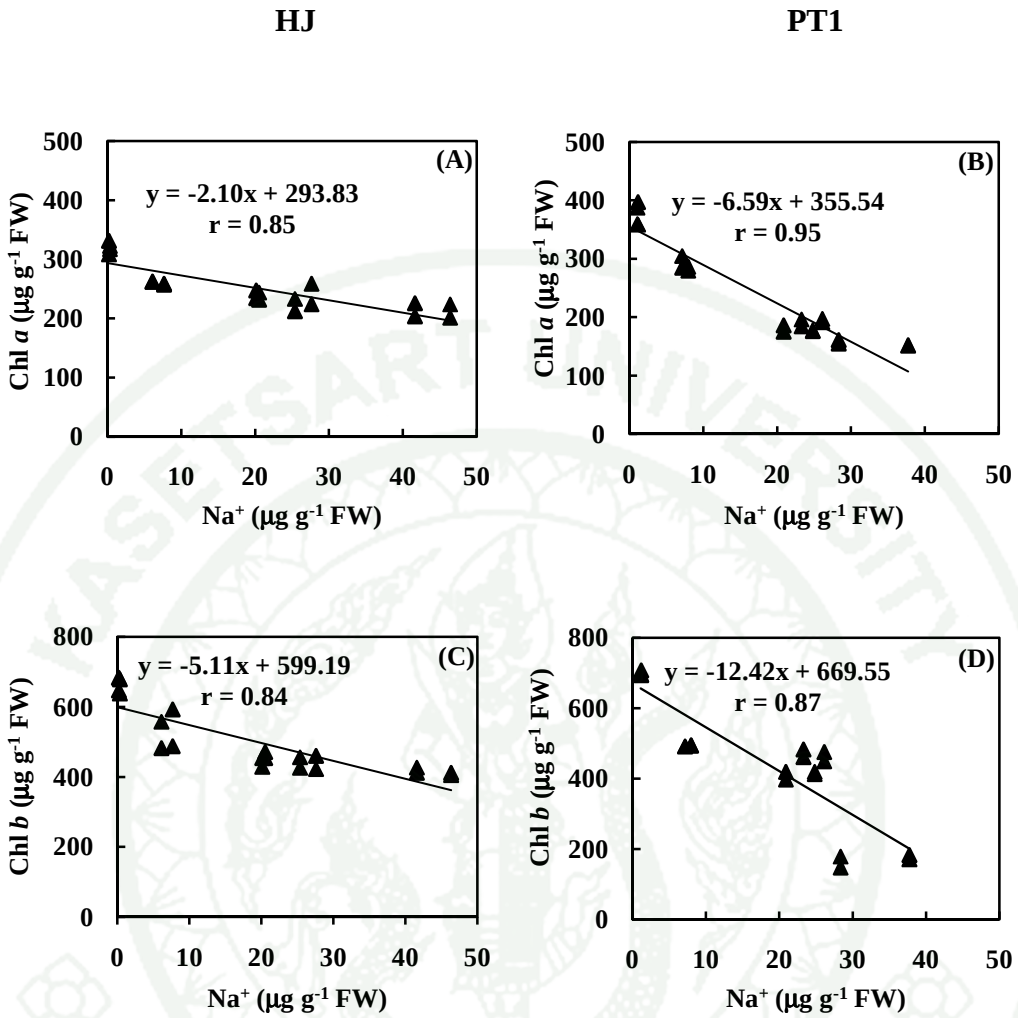

Figure 17 Correlation between Na^+ and Chl *a* and Chl *b* in HJ (A and C) and PT1 (B and D) seedlings when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

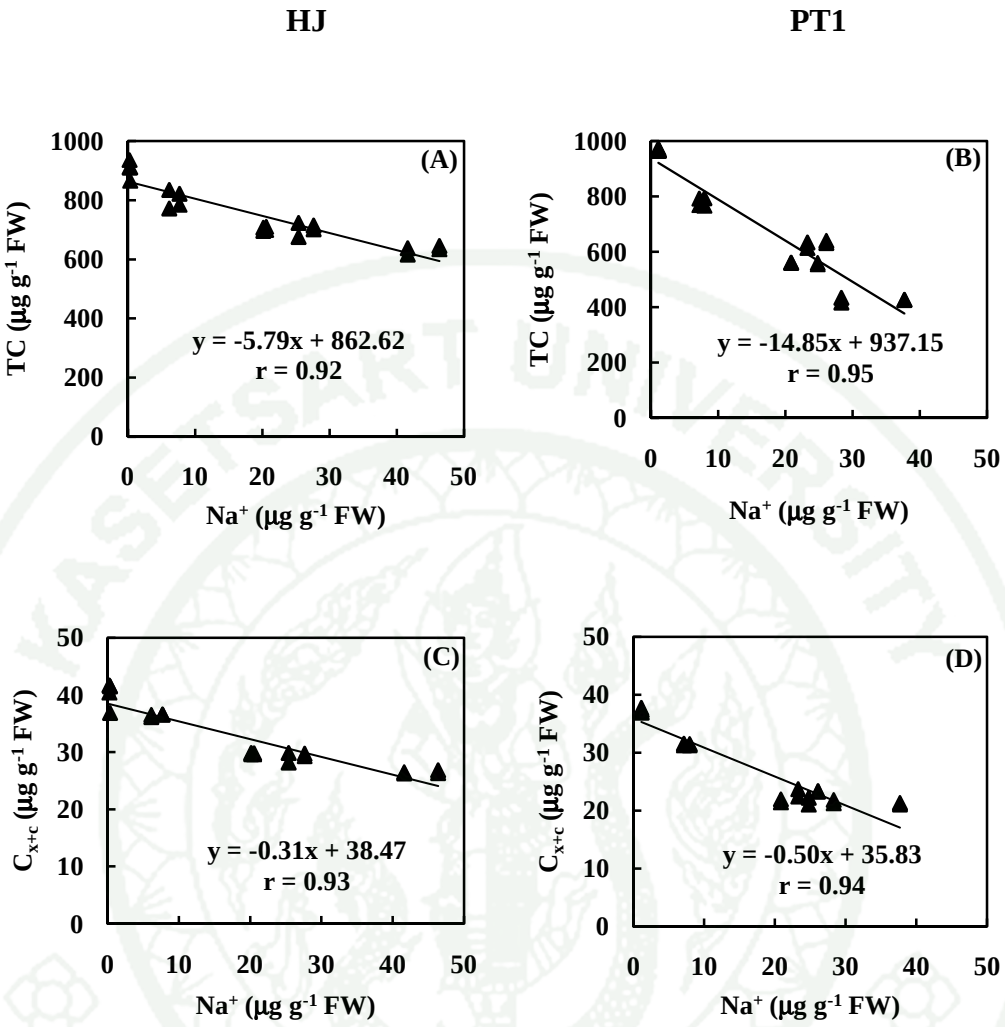

Figure 18 Correlation between Na^+ and TC and C_{x+c} in HJ (A and C) and PT1 (B and D) seedlings when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

Table 15 F_v/F_m, Φ_{PSII}, qP and NPQ in HJ and PT1 rice seedlings after cultured in liquid MS medium for 7 days and subsequently exposed to 0.0, 85.5, 171.0, 256.5 and 342.0 mM NaCl under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

Rice	NaCl (mM)	Mannitol (mM)	F_v/F_m	Φ_{PSII}	qP	NPQ
HJ	0.0	548.9	0.824 a	0.618 a	0.817 a	0.072 a
	85.5	329.4	0.771 b	0.526 b	0.678 b	0.051 b
	171.0	219.6	0.729 cd	0.466 d	0.622 d	0.037 d
	256.5	109.8	0.620 e	0.414 f	0.581 f	0.026 e
	342.0	0.0	0.514 f	0.297 g	0.527 g	0.017 f
PT1	0.0	548.9	0.737 c	0.507 c	0.659 c	0.044 c
	85.5	329.4	0.728 cd	0.497 c	0.594 e	0.037 d
	171.0	219.6	0.709 d	0.446 e	0.528 g	0.011 g
	256.5	109.8	0.627 e	0.411 f	0.516 h	0.010 g
	342.0	0.0	0.620 e	0.403 f	0.508 h	0.010 g
Significant level						
Rice			**	**	**	**
NaCl			**	**	**	**
Rice × NaCl			**	**	**	**

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

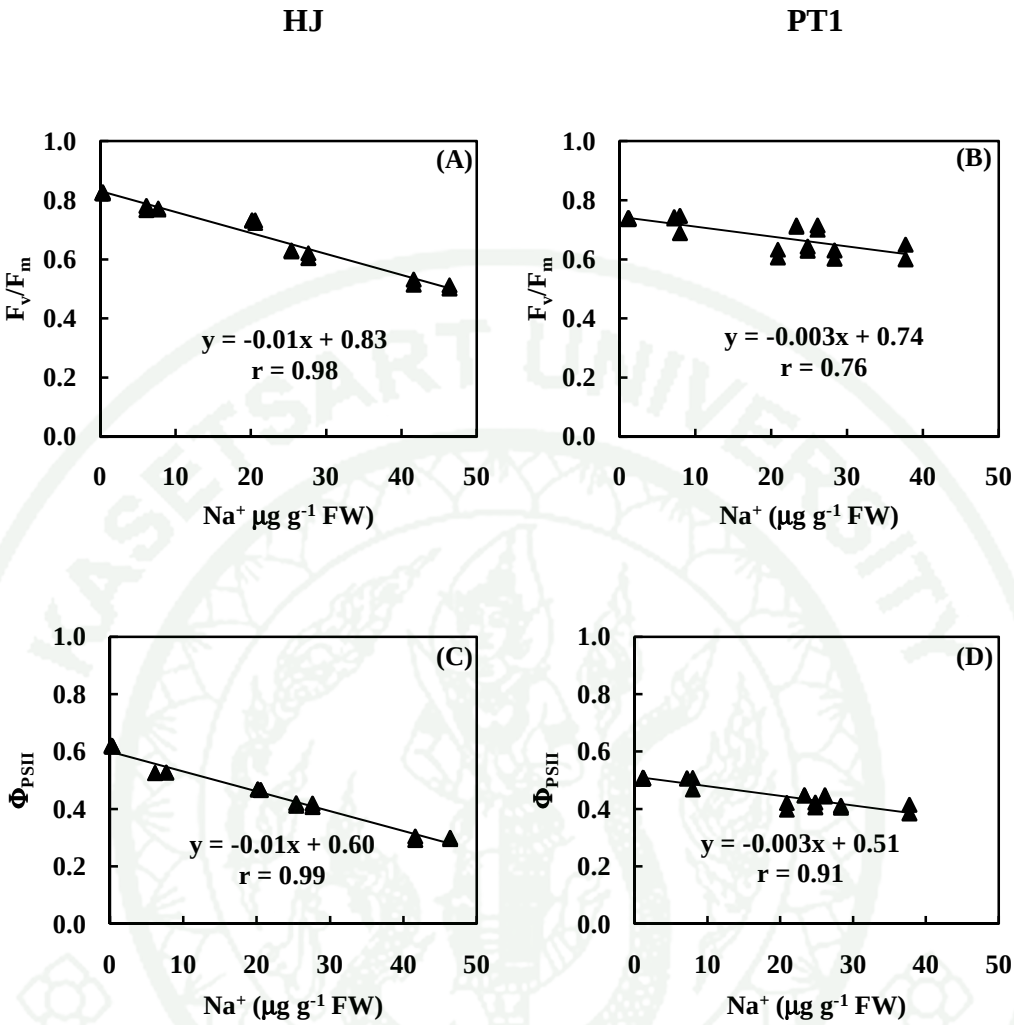

Figure 19 Correlation between Na^+ and F_v/F_m and Φ_{PSII} in HJ (A and C) and PT1 (B and D) seedlings when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

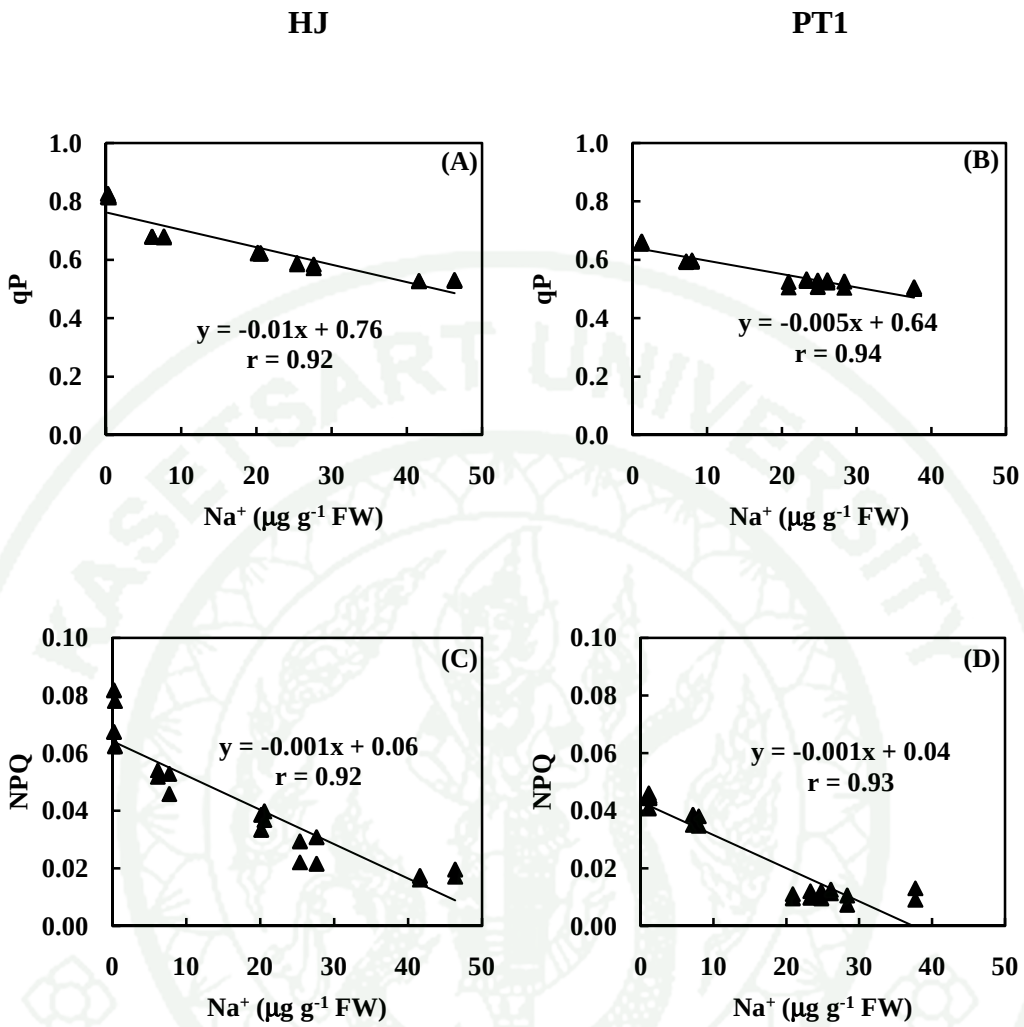

Figure 20 Correlation between Na^+ and qP and NPQ in HJ (A and C) and PT1 (B and D) seedlings when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

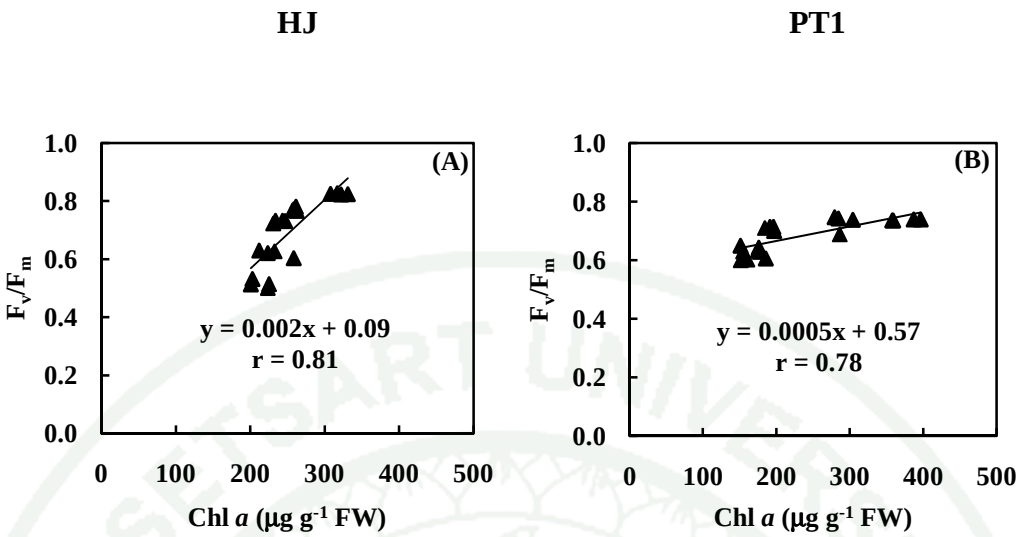

Figure 21 Correlation between Chl *a* and F_v/F_m in HJ (A) and PT1 (B) seedlings when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

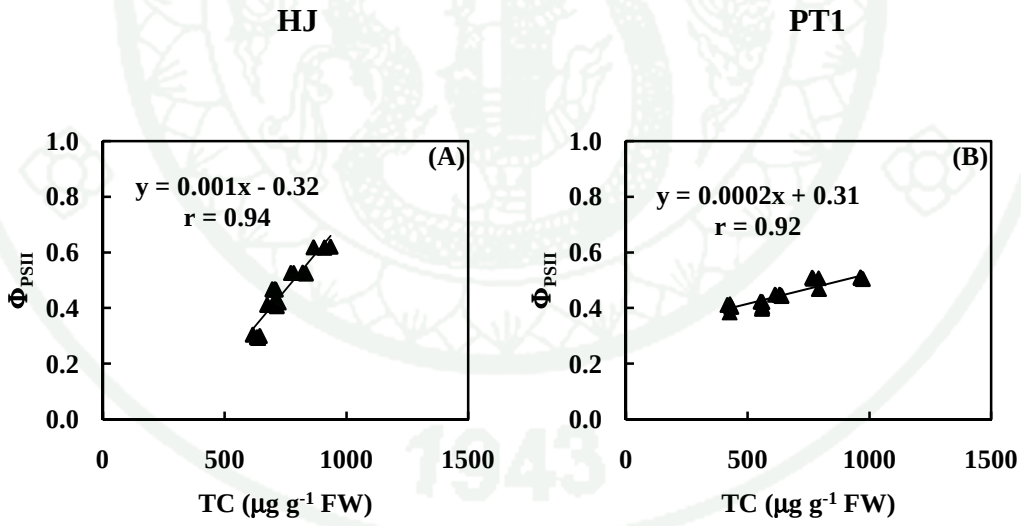

Figure 22 Correlation between TC and Φ_{PSII} in HJ (A) and PT1 (B) seedlings when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

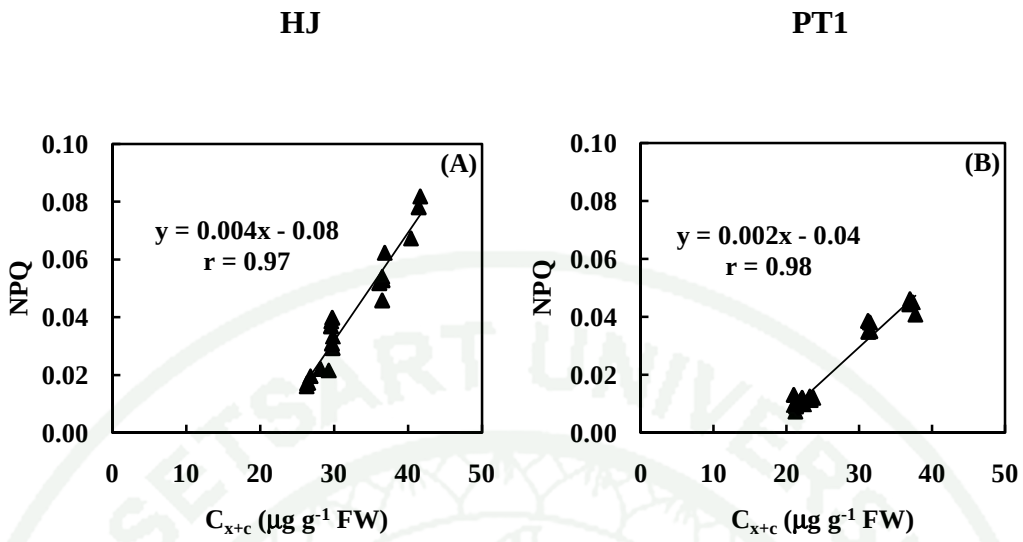

Figure 23 Correlation between C_{x+c} and NPQ in HJ (A) and PT1 (B) seedlings when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

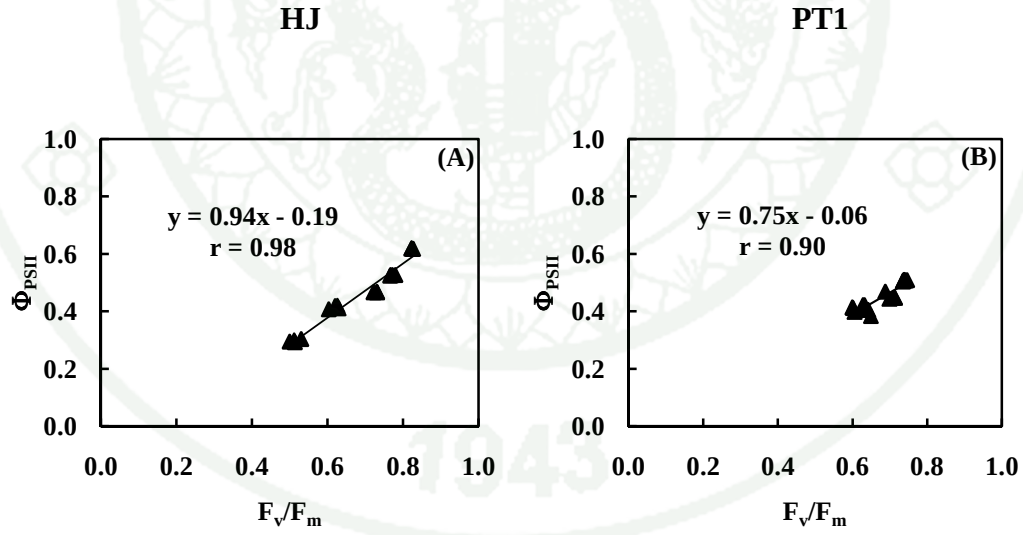

Figure 24 Correlation between F_v/F_m and Φ_{PSII} in HJ (A) and PT1 (B) seedlings when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

Table 16 Fresh weight (FW) and dry weight (DW) in HJ and PT1 roots and shoots after cultured in liquid MS medium for 7 days and subsequently exposed to 0.0, 85.5, 171.0, 256.5 and 342.0 mM NaCl under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

Rice	NaCl (mM)	Mannitol (mM)	Roots		Shoots	
			FW (mg)	DW (mg)	FW (mg)	DW (mg)
HJ	0.0	548.9	35.0 a	7.3 a	145.2 a	47.0 a
	85.5	329.4	28.6 b	4.3 b	132.1 b	33.7 b
	171.0	219.6	28.3 b	4.0 bc	116.4 c	25.4 cd
	256.5	109.8	20.5 cd	3.2 bcd	112.4 cd	23.9 cde
	342.0	0.0	18.4 d	3.0 bcd	110.4 cd	20.4 def
PT1	0.0	548.9	26.7 b	3.3 bcd	108.8 d	28.4 c
	85.5	329.4	21.9 c	3.3 bcd	77.1 e	19.6 ef
	171.0	219.6	12.5 e	2.3 cd	72.7 ef	17.4 f
	256.5	109.8	11.2 e	2.2 d	69.3 f	16.3 f
	342.0	0.0	11.0 e	1.8 d	68.6 f	16.0 f
Significant level						
Rice			**	**	**	**
NaCl			**	**	**	**
Rice × NaCl			**	ns	**	**

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

Under iso-osmotic condition, both HJ and PT1 rice showed the different responses of glucose, sucrose, raffinose and stachyose in roots. These sugars in PT1 salt-stressed roots were increased with the increasing of NaCl concentration while the sugars in HJ roots were slightly changed. Notably, the fructose was not found in both HJ and PT1 seedlings (Table 17). Furthermore, the increase of total soluble sugar in roots was not related to the change of root osmotic potential in HJ (r = 0.08) (Figure 25A) and PT1 (r = 0.63) (Figure 25B). In leaves, the sugars in HJ rice were decreased whereas sugars in PT1 rice were increased (Table 18). Moreover, the total soluble sugar in HJ salt-stressed leaves was not related to leaf osmotic potential (r = 0.77) (Figure 26A) whereas the increasing of total soluble sugar in PT1 salt-stressed leaves reduced the leaf osmotic potential (r = 0.95) (Figure 26B).

Table 17 Glucose, fructose, sucrose, raffinose and stachyose in HJ and PT1 root after cultured in liquid MS medium for 7 days and subsequently exposed to 0.0, 85.5, 171.0, 256.5 and 342.0 mM NaCl under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

Rice	NaCl (mM)	Mannitol (mM)	Sugar contents (μmol g^{-1} FW)				
			Glucose	Fructose	Sucrose	Raffinose	Stachyose
HJ	0.0	548.9	90.4 h	NA	35.7 h	10.9 de	5.5 f
	85.5	329.4	76.9 j	NA	31.4 j	2.8 e	13.3 de
	171.0	219.6	93.5 f	NA	37.3 f	19.3 c	28.1 c
	256.5	109.8	83.6 i	NA	33.3 i	8.6 de	17.5 d
	342.0	0.0	92.5 g	NA	36.8 g	7.7 de	19.0 d
PT1	0.0	548.9	94.1 e	NA	37.8 e	6.0 e	7.8 ef
	85.5	329.4	98.1 d	NA	39.2 d	14.5 cd	19.5 d
	171.0	219.6	119.3 a	NA	48.4 a	42.7 a	46.8 a
	256.5	109.8	110.0 b	NA	43.4 b	28.0 b	39.7 b
	342.0	0.0	109.0 c	NA	43.1 c	33.1 b	38.9 b
Significant level							
Rice			**	ND	**	**	**
NaCl			**	ND	**	**	**
Rice × NaCl			**	ND	**	**	**

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

NA = non-appearance

ND = non-detection

Table 18 Glucose, fructose, sucrose, raffinose and stachyose in HJ and PT1 leaves after cultured in liquid MS medium for 7 days and subsequently exposed to 0.0, 85.5, 171.0, 256.5 and 342.0 mM NaCl under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

Rice	NaCl	Mannitol	Sugar contents (μmol g^{-1} FW)				
	(mM)	(mM)	Glucose	Fructose	Sucrose	Raffinose	Stachyose
HJ	0.0	548.9	236.1 c	42.3 d	65.8 c	90.2 c	103.2 d
	85.5	329.4	155.8 h	25.7 f	43.3 g	74.0 e	85.7 e
	171.0	219.6	160.5 g	34.1 e	41.8 h	89.4 c	99.2 d
	256.5	109.8	151.8 i	41.8 d	36.7 j	38.0 h	44.4 g
	342.0	0.0	189.3 f	74.2 a	40.1 i	51.5 g	67.9 f
PT1	0.0	548.9	206.3 e	33.3 e	59.6 e	80.9 d	98.0 d
	85.5	329.4	215.7 d	30.8 ef	61.5 d	104.4 b	118.7 c
	171.0	219.6	218.3 d	54.3 c	58.6 f	62.7 f	72.5 f
	256.5	109.8	257.1 b	62.4 b	77.8 b	109.9 b	136.8 b
	342.0	0.0	266.4 a	60.6 b	138.1 a	251.6 a	280.2 a
Significant level							
Rice			**	**	**	**	**
NaCl			**	**	**	**	**
Rice × NaCl			**	**	**	**	**

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

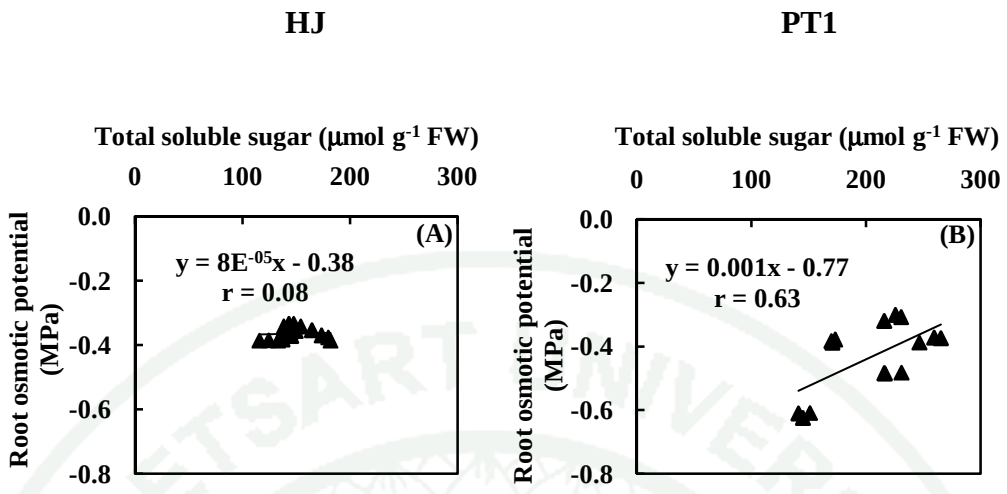

Figure 25 Correlation between total soluble sugar and osmotic potential in HJ (A) and PT1 (B) roots when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

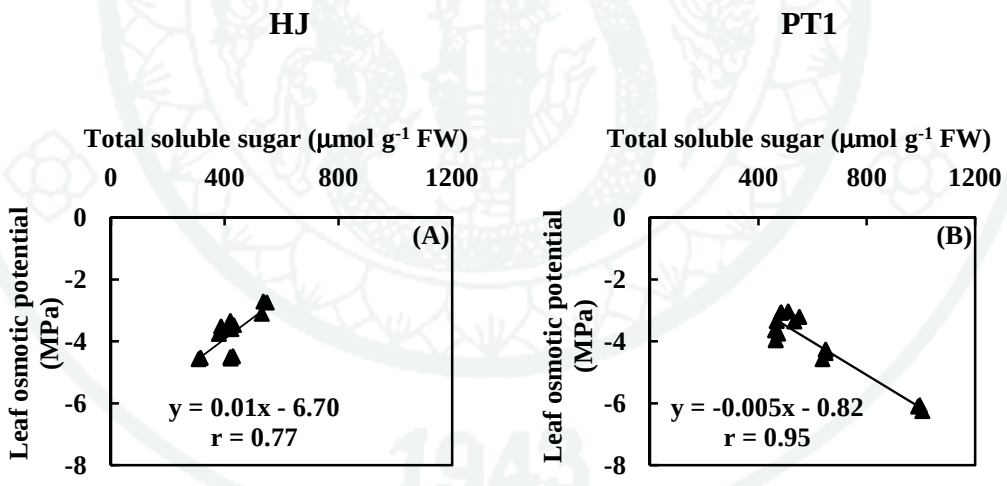

Figure 26 Correlation between total soluble sugar and osmotic potential in HJ (A) and PT1 (B) leaves when exposed to salt stress under iso-osmotic condition (osmotic potential = -1.75 ± 0.20 MPa) for 4 days.

Experiment 3. Salt tolerance ability improvement by exogenous potassium nitrate (KNO_3) and sucrose applications

3.1. Effect of exogenous potassium nitrate (KNO_3) on salt tolerance ability in salt-sensitive rice variety

From the experiment II, the increase of K^+ accumulation played an important role on the salt tolerance ability in HJ salt-stressed seedlings. Therefore, this study was to evaluate the role of exogenous potassium application on physiological responses in salt-sensitive rice.

The study showed that the root osmotic potential of PT1 salt-stressed rice cultured in the medium without KNO_3 was reduced by 1.8 times. There was no difference in HJ salt-stressed rice when compared to the control. Under salt stress, the leaf osmotic potential was severely decreased more than the root osmotic potential in both HJ and PT1 seedlings (Table 19). An increase of KNO_3 in the culture medium decreased the root and leaf osmotic potentials in PT1 salt-stressed seedlings at 11.8 and 14.1 mM KNO_3 (Table 19). Moreover, an increase of KNO_3 concentration in the culture medium reduced the osmotic potential in PT1 salt-stressed roots and leaves (Figure 27).

Chl *a*, Chl *b* and TC in HJ and PT1 salt-stressed seedlings were lower than those in the control (Table 20). However, the photosynthetic pigments in PT1 salt-stressed seedlings were increased by KNO_3 application in the culture medium, these pigment contents were also higher than those in HJ salt-stressed seedlings (Table 20). In addition, C_{x+c} in both HJ and PT1 salt-stressed seedlings was not significantly different from the control. However, higher concentration of KNO_3 application resulted in enhancing the C_{x+c} in the PT1 salt-stressed seedlings. The reduction of the leaf osmotic potential in PT1 salt-stressed seedlings was not related to the Chl *a* and TC (Figure 28).

Table 19 Osmotic potential in PT1 seedlings after cultured in liquid MS medium supplemented with KNO_3 concentration for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

Rice	KNO_3 (mM)	NaCl (mM)	Osmotic potential (MPa)	
			Root	Leaf
HJ	0.0	0	-0.35 a	-1.16 a
HJ	0.0	342	-0.38 a	-5.75 c
PT1	0.0	0	-0.33 a	-2.92 b
PT1	0.0	342	-0.61 b	-6.42 d
PT1	9.4	342	-0.65 b	-7.07 e
PT1	11.8	342	-0.83 d	-9.13 f
PT1	14.1	342	-0.76 c	-10.77 g
Significant level			**	**

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

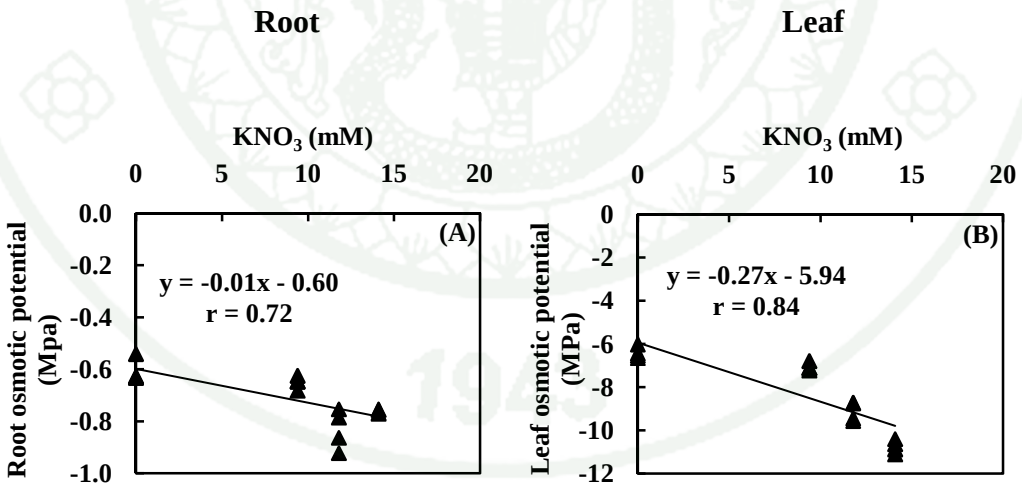

Figure 27 Correlation between KNO_3 concentration and osmotic potential in PT1 roots (A) and leaves (B) after applied with 0.0, 9.4, 11.8 and 14.1 mM KNO_3 for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

Table 20 Photosynthetic pigment concentrations in PT1 seedlings after cultured in liquid MS medium supplemented with 0.0, 9.4, 11.8 and 14.1 mM KNO_3 for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

Rice	KNO_3 (mM)	NaCl (mM)	Pigment concentrations (μg g^{-1} FW)			
			Chl *a*	Chl *b*	TC	C_{x+c}
HJ	0.0	0	184.5 a	59.7 a	243.9 a	60.5 b
HJ	0.0	342	106.9 de	34.4 d	140.7 de	58.8 b
PT1	0.0	0	144.1 bc	42.5 c	186.7 bc	45.0 c
PT1	0.0	342	99.2 e	32.1 d	131.5 e	46.3 c
PT1	9.4	342	112.1 d	38.8 c	151.5 d	48.7 c
PT1	11.8	342	147.0 b	47.0 b	194.3 b	72.1 a
PT1	14.1	342	134.0 c	41.8 c	176.8 c	67.9 a
Significant level			**	**	**	**

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

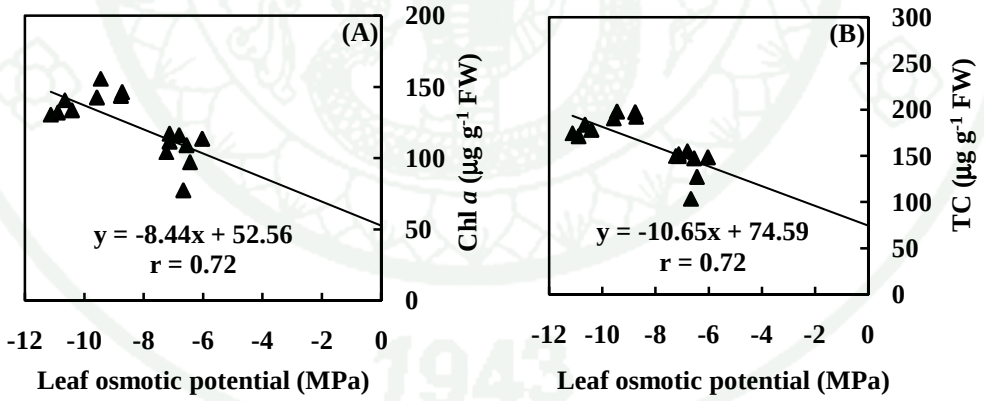

Figure 28 Correlation between leaf osmotic potential and Chl *a* (A) and TC (B) in PT1 seedlings after applied with 0.0, 9.4, 11.8 and 14.1 mM KNO_3 for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

Chl *a* fluorescence parameters, F_v/F_m, Φ_{PSII} and qP in both HJ and PT1 seedlings were reduced when exposed to 342 mM NaCl whereas the KNO_3 application in the culture medium showed that the Chl *a* fluorescence parameters in PT1 salt-stressed seedlings were increased (Table 21). An increase of Chl *a* in PT1 salt-stressed seedlings was positively related to the increasing of F_v/F_m (Figure 29) whereas the increasing of the F_v/F_m was not affected the Φ_{PSII} (Figure 30).

Growth in term of FW and DW of roots and shoots in HJ and PT1 seedlings were significantly reduced when subjected to salt stress. The increasing of KNO_3 concentration in the culture medium increased the shoot growth in PT1 salt-stressed seedlings (Table 22), however the FW and DW in roots were fluctuated.

Increasing of KNO_3 concentration in the culture medium increased growth of PT1 salt-stressed seedlings. This resulted from the increase of photosynthetic pigment concentrations and Chl *a* fluorescence parameters. The table 23 showed the positively correlated coefficients between growth and physiological responses in PT1 salt-stressed seedlings.

Table 21 F_v/F_m, Φ_{PSII} and qP in PT1 seedlings after cultured in liquid MS medium supplemented with 0.0, 9.4, 11.8 and 14.1 mM KNO_3 for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

Rice	KNO_3 (mM)	NaCl (mM)	F_v/F_m	Φ_{PSII}	qP
HJ	0.0	0	0.881 a	0.506 a	0.574 a
HJ	0.0	342	0.728 d	0.321 e	0.414 e
PT1	0.0	0	0.887 a	0.477 b	0.537 bc
PT1	0.0	342	0.731 d	0.368 d	0.502 d
PT1	9.4	342	0.746 d	0.399 c	0.558 ab
PT1	11.8	342	0.849 b	0.413 c	0.519 cd
PT1	14.1	342	0.793 c	0.408 c	0.527 bcd
Significant level			**	**	**

Means with the different letters in each column show significant difference at *p≤0.01* (**) by Duncan's New Multiple Range Test (DMRT).

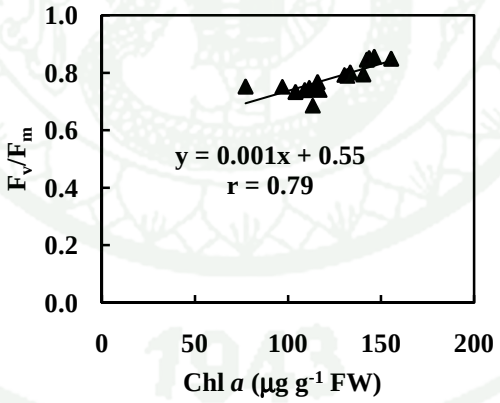

Figure 29 Correlation between Chl *a* and F_v/F_m in PT1 seedlings after applied with 0.0, 9.4, 11.8 and 14.1 mM KNO_3 for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

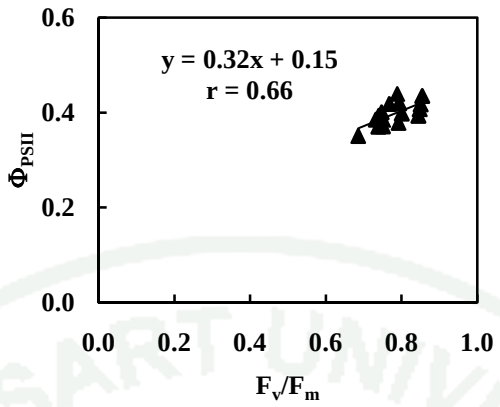

Figure 30 Correlation between F_v/F_m and Φ_{PSII} in PT1 seedlings after applied with 0.0, 9.4, 11.8 and 14.1 mM KNO_3 for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

Table 22 Fresh weight (FW) and dry weight (DW) in PT1 roots and shoots after cultured in liquid MS medium supplemented with 0.0, 9.4, 11.8 and 14.1 mM KNO_3 for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

Rice	KNO_3 (mM)	NaCl (mM)	Roots		Shoots	
			FW (mg)	DW (mg)	FW (mg)	DW (mg)
HJ	0.0	0	36.6 a	4.1 b	192.0 b	29.2 cd
HJ	0.0	342	32.9 bc	3.8 c	165.2 c	31.7 ab
PT1	0.0	0	35.0 ab	4.7 a	217.9 a	33.6 a
PT1	0.0	342	32.1 bc	3.4 de	141.5 d	28.9 d
PT1	9.4	342	30.3 c	3.6 cd	138.5 d	31.2 bc
PT1	11.8	342	36.9 a	3.3 e	156.3 c	31.2 bc
PT1	14.1	342	30.8 c	3.2 e	158.8 c	33.6 a
Significant level			**	**	**	**

Means with the different letters in each column show significant difference at *p≤0.01* (**) by Duncan's New Multiple Range Test (DMRT).

Table 23 Correlation coefficients of the physiological responses in PT1 salt-stressed seedlings after applied with 0.0, 9.4, 11.8 and 14.1 mM KNO_3 for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

	LOP	Chl *a*	Chl *b*	TC	C_{x+c}	F_v/F_m	Φ_{PSII}	qP	NPQ	SFW	SDW
LOP	1	-	-	-	-	-	-	-	-	-	-
Chl *a*	-0.725**	1	-	-	-	-	-	-	-	-	-
Chl *b*	-0.600**	**0.948****	1	-	-	-	-	-	-	-	-
TC	-0.719**	**0.987****	**0.947****	1	-	-	-	-	-	-	-
C_{x+c}	**-0.821****	**0.963****	**0.877****	**0.961****	1	-	-	-	-	-	-
F_v/F_m	-0.667**	0.791**	0.722**	0.795**	0.820**	1	-	-	-	-	-
Φ_{PSII}	-0.524*	0.565*	0.539*	0.558*	0.502*	0.660**	1	-	-	-	-
qP	0.021^{ns}	-0.005^{ns}	0.088^{ns}	0.008^{ns}	-0.128^{ns}	-0.014^{ns}	0.664**	1	-	-	-
NPQ	0.305^{ns}	-0.037^{ns}	-0.018^{ns}	-0.047^{ns}	-0.141^{ns}	-0.477^{ns}	-0.157^{ns}	0.051^{ns}	1	-	-
SFW	**-0.911****	**0.820****	0.657**	0.797**	0.921**	0.784**	0.494^{ns}	-0.176^{ns}	-0.249^{ns}	1	-
SDW	-0.771**	0.486^{ns}	0.463^{ns}	0.487^{ns}	0.540*	0.485^{ns}	0.613*	0.394^{ns}	-0.393^{ns}	0.623**	1

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

3.2. Improvement salt tolerance ability in salt-sensitive rice variety by exogenous sucrose application

The glucose and fructose in both HJ and PT1 seedlings were reduced when exposed to 342 mM NaCl (Table 24). However, exogenous sucrose application increased the glucose and fructose in PT1 salt-stressed roots. In this study, the sucrose was not detected (Table 24). In contrast to the roots, the salt stress remarkably induced the glucose, fructose and sucrose accumulations in HJ and PT1 leaves (Table 25). The exogenous sucrose concentration increased glucose and fructose in PT1 salt-stressed leaves, but not sucrose (Table 25). Furthermore, the total soluble sugar in PT1 salt-stressed seedlings cultured in the culture medium supplemented with sucrose reduced the root osmotic potential while leaf osmotic potential was increased (Figure 31).

Table 24 Sucrose, glucose and fructose in PT1 roots after cultured in liquid MS medium supplemented with 0.0, 29.2, 58.4 and 116.8 mM sucrose for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

Rice	Sucrose (mM)	NaCl (mM)	Sugar contents (μmol g^{-1} FW)		
			Sucrose	Glucose	Fructose
HJ	0.0	0	NA	53.7 c	50.0 c
HJ	0.0	342	NA	48.8 e	49.7 c
PT1	0.0	0	NA	47.8 f	48.4 d
PT1	0.0	342	NA	37.4 g	39.6 e
PT1	29.2	342	NA	51.5 d	40.7 e
PT1	58.4	342	NA	92.2 b	84.1 b
PT1	116.8	342	NA	242.0 a	237.0 a
Significant level			ND	**	**

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

NA = non-appearance

ND = non-detection

Table 25 Sucrose, glucose and fructose in PT1 leaves after cultured in liquid MS medium supplemented with 0.0, 29.2, 58.4 and 116.8 mM sucrose for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

Rice	Sucrose (mM)	NaCl (mM)	Sugar contents (μmol g^{-1} FW)		
			Sucrose	Glucose	Fructose
HJ	0.0	0	NA	155.9 g	133.5 f
HJ	0.0	342	195.9	369.0 e	397.0 d
PT1	0.0	0	NA	194.5 f	193.9 e
PT1	0.0	342	386.6	411.0 d	430.2 c
PT1	29.2	342	183.5	481.7 c	446.1 c
PT1	58.4	342	714.0	989.5 b	995.7 a
PT1	116.8	342	3.6	1084.8 a	619.4 b
Significant level			ND	**	**

Means with the different letters in each column show significant difference at $P \leq 0.01$ (**) by Duncan's New Multiple Range Test.

NA = non-appearance

ND = non-detection

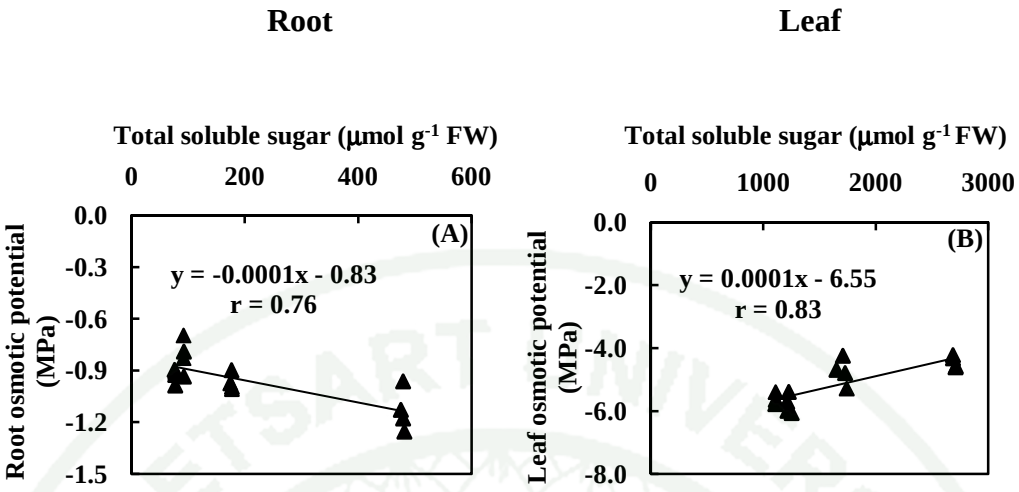

Figure 31 Correlation between total soluble sugar and osmotic potential in roots (A) and leaves (B) in PT1 seedlings after applied with 0.0, 29.2, 58.4 and 116.8 mM sucrose for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

Osmotic potential of root and leaf tissues in HJ and PT1 rice was decreased by salt stress, however the osmotic potential in PT1 salt-stressed seedlings was reduced more than that in the HJ salt-stressed seedlings, especially osmotic potential in leaf (Table 26). Interestingly, the increasing of sucrose concentration in the culture medium reduced the osmotic potential in PT1 salt-stressed roots more than that in PT1 salt-stressed roots without sucrose, but the osmotic potential was inversely increased in leaves (Table 26).

Table 26 Osmotic potential in PT1 seedlings after cultured in liquid MS medium supplemented with 0.0, 29.2, 58.4 and 116.8 mM sucrose for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

Rice	Sucrose (mM)	NaCl (mM)	Osmotic potential (MPa)	
			Root	Leaf
HJ	0.0	0	-0.58 a	-1.54 a
HJ	0.0	342	-0.88 bc	-3.82 b
PT1	0.0	0	-0.68 a	-1.65 a
PT1	0.0	342	-0.93 c	-5.79 d
PT1	29.2	342	-0.81 b	-5.63 d
PT1	58.4	342	-0.97 c	-4.43 c
PT1	116.8	342	-1.13 d	-4.75 c
Significant level			**	**

Means with the different letters in each column show significant difference at $P \leq 0.01$ (**) by Duncan's New Multiple Range Test.

The Chl *a*, Chl *b* and TC in PT1 salt-stressed seedlings were severely reduced more than those in the HJ salt-stressed seedlings. Exogenous sucrose application increased Chl *a*, Chl *b* and TC in PT1 salt-stressed seedlings (Table 27). Additionally, the C_{x+c} in HJ and PT1 salt-stressed seedlings was induced comparing to the control, however application of sucrose to PT1 salt-stressed rice resulted in the reduction of C_{x+c} (Table 27). Furthermore, an increase of leaf osmotic potential was positively related to TC (Figure 32).

Table 27 Photosynthetic pigments (Chl *a*, Chl *b*, TC and C_{x+c}) in PT1 seedlings after cultured in liquid MS medium supplemented with 0.0, 29.2, 58.4 and 116.8 mM sucrose for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

Rice	Sucrose (mM)	NaCl (mM)	Pigment concentrations (μg g^{-1} FW)			
			Chl *a*	Chl *b*	TC	C_{x+c}
HJ	0.0	0	155.0 a	62.8 ab	218.5 a	24.1 c
HJ	0.0	342	135.9 b	50.4 d	186.6 d	28.2 b
PT1	0.0	0	156.5 a	66.3 a	223.0 a	24.1 c
PT1	0.0	342	125.3 c	29.4 e	154.3 e	34.2 a
PT1	29.2	342	138.5 b	51.7 d	190.2 cd	27.8 b
PT1	58.4	342	137.0 b	62.1 b	199.3 b	25.0 c
PT1	116.8	342	139.2 b	55.7 c	194.0 c	27.1 b
Significant level			**	**	**	**

Means with the different letters in each column show significant difference at $P \leq 0.01$ (**) by Duncan's New Multiple Range Test.

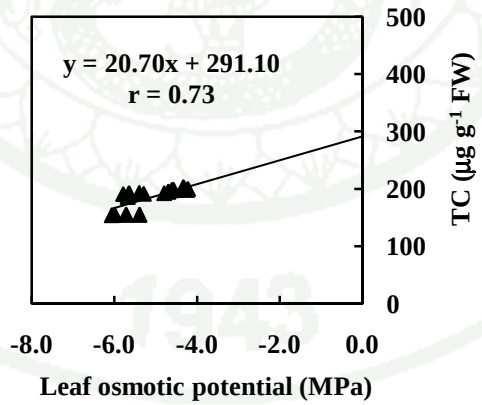

Figure 32 Correlation between osmotic potential and TC in PT1 leaves after applied with 0.0, 29.2, 58.4 and 116.8 mM sucrose for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

Chl *a* fluorescence parameters i.e. F_v/F_m, Φ_{PSII} and qP in HJ and PT1 seedlings exposed to salt stress for 4 days were significantly reduced when compared to the control. In PT1 salt-stressed seedlings cultured in the culture medium supplemented with sucrose, the F_v/F_m, was increased whereas Φ_{PSII} and qP were reduced (Table 28).

Table 28 F_v/F_m, Φ_{PSII} and qP in PT1 seedlings after cultured in liquid MS medium supplemented with 0.0, 29.2, 58.4 and 116.8 mM sucrose for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

Rice	Sucrose (mM)	NaCl (mM)	F_v/F_m	Φ_{PSII}	qP
HJ	0.0	0	0.901 a	0.585 b	0.651 b
HJ	0.0	342	0.803 c	0.503 c	0.611 c
PT1	0.0	0	0.914 a	0.622 a	0.682 a
PT1	0.0	342	0.795 c	0.497 c	0.624 c
PT1	29.2	342	0.804 c	0.444 e	0.561 d
PT1	58.4	342	0.856 b	0.494 c	0.540 d
PT1	116.8	342	0.857 b	0.460 d	0.542 d
Significant level			**	**	**

Means with the different letters in each column show significant difference at $P \leq 0.01$ (**) by Duncan's New Multiple Range Test.

Salt stress caused a significant reduction in the FW and DW in HJ and PT1 seedlings, however exogenous sucrose application increased the growth in PT1 salt-stressed seedlings (Table 29).

Increasing of sucrose concentration in the culture medium increased growth of PT1 salt-stressed seedlings. This was resulted from the increase in osmotic potential and photosynthetic pigment concentrations. The table 30 showed the positively correlated coefficients between growth and physiological responses in PT1 salt-stressed seedlings.

Table 29 Fresh weight (FW) and dry weight (DW) in PT1 roots and shoots after cultured in liquid MS medium supplemented with 0.0, 29.2, 58.4 and 116.8 mM sucrose for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

Rice	Sucrose (mM)	NaCl (mM)	Roots		Shoots	
			FW (mg)	DW (mg)	FW (mg)	DW (mg)
HJ	0.0	0	48.3 a	4.5 c	257.5 a	38.6 b
HJ	0.0	342	24.0 c	3.1 de	212.5 b	37.4 b
PT1	0.0	0	49.1 a	4.0 cd	201.4 b	33.1 b
PT1	0.0	342	15.7 d	1.8 e	154.0 c	31.7 b
PT1	29.2	342	40.4 b	5.1 c	159.2 c	35.4 b
PT1	58.4	342	41.3 b	9.2 a	219.2 ab	50.8 a
PT1	116.8	342	40.6 b	6.4 b	231.6 ab	56.5 a
Significant level			**	**	**	**

Means with the different letters in each column show significant difference at $P \leq 0.01$ (**) by Duncan's New Multiple Range Test.

Table 30 Correlation coefficients of the physiological responses in PT1 salt-stressed seedlings after applied with 0.0, 29.2, 58.4 and 116.8 mM sucrose for 14 days and subsequently exposed to 342 mM NaCl for 4 days.

	LOP	Chl *a*	Chl *b*	TC	C_{x+c}	F_v/F_m	Φ_{PSII}	qP	NPQ	SFW	SDW
LOP	1	-	-	-	-	-	-	-	-	-	-
Chl *a*	0.597*	1	-	-	-	-	-	-	-	-	-
Chl *b*	0.783**	**0.828****	1	-	-	-	-	-	-	-	-
TC	0.728**	**0.844****	**0.976****	1	-	-	-	-	-	-	-
C_{x+c}	-0.654**	-0.653**	**-0.934****	**-0.945****	1	-	-	-	-	-	-
F_v/F_m	0.791**	0.462^{ns}	0.676**	0.657**	-0.663**	1	-	-	-	-	-
Φ_{PSII}	0.084^{ns}	-0.535*	-0.309^{ns}	-0.426^{ns}	0.308^{ns}	0.089^{ns}	1	-	-	-	-
qP	-0.701**	-0.734**	**-0.893****	**-0.925****	**0.873****	-0.539*	0.402^{ns}	1	-	-	-
NPQ	0.469^{ns}	0.362^{ns}	0.583*	0.613*	-0.651**	0.310^{ns}	0.023^{ns}	-0.622**	1	-	-
SFW	**0.854****	0.614*	0.685**	0.662**	-0.560*	0.711**	0.029^{ns}	-0.703**	0.425^{ns}	1	-
SDW	**0.870****	0.634**	0.743**	0.735**	-0.653**	0.787**	-0.083^{ns}	-0.779**	0.367^{ns}	**0.958****	1

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

DISCUSSIONS

1. Physiological responses of salt-tolerant and salt-sensitive rice varieties to different NaCl concentrations and salt exposure times

Reduction of the osmotic potential in the salt-stressed plants depended mainly on varieties (de Azevedo Neto *et al.*, 2004), organ (Cha-um *et al.*, 2007b), salt concentration (Nakamura *et al.*, 2002) and salt exposure time (de Herralde *et al.*, 1998). From the result in figure 5 the osmotic potential in roots and leaves of the control treatment was reduced at 4 and 8 days. This is 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 (Ahmad *et al.*, 2007; Cha-um *et al.*, 2010b). In order to uptake water from the medium, the seedlings have to adjust themselves by lowering osmotic potential (Taiz and Zeiger, 2002; Musacchi *et al.*, 2006). This result confirmed the reduction of the osmotic potential in salt-tolerant HJ and salt-sensitive PT1 rice under salt stress, especially leaf tissues (Figure 5C and 5D). This fact was supported by Cha-um *et al.* (2007b) and Giaveno *et al.* (2007) 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 salt-sensitive PT1 seedlings was severely decreased more than those in salt-tolerant HJ seedlings (Figure 5C and 5D). 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 (Dionisio-Sese and Tobita, 1998; Lefèvre *et al.*, 2001; Cha-um *et al.*, 2009a). Moreover, relationship between NaCl concentration and osmotic potential reduction was found in the other plants such as barley (Chen *et al.*, 2007), *Rumex* (Chen *et al.*,

2004), red raspberry (Neocleous and Vasilakakis, 2007) and safflower (Siddiqi and Ashraf, 2008).

Beside the reduction of osmotic potential, toxicity of Na^+ and Cl^- was reported (Chen *et al.*, 2004; Chen *et al.*, 2007; Neocleous and Vasilakakis, 2007; Siddiqi and Ashraf, 2008). This ionic effect disturbed plant metabolism, especially photosynthesis which affected plant growth and development (Taiz and Zeiger, 2002). 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 salt-tolerant HJ and salt-sensitive PT1 seedlings. However, the photosynthetic pigment concentrations in salt-tolerant HJ seedlings were higher than those in salt-sensitive PT1 seedlings (Figure 6 and Figure 7). Boriboonkaset (2007) reported that the chloroplast ultrastructure in salt-sensitive IR29 and PT1 rice was severely disordered more than that in salt-tolerant Pokkali and HJ rice after exposing to 342 mM NaCl.

At 171 mM NaCl, the increase of Chl *a* (Figure 6A and 6B) and TC (Figure 7A and 7B) in salt-tolerant HJ rice might relate to the higher salt tolerance ability of salt-tolerant HJ rice than salt-sensitive PT1 rice. Similar result was reported by Wanichananan *et al*. (2003) who demonstrated that the chlorophyll index (CI) in salt-sensitive KDML105 rice was declined by 4 times more than that in salt-tolerant Hawm Naipon rice 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* (de Herralde *et al.*, 1998), canola (Kauser *et al*., 2006) and rice (Cha-um *et al*., 2007b). Moreover, the degradation of photosynthetic pigments depended on lipid protein ratio of pigment-protein complexes alteration and increasing of chlorophyllase activity (Iyengar and Reddy, 1996; Taffouo *et al.*, 2010).

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 salt-tolerant HJ and salt-sensitive PT1 seedlings, however the reduction of C_{x+c} in salt-tolerant HJ seedlings at 171 mM NaCl was less than that in salt-sensitive PT1 seedlings (Figure 7C and 7D). It was reported that the salt stress generated reactive oxygen species which caused lipid peroxidation and photo-oxidation of chlorophyll (Dionisio-Sese and Tobita, 1998; Santos, 2004). The higher C_{x+c} in salt-tolerant HJ seedlings prevented chlorophyll degradation (Figure 6 and Figure 7) and resulted in the increasing of salt tolerance in salt-tolerant HJ more than in salt-sensitive PT1 seedlings. This result was similar to Cha-um *et al.* (2004) who reported that the C_{x+c} in KDML105 rice which is a salt-sensitive variety, was reduced by 12 times compared to the control when subjected to 342 mM NaCl for 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 (Maxwell and Johnson, 2000; Loreto *et al.*, 2003). The water oxidation in the light reaction was used as a tool to indicate the sensitivity to stress conditions such as chilling (Greaves and Wilson, 1987), heat (Liu and Huang, 2000; Wang and Huang, 2004), drought (Wang and Huang, 2004), light intensity (Drozak and Romanowska, 2006), submergence (Panda *et al.*, 2008) and salt (Cha-um and Kirdmanee, 2008; Cha- um *et al.*, 2009a; Cha-um and Kirdmanee, 2009; Mehta *et al.*, 2010).

In salt-tolerant HJ and salt-sensitive PT1 seedlings, the 171 mM NaCl was slightly changed F_v/F_m, Φ_{PSII} and qP. However, the F_v/F_m was severely affected by 342 mM NaCl (Figure 8 and 9). At 342 mM NaCl, the F_v/F_m in salt-tolerant HJ rice was higher than that in salt-sensitive PT1 rice (Figure 8A and 8B). This might play a role on higher salt tolerance of salt-tolerant HJ rice than salt-sensitive PT1 rice. 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. As a result, photosynthesis was reduced (Havaux, 1993; Wen *et al.*, 2005; Murata *et al.*, 2007).

This result agreed with the report of Cha-um *et al.* (2007a) who demonstrated that the stabilization of photosynthetic pigments, especially chlorophyll in salt-tolerant GS No. 4371 and salt-sensitive GS No. 7032 rice lines 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 rose (Jiminez *et al.*, 1997), sorghum (de Lacerda *et al.*, 2003), olive (Loreto *et al.*, 2003), sunflower (Santos, 2004), canola (Kauser *et al.*, 2006), sweet almond (Ranjbarfordoei *et al.*, 2006), cucumber (Stępień and Klobus, 2006), *Ramonda serbica* (Degl'Innocenti *et al.*, 2008), barley (Degl'Innocenti *et al.*, 2009) and rice (Cha- um *et al.*, 2009a) were severely reduced by salt stress.

NPQ is a non-photochemical quenching which plays an important role on anti-oxidative system. After salt treatment, the NPQ in salt-tolerant HJ and salt-sensitive PT1 seedlings was decreased, but the decreasing of the NPQ in salt-tolerant HJ rice was less than in salt-sensitive PT1 rice (Figure 9C and 9D). This higher NPQ is correlated with the better salt tolerance ability in salt-tolerant HJ rice. This result was similar to Cha-um *et al.* (2007a) 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.

In this study, the shoot growth in salt-tolerant HJ and salt-sensitive PT1 seedlings were severely reduced when NaCl concentration was increased. However, the shoot growth in salt-tolerant HJ seedlings was higher than that in salt-sensitive PT1 seedlings whereas salt did not affect the root growth (Table 4 and Table 5). Similarly, the report of Cha-um *et al.* (2010a) 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, 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 (Almodares *et al.*, 2008b), tomato (Chookhampaeng *et al.*, 2008), cotton (Ashraf and Ahmad, 2000) and quinoa (Prado *et al.*, 2000).

NaCl reduced shoot growth in both salt-tolerant HJ and salt-sensitive PT1 rice. Leaf osmotic potential, photosynthetic pigment concentrations and Chl *a* fluorescence parameters played important roles on shoot growth under salt stress (Table 6-11). Salt ion accumulation reduced leaf osmotic potential, meanwhile the ions damaged photosynthetic pigment apparatus.

2. Physiological responses of salt-tolerant and salt-sensitive rice varieties to salt stress under iso-osmotic condition

From the experiment I, growth of salt-tolerant HJ and salt-sensitive PT1 seedlings was reduced by salt stress. However, the previous experiment could not clearly separate the ionic effect from osmotic effect on the physiological responses. In this study, an experiment about ionic effect of NaCl was carried out under iso-osmotic condition.

Under iso-osmotic condition, this research showed that the increasing of Na^+ in salt-tolerant HJ and salt-sensitive PT1 roots and leaves was similar to the result of the salt stress treatment without osmotic control in many species such as rice (Dionisio-Sese and Tobita, 1998; Lefèvre *et al.*, 2001; Nakamura *et al.*, 2002; Cha-um *et al.*, 2007b), wheat (Zheng *et al.*, 2008b; El-Hendawy *et al.*, 2009; Mahmood, 2009), maize (de Azevedo Neto *et al.*, 2004) and sorghum (de Lacerda *et al.*, 2005).

This result indicated that the osmotic control in the culture medium has no different effect on the Na^+ uptake (Table 12 and 13). Na^+ and K^+ have equal electric charge, however molecular weight (size) of Na^+ is less than that of K^+. Thus, Na^+ can

diffuse through the cell membrane faster than K^+. Ion transport through channels is always passive and the ion transport specificity depends on pore size and electric charge more than on selective binding. Therefore, the channel transport is limited mainly to ions or water (Taiz and Zeiger, 2002).

Furthermore, Na^+ uptake involved with the type of channel, especially non-selective cation channels (NSCCs). Kader and Lindberg (2005) found that the function of NSCCs in salt-tolerant Pokkali and salt-sensitive BRRI Dhan29 rice varieties were inhibited by applying with the inhibitor of NSCCs (Ca^{2+}, Zn^{2+} and La^{3+}). This inhibition resulted in the reduction of the Na^+ uptake.

This result showed that the increasing of the Na^+ in salt-tolerant HJ roots and leaves was higher than that in salt-sensitive PT1 rice. Not only the increasing of Na^+ but also K^+ was increased in salt-tolerant HJ roots and leaves, while the K^+ in salt-sensitive PT1 roots and leaves was unchanged (Table 12 and 13). The salt-tolerant HJ rice may have higher efficiency of the K^+ selectivity than that the salt-sensitive PT1 rice. This result was contrast to the result of the non-osmotic control which showed the increase of NaCl concentration induced Na^+ while K^+ was decreased (Hernández *et al*., 1995; Levigneron *et al*., 1995; Hasegawa *et al*., 2000; Aziz and Khan, 2001; Romero-Aranda *et al*., 2001; Bayuelo-Jimenez *et al*., 2003; Parida *et al*., 2004; Cha-um *et al*., 2007b; Hu *et al*., 2007; Maggio *et al*., 2007; Sabir and Ashraf, 2007; Perica *et al*., 2008; Zia *et al*., 2008). In rice plant, an increase in Na^+ reduced K^+ in many rice varieties such as Hitomebore and IR28 (Dionisio-Sese and Tobita, 1998; Nakamura *et al*., 2002), I Kong Pao (IKP) (Lefèvre *et al*., 2001), PT1 and HJ (Cha-um *et al*., 2007b) and IR20 (Krishnamurthy *et al*., 2009).

Under salt stress, inward rectifying potassium channels are highly selective for K^+ uptake (Maathius and Amtmann, 1999), moreover capacity K^+ uptake depended on salt stress level and ion transporters such as HKT, AKT, HAK, KUP, KT and CPA (Horie *et al*., 2001; Golldack *et al*., 2002; Garciadeblás *et al*., 2003; Fuchs *et al*., 2005; Kader *et al*., 2006; Gierth and Mäser, 2007; Takahashi *et al*., 2007; Szczerba *et al*., 2009) which played important role to select or control ion transportation in the

plants. In this study, the K^+ in salt-tolerant HJ seedlings was increased while it was unchanged in salt-sensitive PT1 seedlings under iso-osmotic condition. Kader and Lindberg (2005) demonstrated that the K^+-selective channels in salt-sensitive BRRI Dhan29 seedlings was inhibited by applying inhibitor for K^+-selective channels (TEA, CS^+ and Ba^{2+}) while salt-tolerant Pokkali seedlings was not affected. This indicated that the differences of the K^+-selective channels capabilities of rice varieties were important on the ion selectivity and uptake.

The increase of NaCl concentration in the culture medium increased Na^+ uptake which correlated to the increasing of EL_{root} (Figure 14). This result was similar to the salt stress without osmotic control (Dionisio-Sese and Tobita, 1998; Yasar *et al*., 2006; da Silva *et al*., 2008; Zheng *et al*., 2008b). The EL_{root} in salt-tolerant HJ roots was lower than that in salt-sensitive PT1 roots (Table 12). It was possible that the increasing of the K^+ was higher than the Na^+. This increasing led to the reduction of the damage of the composition and structure of membrane such as phospholipids and protein (Zhao and Qin, 2005; Farooq and Azam, 2006; Perica *et al.*, 2008). This result was similar to the report of Dionisio-Sese and Tobita (1998) which showed that the electrolyte leakage in salt-sensitive IR28 seedlings enhanced by 8.5 times and higher than that in the salt-tolerant Pokkali seedlings when exposed to 120 mM NaCl for 7 days.

This result showed that the increasing of the Na^+ increased Na^+:K^+ ratio in salt-tolerant HJ and salt-sensitive PT1 seedlings. Salt tolerance of the salt-tolerant HJ rice correlated to the lower Na^+:K^+ ratio (Table 12 and 13). Similarly, the result of the salt stress without osmotic control showed that the increasing of Na^+ led to the enhancement of Na^+:K^+ ratio which resulted in ion imbalance (Dionisio-Sese and Tobita, 1998; Yasar *et al*., 2006; da Silva *et al*., 2008; Zheng *et al*., 2008b). Moreover, the Na^+:K^+ ratio had been utilized to identify the salt tolerance ability in rice (Dionisio-Sese and Tobita, 1998; Nakamura *et al*., 2002; Zeng, 2005), maize (Çiçek and Çakirlar, 2002), sorghum (Netondo *et al*., 2004), green bean (Yasar *et al*., 2006), *Arbutus unedo* (Navarro *et al*., 2007), sunflower (Quintero *et al*., 2007), jojoba

(Roussos *et al*., 2007), umbu plant (da Silva *et al*., 2008) and wheat (Zheng *et al*., 2008a).

An increase in Na^+ content in salt-tolerant HJ and salt-sensitive PT1 seedlings was inversely related to the osmotic potential, especially leaf water potential (Figure 15). Salt accumulation reduced the osmotic potential in the soil solution, culture medium and plants. This reduction resulted in the water influx into the plants (Munns, 2002; Yokoi *et al.*, 2002; Ranathunge *et al*., 2003). Reduction of osmotic potential in many species such as maize (Çiçek and Çakirlar, 2002), barley (Garthwaite *et al*., 2005) and rice (Ahmad *et al*., 2007) was stimulated by salt stress. Furthermore, the reduction of osmotic potential depended on rice varieties and salt concentrations (Lefèvre *et al.*, 2001; Ueda *et al*., 2006). In this study, the osmotic potential in salt-sensitive PT1 leaves was more severely reduced than the salt-tolerant HJ leaves (Figure 15). It might be possible that the increasing of Na^+ in the cytosol of salt-sensitive PT1 leaves may accumulate more than in salt-tolerant HJ leaves. The Na^+ in salt-tolerant HJ leaves may be transported into vacuole which accumulated the higher concentration of Na^+ without interferring the cell osmotic potential. Kader and Lindberg (2005) showed that the Na^+ content in the cytosol of the salt-sensitive BRRI Dhan29 leaves was accumulated by 1.5 times more than that in the salt-tolerant Pokkali leaves when subjected to 100 mM NaCl.

Under salt stress with iso-osmotic condition, the increasing of Na^+ in salt-tolerant HJ and salt-sensitive PT1 seedlings induced reactive oxygen species (ROSs) generation (Vaidyanathan *et al.*, 2003) that disrupted the ultrastructure and chloroplast functions and degraded the photosynthetic pigments (Hernández *et al.*, 1995; Demiral and Türkan, 2005). In this study, the reduction of the photosynthetic pigment concentrations in salt-tolerant HJ and salt-sensitive PT1 leaves was similar to the result of the salt stress treatment without osmotic control in many species such as rice (Lefèvre *et al.*, 2001; Cha-um *et al.*, 2007b), wheat (Zheng *et al.*, 2008b) and maize (de Azevedo Neto *et al.*, 2004). Photosynthetic pigments are related to the light harvesting complexes and water oxidation in photosystem II (PSII) which can be evaluated by chlorophyll *a* fluorescence parameters. This parameter measurement is a

simple, rapid and sensitive procedure in the plant responses to abiotic stresses (Gray *et al*., 1997; Sudhir *et al*., 2005; Cha-um *et al*., 2009a). The chlorophyll *a* fluorescence reductions in salt-tolerant HJ and salt-sensitive PT1 leaves were related to the decreasing of photosynthetic pigment concentrations when Na^+ was increased (Figure 17-20).

The chlorophyll *a* fluorescence parameters in salt-stressed bean (Hernández *et al*., 1995; Parida and Das, 2005), tomato (Khavari-Nejad and Mostofi, 1998), winter wheat (Sairam and Srivastava, 2002; Zheng *et al*., 2008b), cotton (Meloni *et al*., 2003), sunflower (Santos, 2004), rice (Cha-um *et al*., 2007b; Cha-um *et al*., 2009b) and castor bean (Pinheiro *et al*., 2008), red raspberry (Neocleous and Vasilakakis, 2007) and citrus (López-Climent *et al*., 2008) were decreased under salt stress. The photosynthetic pigment degradations were the main cause of the efficiency reduction of the photochemistry in photosystemII (PSII).

The photosynthetic pigment, especially chlorophyll which is a major pigment that plays an important role on the light absorption and electron transportation from photosystemII (PSII) to photosystemI (PSI). Cha-um *et al.* (2009c) showed that the total chlorophyll concentration in salt-tolerant HJ and salt-sensitive KDML105 rice was degraded by 29% and 35%, respectively when subjected to 342 mM NaCl for 7 days. Furthermore, the reduction of photosynthetic performances in light reaction of PSII indicated that the photosynthetic apparatus such as thylakoid membrane was damaged by toxic ion such as Na^+ which inhibited the electron transport site and reduced the energy transfer from light harvesting antenna to PSII (Sudhir *et al*., 2005).

Boriboonkaset (2007) demonstrated that chloroplast ultrastructure in salt-sensitive IR29 and PT1 rice was severely damaged more than that in salt-tolerant Pokkali and HJ rice when exposed to 342 mM NaCl for 4 days. The chloroplast ultrastructure of salt-stressed seedlings was damaged through the swelling of thylakoid and granum. In addition, the reduction of the chlorophyll *a* fluorescence in salt-tolerant HJ leaves was more than those in salt-sensitive PT1 leaves under iso-osmotic condition (Table 15). Furthermore, an increase in Na^+ caused the reduction of

the carbon assimilation which increased internal CO_2 concentration and reduced the stomatal conductance (Maxwell and Johnson, 2000). The photosynthetic rate, stomatal conductance and transpiration rate in the salt-tolerant IR651 rice were maintained by 50%, 100% and 50%, respectively when exposed to salt stress (EC = 6 mS cm^{-1}) for 35 days and these activities were higher than those in salt-sensitive IR29 rice (Moradi and Ismail, 2007). Alternatively, the reduction of stomatal conductance and transpiration rate may help the reduction of Na^+ uptake which occurred by passive transportation through the transpiration stream (Yeo and Flowers, 1986).

Reduction of the photosynthetic pigment concentrations and chlorophyll *a* fluorescence parameters indicated the loss of photosynthetic performances in gaining the energy from light in the light reaction of photosynthesis, resulting in growth reduction in many species such as pea (Hernández *et al*., 1995), rice (Dionisio-Sese and Tobita, 1998; Nguyen *et al*., 2005; Cha-um *et al*., 2007a; Morsy *et al*., 2007), guava (Ali-Dinar *et al*., 1999), *Rumex* (Chen *et al*., 2004), sunflower (Santos, 2004), red raspberry (Neocleous and Vasilakakis, 2007), safflower (Siddiqi *et al*., 2007), cotton (Chachar *et al*., 2008) and citrus (López-Climent *et al*., 2008). The increasing of Na^+ competed with the essential nutrient uptake, especially K^+ which played an important role on cell turgor and cell expansion and resulted in plant growth reduction (Taffouo *et al.*, 2010).

In this study, the accumulation of sugars in salt-tolerant HJ and salt-sensitive PT1 roots and leaves under iso-osmotic condition was increased comparing to the control (0 mM NaCl + 548.9 mM mannitol). However, the sugar accumulation in salt-sensitive PT1 roots and leaves was higher than that in salt-tolerant HJ roots and leaves (Table 17 and 18). The result was similar to the study without osmotic control in many species such as barley (Ahmad *et al*., 2006), tomato (Khelil *et al*., 2007; Chookhampaeng *et al*., 2008), sorghum (Almodares *et al*., 2008a; Almodares *et al*., 2008b), rice (Cha-um *et al*., 2009b) and eggplant (Abbas *et al*., 2010).

Sugar acted as osmotic adjustment, membrane stabilization and reactive oxygen species scavengers when exposed to abiotic stresses such as drought, salt, extreme temperature and light intensity (Bohnert and Jensen, 1996). Under salt stress, the Na^+ accumulation reduced the osmotic potential. Plants have to adapt themselves by sugar accumulation to maintain water balance. The salt-sensitive PT1 roots and leaves accumulated glucose and fructose higher than the salt-tolerant HJ roots and leaves. Although, the Na^+ and K^+ accumulation in salt-tolerant HJ rice were higher than that in salt-sensitive PT1 rice, it was found that the glucose and fructose accumulation in salt-tolerant HJ rice was lower than that in salt-sensitive PT1 rice (Table 17 and 18). It was possible that the salt-tolerant HJ rice utilized K^+ for osmotic adjustment. In the same time, the salt-sensitive PT1 rice required sugar to adjust the osmotic pressure.

This result was similar to the report of Pattanagul and Thitisaksakul (2008) which showed that one fold of the total soluble sugar in salt-sensitive KDML105 rice was induced comparing to the salt-tolerant Pokkali and Luang Anan rice when subjected to 150 mM NaCl without osmotic control. In contrast, the total soluble sugar contents in salt-tolerant HJ roots and leaves were accumulated by 0.5 and 4.6 folds, respectively and higher than that in salt-sensitive PT1roots and leaves when exposed to 342 mM NaCl under non-osmotic control (Cha-um *et al*., 2009b).

Furthermore, there was other compatible solute accumulations such as glycinebetaine (Meloni *et al.*, 2004), proline and carbohydrate (Jouve *et al.*, 2004; Tatar *et al.*, 2010) which involved in the osmotic control under salt stress. Makela *et al.* (2000) and Cha-um *et al.* (2007a) reported that the photosynthetic pigment degradation was alleviated by glycinebetaine which functioned to protect the photosynthetic machinery by stabilizing the ultrastructure of the chloroplast.

3. Salt tolerance ability improvement by exogenous potassium nitrate (KNO_3) and sucrose applications

3.1. Effect of exogenous potassium nitrate (KNO_3) application on salt tolerance ability in salt-sensitive rice

The result in the previous experiment showed that the increasing of NaCl concentration induced Na^+ accumulation whereas the accumulation of K^+ was decreased in PT1 salt-sensitive rice. In this experiment, the exogenous K^+ (KNO_3) application enhanced salt tolerance ability of the salt-sensitive PT1 rice.

The increasing of the NaCl concentration in the culture medium reduced the osmotic potential in salt-tolerant HJ and salt-sensitive PT1 seedlings (Table 19). Similar results to this study were found in many species such as tomato (Romero-Aranda *et al.,* 2001), maize (Çiçek and Çakirlar, 2002), sorghum (Netondo *et al.,* 2004), safflower (Siddiqi *et al.,* 2007), rice (Nakamura *et al.,* 2002; Cha-um *et al.,* 2009b), winter wheat (Zheng *et al.,* 2008a) and barley (Degl'Innocenti *et al.,* 2008). Cha-um *et al.* (2007b) reported that the osmotic potential in salt-tolerant HJ and salt-sensitive PT1 rice exposed to 427 mM NaCl for 5 days were reduced by 91% and 98% (roots) and 177% and 203% (leaves), respectively when compared to the control. However, the Na^+ is a toxic ion which disturbs ion homeostasis and injures plant organs (Hasegawa *et al.,* 2000; Tester and Davenport, 2003; Ashraf, 2004; Khelil *et al.,* 2007). The K^+ which competes to Na^+ could reduce damage caused by Na^+.

In this experiment, the result showed that the osmotic potential in salt-sensitive PT1 seedlings cultured on the culture medium supplemented with KNO_3 application and subsequently exposed to 342 mM NaCl were reduced (Table 19). The K^+ uptake and accumulation were enhanced by exogenous potassium application in the culture medium (Chartzoulakis *et al.,* 2006; Khayyat *et al.,* 2009) and nutrient solution (Kaya and Higgs, 2003; Kaya *et al.,* 2003; Akinci and Simsek, 2004; Umar, 2006; Kaya *et al.,* 2007; Collins *et al.,* 2008; Tabatabaei and Fakhrzad, 2008; Zheng *et al.,* 2008a; Akram *et al.,* 2009; Khayyat *et al.,* 2009). An increase in K^+ in salt-

sensitive PT1 seedlings led to the adjustment of osmotic potential and preserved water balance under salt stress. Moreover, the water content in salt-sensitive winter wheat (JN17) was maintained by application of 11, 16 and 21 mM potassium nitrate (KNO_3) (Zheng *et al.,* 2008a).

Photosynthetic pigment concentrations and chlorophyll *a* fluorescence parameters in salt-tolerant HJ and salt-sensitive PT1 seedlings were reduced by salt stress (Table 20). This result was similar to the result in many salt-stressed species such as rice (Nakamura *et al.,* 2002; Cha-um *et al.,* 2004; Cha-um *et al.,* 2007b), pepper and cucumber (Kaya *et al.,* 2003), *Eucalyptus*, Rain tree and Thai neem (Cha-um and Kirdmanee, 2008), olive (Ben Ahmed *et al.,* 2008) and sunflower (Noreen and Ashraf, 2008). This result was similar to the result of the salt-stressed species such as *Rumex* (Chen *et al.,* 2004), sunflower (Santos, 2004), citrus (López-Climent *et al.,* 2008) and rice (Cha-um *et al.,* 2009a). An increase in salinity enhanced toxic ions particularly Na^+ and led to the disorganization and damaging of cell (Kader and Lindberg, 2005). In this study, the photosynthetic pigment degradations in salt-sensitive PT1 seedlings were alleviated by applying 11.8 and 14.1 mM KNO_3 in the culture medium (Table 20).

The exogenous KNO_3 application resulted in maintaining the photosynthetic efficiencies (F_v/F_m, Φ_{PSII} and qP) (Table 21) and improved growth (cell turgor pressure and cell enlargement) (Table 22). The application of potassium nitrate (KNO_3) 11.8 and 14.1 mM KNO_3 protected the photosynthetic pigments from toxic ion (Na^+) and enhanced K^+ in the guard cells for maintaining stomatal conductance and CO_2 assimilation in the dark reaction of photosynthesis. The application of potassium nitrate (KNO_3) alleviated the photosynthetic pigment degradations in many species such as bell pepper (Kaya *et al.,* 2003), cucumber (Akinci and Simsek, 2004), *Lagenaria siceraria* (Ahmad and Jabeen, 2005), winter wheat (Zheng *et al.,* 2008a) and strawberry (Khayyat *et al.,* 2009).

Alternatively, the exogenous application of various potassium fertilizers such as potassium phosphate (KH_2PO_4) (Kaya *et al.,* 2003), potassium sulfate

(K_2SO_4) (Chartzoulakis *et al.*, 2006; Akram *et al.*, 2009; Khayyat *et al.*, 2009) and potassium hydroxide (KOH) (Akram *et al.*, 2007) maintained photosynthetic pigment concentrations which affected the growth promotion. Zheng *et al.* (2008a) reported that the high K^+ accumulation in salt-tolerant wheat (DK961) and salt-sensitive wheat (JN17) varieties maintained photosynthetic abilities and resulted in maintaining growths under salt stress.

3.2. Improvement of salt tolerance ability in salt-sensitive rice variety by exogenous sucrose application

From the experiment 2, the result showed that the increasing of NaCl concentration in the culture medium induced sugar accumulation, especially in salt-sensitive PT1 seedlings. In this experiment, the role of exogenous sugar application on salt tolerance ability of salt-sensitive seedlings was evaluated. This result showed that the sugar contents in salt-sensitive PT1 seedlings cultured on the culture medium supplemented with different sucrose concentrations were enhanced, especially glucose and fructose (Table 24 and 25). An increase in sugar contents involves in salt defensive mechanism (Kerepesi and Galiba, 2000; Prado *et al.*, 2000; Khelil *et al.*, 2007; Yin *et al.*, 2010).

An increase in sugar contents in this experiment was similar to the result of the increasing of the soluble sugars in cyanobacterium (*Microcoleus vaginatus* Gom.) (Chen *et al.*, 2003), barley (Tabaei-Aghdaei *et al.*, 2003) and wheat (Javed and Ikram, 2008) when they were cultured on the culture medium supplemented with exogenous sucrose. However, an increase in sucrose content in salt-sensitive PT1 seedlings was less than the glucose and fructose during salt stress (Table 24 and 25). The sucrose concentration in the culture medium is changed to the monosaccharide (glucose and fructose) by heat and pressure from the procedure to prepare the culture medium. Ball (1953) compared the sugar contents in the culture medium supplemented with 3% (w/v) sucrose which was sterilized by autoclaving or filtration. The sucrose was found in filtration sterilization, while sucrose and smaller proportion of glucose and levulose were found in autoclaving sterilization.

In this experiment, the enhancement of total soluble sugars in salt-sensitive PT1 seedlings cultured on the culture medium supplemented with different sucrose concentrations was related to the reduction of the root osmotic potential (Figure 31A). It is possible that the root is the first organ which is primarily affected by salt stress, thus the root tissue may adapt to salt stress by adjustment the water influx ability (Liu *et al.*, 2004; Ben Ahmed *et al.*, 2008; Hajlaoui *et al.*, 2010).

In contrast to root, the leaf osmotic potential in salt-sensitive PT1 seedlings cultured on the culture medium supplemented with different sucrose concentrations was maintained (Figure 31B). Glucose and fructose have less effect on the alteration of osmotic potential than sucrose. Thus, the increasing of glucose and fructose did not affect the changing of leaf osmotic potential (Table Figure). Kerepesi and Galiba, (2000) suggested that sugars played an important role on water replacement to the maintaining phospholipids in the liquid-crystalline phase structural changes in soluble proteins, especially chloroplast structure. Beside the role of sugar on osmotic control, Boriboonkaset (2007) found that the chloroplast structure was damaged by the increasing of salt stress without exogenous sugar application. Moreover, other possible roles of sugars may be as a readily available energy source (Ben Dkhil and Denden, 2010).

The exogenouse sucrose application enhanced the photosynthetic pigment concentrations and water oxidation in photosystemII (PSII), especially the maximum quantum yield of PSII (F_v/F_m) (Table 27 and 28). In this study, the sugar accumulation in salt-sensitive PT1 seedlings improved water use ability which led to the maintaining water uptake capability, photosynthetic pigment stabilizations, water oxidation in PSII, electron transportation capacity and growth promotion (Table 29).

The quantum efficiency of PSII (Φ_{PSII}) and photochemical quenching (qP) decreased in the salt-sensitive PT1 seedlings cultured on the culture medium with sucrose application (Table 28). The increasing of sugar contents in leaf tissues may cause a feed-back inhibition on carbon metabolism. This result was similar to the report of Mosaleeyanon *et al*. (2004) who reported that the photosynthetic efficiencies

of rain tree was reduced when cultured on the culture medium supplemented with 29, 58 and 88 mM sucrose. Alternatively, the sugars are also the precursors of antioxidant compounds for protecting against the oxidative stress which causes damage of photosynthetic performances. This resulted in maintaining the PSII and photosynthetic pigment concentrations of *Arabidopsis* seedlings applied sucrose during exposing to atrazine (Sulmon *et al.*, 2004).

In conclusion**,** there was no difference between Na^+ effect on physiological responses of salt-tolerant and salt-sensitive rice grown under osmotic control and without osmotic control. Under osmotic control, the salt-tolerant rice accumulated high Na^+ and K^+, while the salt-sensitive rice accumulated high Na^+ and low K^+. The reduction of K^+ in salt-sensitive rice was also reported by Lefèvre *et al.* (2001), Nakamura *et al.* (2002), Cha-um *et al.* (2007b). The increasing of K^+ led to the increasing of salt tolerance ability in salt-tolerant rice. In salt-sensitive rice, the exogenous KNO_3 application increased K^+ uptake and accumulation (Chartzoulakis *et al.*, 2006; Khayyat *et al.*, 2009). The increasing of K^+ in salt-sensitive seedlings led to the adjustment of osmotic potential and preserved water balance under salt stress.

Sugar acted as osmotic adjustment, membrane stabilization and reactive oxygen species scavengers when exposed to abiotic stresses such as drought, salt, extreme temperature and light intensity (Bohnert and Jensen, 1996). Raffinose and stachyose which are members of the raffinose family oligosaccharide (RFOs) accumulated in plant exposed to cold or dessication stress (Morsy *et al.*, 2007). However, in this study the raffinose and stachyose contents were unchanged when subjected to salt stress. Alternatively, the glucose, fructose and sucrose were clearly increased in salt-sensitive rice. Pattanagul and Thitisaksakul (2008) showed that one fold of the total soluble sugar in salt-sensitive KDML105 rice was induced comparing to the salt-tolerant Pokkali and Luang Anan rice. As salt concentration increased, sucrose decreased (Table 17 and 18) while glucose and fructose increased. It seems that sucrose was cleaved into two monosaccharides (Scholes *et al*., 1996). Glucose and fructose are easily utilized by plant.

CONCLUSIONS

With and without iso-osmotic control, the physiological responses i.e. osmotic potential, photosynthetic pigment concentrations, Chl *a* fluorescence parameters and growth in salt-tolerant (HJ) and salt-sensitive (PT1) seedlings were significantly reduced with the increasing of NaCl concentrations and salt exposure times. This indicated the ionic effect of NaCl. Moreover, the physiological responses in PT1 seedlings were severely reduced more than that the HJ seedlings.

The increasing of the K^+ accumulation in HJ salt-stressed seedlings played an important role on the reduction of Na^+:K^+ ratio and root electrolyte leakage while photosynthetic pigments and Chl *a* fluorescence parameters were reduced. In contrast, the exogenous KNO_3 application increased photosynthetic pigments and Chl *a* fluorescence parameters which resulted in the increasing of fresh and dry weights.

Under iso-osmotic condition, the glucose, fructose, sucrose, raffinose and stachyose in PT1 seedlings were significantly accumulated when NaCl concentration was increased. The sugar accumulation in PT1 salt-stressed seedlings played a crucial role on the reduction of leaf osmotic potential whereas the exogenous sucrose application significantly increased leaf osmotic potential, glucose and fructose contents and fresh and dry weights.

These results could be concluded that there was no difference of the physiological responses in salt-stressed seedlings between with and without osmotic controls. Moreover, the exogenous KNO_3 and sucrose applications could improve salt tolerance ability of rice.

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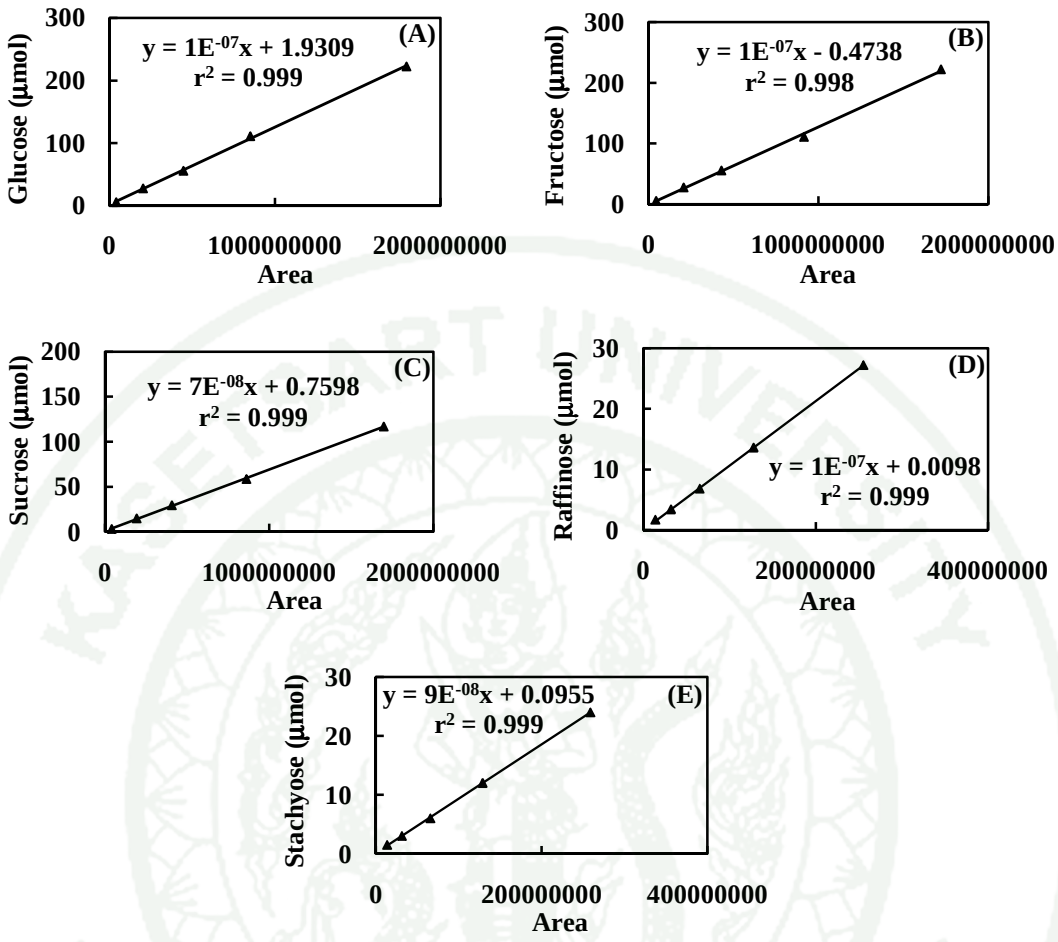
APPENDIX

Appendix Figure 1 Rice seedlings were cultured on MS semi-solid medium (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose (A) and fourteen-day-old rice seedlings were aseptically transferred to 60 mL MS sugar-free liquid medium by using vermiculite as supporting material (B) 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 for 16 h d^{-1} photoperiod.



Appendix Figure 2 Sugar content was calculated according to equation in the standard curve of glucose (A), fructose (B), sucrose (C), raffinose (D) and stachyose (E), respectively.

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PUBLICATION

1) Siringam, K., N. Juntawong, S. Cha-um and C. Kirdmanee. 2009. Relationships between sodium ion accumulation and physiological characteristics in rice (*Oryza sativa* L. spp. *indica*) seedlings grown under iso-osmotic salinity stress. **Pak. J. Bot.** 41: 1837-1850.
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1) Siringam K., N. Juntawong, S. Cha-um and C. Kirdmanee. 2007. **Na^+ accumulation, pigment concentrations, water oxidation and growth abilities of salt-tolerant and salt-sensitive cultivars of indica rice (*Oryza sativa* L. ssp. *indica*) in responses to salt stress.** Poster Presented at The 2nd International Conference on Rice for the Future (BioAsia 2007).
2) Siringam K., N. Juntawong, S. Cha-um and C. Kirdmanee. 2009. **Ion contents, membrane injury, raffinose and stachyose and growth characters of indica rice (*Oryza sativa* L. ssp. *indica*) roots in responses to iso-osmotic salt stresses.** Poster Presented at Agricultural Biotechnology for Better Living and a Clean Environment (ABIC 2009).