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Physiological Responses of Thai neem (*Azadirachta siamensis* Val.) to Salt Stress for Salt-tolerance Screening Program

Suriyan Cha-um[a,b*], Kriengkrai Mosaleeyanon[a], Kanyaratt Supaibulwatana[b], Chalermpol Kirdmanee[a]

[a] National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Thailand Science Park, Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand.

[b] Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand.

[*] Corresponding author, E-mail: suriyanc@biotec.or.th

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ABSTRACT: The objective of this investigation is to study the physiological responses of Thai neem (*Azadirachta siamensis* Val.) to salt stress for salt tolerance screening and for establishing a salt tolerance index (*STI*). The physiological responses of photoautotrophic seedlings under salt stress condition, especially, net photosynthetic rate (*NPR*) were evaluated as *STI*. Fifty-six-day-old photoautotrophic seedlings were treated *in-vitro* with 0 (control), 0.34, 0.68 and 1.02 M NaCl solution contained in the culture media. Physiological characteristics, namely water use efficiency [water potential (Ψ_w), solution potential (Ψ_s) and pressure potential (Ψ_p)], photosynthetic ability [chlorophyll a (Chl_a), chlorophyll b (Chl_b) and *NPR*] and growth efficiency [fresh weight, dry weight, leaf area and leaf area ratio (*LAR*)], were measured after treatment for 28 days. The increase of NaCl concentrations in the culture media decreased Ψ_w and Ψ_s, but, increased Ψ_p in the leaf tissues. Chlorophyll *a* and *b* concentrations of seedlings cultured under salt stress condition (0.34-1.02 M NaCl) were significantly lower than those of seedlings cultured under control conditions (0 M NaCl). The chlorophyll degradation is positively related to *NPR* reduction (r = 0.98), resulting in the low survival percentage (r = 0.95). Thus, the lower water use efficiency and lower photosynthetic ability of seedlings cultured under salt stress condition led to growth reduction as shown by fresh weight, dry weight, leaf area and *LAR*. The photoautotrophic seedlings of Thai neem were approximately 50% inhibited at 0.34 M NaCl concentration. So this concentration, which was the 50% lethal dose (LD_{50}), was applied for the salt-tolerant screening program. Six hundred and sixty clones of Thai neem were collected, disinfected and were then germinated *in-vitro* on Phytagel®-solidified MS sugar-free media (photoautotrophic condition). The culture media of those clones were adjusted the NaCl concentration to LD_{50}. Nine clones of Thai neem still survived under salt stress condition (0.34 M NaCl) for 28 days and were classified as the salt tolerant clones. Salt tolerant ability was evaluated by the *NPR* reduction under salt stress condition. Salt tolerant index (*STI* = *NPR* at 1.02 M NaCl / *NPR* at 0 M NaCl) was investigated by the ratio of *NPR* under salt stress (1.02 M NaCl) to control (0 M NaCl) conditions. Nine salt tolerant clones and one salt sensitive clone were assayed for salt tolerant ability using *STI*. The *STI* of the salt tolerant clones were higher than those of the salt sensitive clone by a factor of 1.3-1.5 times. The *STI* is a rapid technique to assay the salt tolerant ability of many clones of Thai neem.

KEYWORDS: growth efficiency, net photosynthetic rate, salt tolerant index, sodium chloride, water use efficiency.

INTRODUCTION

Saline soil area is a serious problem in many areas of the world, distributed over some 4×10^8-9×10^8 hectares, which is three times larger than agricultural area. It can be increased due to use of the low quality irrigation water and non-sustainable agricultural processes. In saline soil, a plant is exposed to increasing levels of both water and osmotic stress, because the metric potential and the osmotic potential decrease simultaneously with decreasing soil moisture content.[1] Salt ions associated with saline soil attract water, raising the suction (osmotic potential) of water head in the soil, thereby reducing the water potential of plant. The water potential limits efficiency of water use, because plants need to develop more negative water potential to maintain a downhill gradient of water potential between the soil and the plant.[2] Moreover, salts in saline soil directly damage the plant cells, tissues and organelles. Salt sensitive species are susceptible to injury

by salt stress, resulting in reduction in the leaf expansion and chlorophyll synthesis prior to plant mortality. In addition, many plant species cultivated under salt stress condition show the damage symptoms such as wilting, chlorosis, necrosis, burn and senescence, causing growth reduction and low productivity.[3]

Reforestation is one of the most practical and effective strategies to solve the saline soil problems. Trees cause remediation of saline soil area by absorbing precipitation, using underground water and protecting water-rise.[4] However, the lack of salt-tolerant species is the most important obstacle to solve this problem. Screening for salt tolerance has been investigated in many crop species such as rice,[5] barley,[6] tomato,[7] tobacco[8] and mulberry.[9] Most previous reports generally used the photomixotrophic system (sugar as a carbon source). This system generally generates abnormal plantlets with the morphological disorders [hyperhydricity of leaves and shoots, curled leaves, poor leaf expansion, incomplete rooting and poor secondary root], anatomical disorders [low stomata density, poor spongy and palisade tissues, inferior vascular connection between shoot and root] and physiological disorders [low stomatal conductance, chlorophyll concentration, net photosynthetic rate and high transpiration rate]. These characteristics are reduced by using a photoautotrophic system (CO_2 as a carbon source).[10] This investigation herein has used a photoautotrophic system as a prototype for the realistic phenotypic responses to salt stress for salt tolerant screening. Thai neem (*Azadirachta simensis* Val.) was chosen as a model of the woody species, as it widely distributed in several parts of Thailand. It can grow in nutrient deficiency, drought and saline soil conditions.[11] The objective of this investigation is to study the physiological responses, water use efficiency, photosynthetic ability and growth efficiency, of Thai neem to salt stress for salt tolerance screening and for establishing a salt tolerance index (*STI*).

Materials and Methods

Physiological Responses to Salt Stress

Plant materials and experimental design

Seeds of Thai neem (*Azadirachta siamensis* Val.) were collected from Chainat province, disinfected by 20% Clorox® (0.25% (w/v) sodium hypochlorite, The Clorox Co., USA) for 30 min, subsequently rinsed thrice with sterile distilled water. The pericarp of the disinfected seeds was removed and then embryo composing of endosperm was germinated on 0.25% Phytagel®-solidified MS sugar-free media (photoautotrophic condition). Air exchange rate was adjusted to 2.3±0.2 h^{-1} by punching a plastic cap (∅ 1 cm) and replaced with a gas-permeable microporous polypropylene film (0.22 μm pore size, Nihon Millipore Ltd., Japan) over a hole. Glass vessels (250 ml) containing embryo were incubated at 25±2°C ambient temperature, 60±5% relative humidity (RH), 60±5 μmol $m^{-2} s^{-1}$ photosynthetic photon flux density (PPF). The PPF was provided by fluorescence lamps (TDL 36 W/84 Cool White 3350 Im, Philips, Thailand) with 16 hd^{-1} photoperiod for 56 days. The culture media of seedlings were adjusted to contain 0 (control), 0.34, 0.68 and 1.02 M NaCl. Glass vessels containing seedlings were transferred to a Plant Growth Incubator (EYELA, Model EYELATRON FLI-301LH, Japan) under temperature shift (25±2°C darkperiod /28±2°C photoperiod), 60±5% RH, 100±5 μmol $m^{-2} s^{-1}$ PPF provided by fluorescence lamps with 16 hd^{-1} photoperiod. Survival percentage, physiological characteristics and growth efficiency were recorded at day 28. The experiment was designed as completely randomized design (CRD) with ten replicates and four seedlings per replicates. The mean values of each treatment were compared by the least significant difference (LSD) and analyzed by using SPSS software (SPSS for Windows, SPSS Inc., USA). The correlation between chlorophyll concentration and net photosynthetic rate as well as net photosynthetic rate and survival percentage were evaluated by Pearson's correlation coefficient.

Physiological characteristics

Water use efficiency was analyzed according to Warne *et al* method.[12] Leaves from the second and the third node of the top were cut and rapidly placed in Tru Psi chambers (Tru Psi, Model SC10X, Decagon, USA) to measure the leaf water potentials (Ψ_w). Equilibration times ranged from 0.5 h for 0 M NaCl (control) to 1.5 h for plant grown at 0.34, 0.68 and 1.02 M NaCl. In addition, the fresh leaves were placed in cryogenic-tube and were then frozen in liquid nitrogen for 2-3 h. The frozen leaves were thawed at 40 °C in water bath and rapidly put in the Tru Psi chambers to measure the leaf solution potential (Ψ_s). Leaf pressure potential (Ψ_p) was calculated using the equation:

$$\Psi_p = \Psi_w - \Psi_s$$

Chlorophyll concentration was analyzed following the method of Shabala *et al*.[13] One hundred milligrams of the second and third leaves from the top were cut, placed into 25 ml glass vial (Opticlear® KIMBLE, USA) containing with 10 ml 99.5% (v/v) acetone and then blended with a homogenizer (T-25 Basic ULTRA-TURRAX®, IKA, Japan). The glass vials were capped sealed with Parafilm® (Laboratory Film 4 in. × 125ft. Roll, American National Can™ Menasha, WI 54952, Chicago, USA) to prevent evaporation and then stored at 4°C for 2 days. Chlorophyll *a* (Chl_a) and chlorophyll *b* (Chl_b) concentrations were measured using an UV-

visible Spectrophotometer (DR/4000, Model 48000, HACH Company, USA) at 662 nm (D_{662}) and 644 nm (D_{644}), respectively. A solution of 95% acetone (v/v) was used as a blank. The concentrations of Chl_a and Chl_b (μg g^{-1} FW) in leaf tissues were calculated using the following equations:

$$Chl_a = 9.784D_{662}\text{-}0.99D_{644}$$
$$Chl_b = 21.426D_{644}\text{-}4.65D_{662}$$

$$\text{Total Chlorophyll} = Chl_a + Chl_b$$

where D_i is an optical density at the wavelength i.

Net photosynthetic rate (*NPR*) was calculated by the different concentrations of CO_2 inside and outside of glass vessel containing with seedlings. The inside and outside CO_2 concentrations of the glass vessel (C_{in} and C_{out}) at steady state were measured by Gas Chromatography (GC; Model GC-17A, Shimadzu Co. Ltd., Japan). The GC capillary column and detector were GS-Q (J&W Scientific®, Germany) and thermal conductivity detector (TCD), respectively. The detector and injector were set the temperature at 250 °C. The temperature program of column was set at 30 °C for 1 min at initial state, and increased to 100 °C at a rate of 20 °C per min and kept for 1 min. The *in vitro* net photosynthetic rate was calculated according to Fujiwara et al[14], as follow:

$$NPR = \frac{K.E.V\,(C_{in} - C_{out})}{L}$$

where K is the conversion factor converting CO_2 amount from volume to mole (-40.9 mol m^{-3} at 28 °C); E, the number of air exchanges per hour of the vessel (2.32 h^{-1}); V, the air volume of the vessel (0.0025 m^3); C_{in} and C_{out}, inside and outside CO_2 concentrations (μmol mol^{-1}) of the glass vessel at steady state condition, respectively; and L, the leaf area (m^2).

Growth efficiency

Fresh weight, dry weight, and leaf area of seedlings were taken as a growth efficiency using the method described by Lutts *et al.*[5] Dry weight was measured after drying the tissues at 110 °C in a hot-air oven (Memmert, Model 500, Germany) for 2 days. The leaf area was measured by using Leaf Area Meter DT-scan (Delta-Scan Version 2.03, Delta-T Devices, Ltd., England). Leaf area ratio (*LAR*) was calculated using the equation:

$$LAR = \frac{\text{Leaf area}}{\text{Total dry weight of plantlets}}$$

Screening for salt tolerance

Plant materials and salt tolerant screening

Seeds of 660 Thai neem clones were collected from north, central and northeastern parts of Thailand and disinfected with 20% Clorox® (0.25% (w/v) sodium hypochlorite) for 30 min, subsequently rinsed thrice with sterile distilled water. The pericarp of the disinfected seeds was removed and the embryo composing of endosperm was germinated on 0.25% Phytagel®-solidified MS sugar-free medium (photoautotrophic condition). Air exchange rate was adjusted to 2.3±0.2 h^{-1} by punching a plastic cap (∅ 1 cm) and replaced with a gas-permeable microporous polypropylene films (0.22 μm pore size) over a hole. Glass vessel containing embryo was incubated at 25±2°C ambient temperature, 60±5% RH, 60±5 μmol m^{-2} s^{-1} PPF provided by fluorescence lamps with 16 hd^{-1} photoperiod for 56 days. The seedlings were treated with 50% lethal dose of NaCl concentration (0.34 M NaCl) in the culture media for 28 days.

Establishment of salt tolerant index

Plant materials and experimental design

Nine salt tolerant clones and one salt sensitive clone from screening program were propagated according to Joshi and Thengane[15] method. The nodes of Thai neem seedlings were used as the plant materials for proliferation on the MS medium supplemented with 8.9 mM N^6-benzyladenine (BA) for 42 days, and then shoots were elongated after transferring to MS medium for 14 days. The multiple shoots (length 2.50±0.25 cm) initiated roots on MS medium supplemented with 5 mM 1*H*-indole-3-butyric acid (IBA) for 14 days and were then transferred to glass vessels (250 ml) containing 30 ml MS sugar-free medium using 2 g vermiculite as supporting material. Air exchange rate in the culture vessel was adjusted to 2.3±0.2 h^{-1} by punching a plastic cap (∅ 1 cm) and replacing it with a gas-permeable microporous polypropylene film (0.22 μm pore size). Glass vessels containing plantlets were incubated under temperature shift 28±2°C / 25±2°C (16 h photoperiod/8 h darkperiod), 60±5% RH, 100±5 μmol m^{-2} s^{-1} PPF and CO_2-enrichment (1,000±100 μmol mol^{-1}) conditions in a Plant Growth Incubator (EYELA, Model EYELATRON FLI-301LH, Japan) for 56 days. The culture media of plantlets were adjusted to 0, 0.17, 0.34, 0.68 and 1.02 M NaCl. The *NPR* of each treatment was measured as in the previous experiment. The experiment was designed as CRD with ten replications and four seedlings per replicate. The mean values of each treatment were compared by LSD and analyzed by SPSS software.

$$STI = \frac{NPR \text{ at } 1.02 \text{ M NaCl}}{NPR \text{ at } 0 \text{ M NaCl}}$$

The salt tolerant and salt sensitive plantlets were used for calculation of salt tolerance index (*STI*) in a salt-tolerant ability assay by the *NPR* reduction under salt stress condition. The ratio of *NPR* values of plantlets

cultured on MS medium supplemented with 1.02 M NaCl (salt stress) to 0 M NaCl (control) were taken as *STI*. The *STI* was calculated for each clone using the following equation:

Results and Discussion

Physiological Responses to Salt Stress

The water use efficiency, water potential (Ψ_w), and solute potential (Ψ_s), was measured as an osmotic adjustment of leaf tissue. The Ψ_w and Ψ_s of leaf decreased significantly with increasing NaCl concentrations in the culture media. High salt concentration in the culture media strongly decreased Ψ_w and Ψ_s, as well as increasing pressure potential (Ψ_p) of leaf. Ψ_w and Ψ_s decreased to the lowest level at 1.02 M NaCl, while the Ψ_p increased to the highest level (Fig 1). Chlorophyll *a* (Chl_a) and chlorophyll *b* (Chl_b) concentrations of the Thai neem seedlings decreased strongly with increasing NaCl concentration (Fig 2). The Chl_a and Chl_b of seedlings cultured under 1.02 M NaCl conditions reduced significantly 9- and 6-folds, respectively, compared to control seedlings. The degradation of chlorophyll concentrations related positively to *NPR* (r = 0.98) of Thai neem seedlings (Fig 3). Subsequently, the *NPR* reduction also correlated significantly with survival percentage (r = 0.95, Fig 4). The reduction on *NPR* of seedlings cultured under salt stress conditions affected directly low growth efficiency, leading to the low survival percentage. The growth efficiency of the seedlings decreased significantly with increasing NaCl concentration in the culture media. Fresh weight, dry weight, leaf area and *LAR* of seedlings treated with 1.02 M NaCl decreased strongly 1.23-, 1.52-, 1.90- and 1.26-times, respectively compared to control seedlings (Table 1). Leaves of salt stressed seedlings normally expressed damage symptoms such as chlorosis with patches of necrosis, leaf burn, and senescence as well

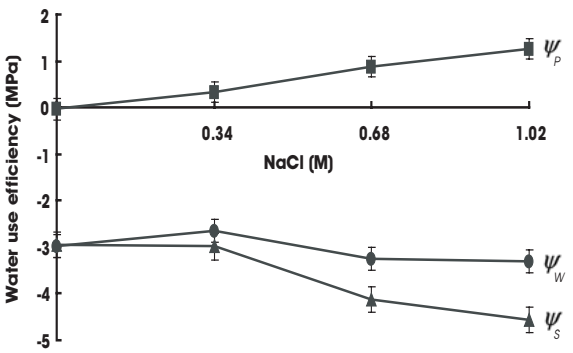

Fig 1. Water use efficiency of Thai neem seedlings cultured *in vitro* on Murashige and Skoog medium supplemented with 0, 0.34, 0.68 and 1.02 mM NaCl concentrations for 28 days, showing leaf water potential (Ψ_w), solution potential (Ψ_s), and pressure potential (Ψ_p). Error bars represent ± SE.

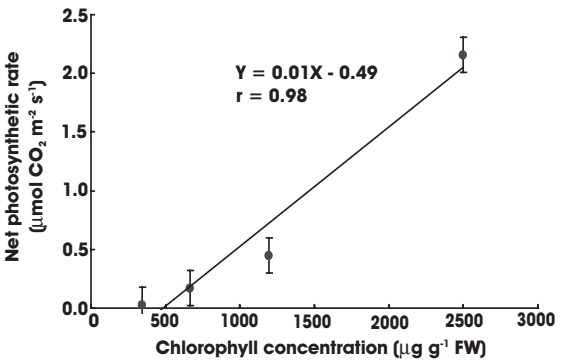

Fig 3. Correlation between total chlorophyll concentration and net photosynthetic rate of Thai neem seedlings cultured *in vitro* on Murashige and Skoog medium supplemented with 0, 0.34, 0.68 and 1.02 mM NaCl concentrations for 28 days. Error bars represent ± SE.

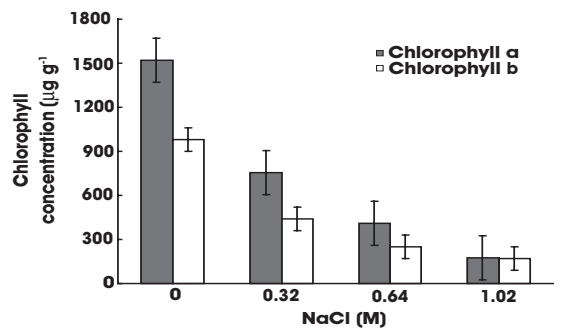

Fig 2. Chlorophyll *a* and chlorophyll *b* concentrations (µg/g FW) in the leaf tissues of Thai neem seedlings cultured *in vitro* on Murashige and Skoog medium supplemented with 0, 0.34, 0.68 and 1.02 mM NaCl concentrations for 28 days. Error bars represent ±SE.

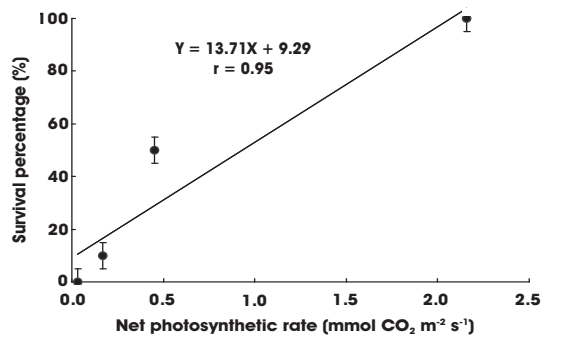

Fig 4. Correlation between net photosynthetic rate and survival percentage of Thai neem seedlings cultured *in vitro* on Murashige and Skoog medium supplemented with 0, 0.34, 0.68 and 1.02 mM NaCl concentrations for 28 days. Error bars represent ± SE.

Table 1. Growth efficiency, as determined by fresh weight,dry weight, leaf area, and leaf area ratio (*LAR*), of Thai neem seedlings cultured *in vitro* on Murashige and Skoog medium supplemented with 0, 0.34, 0.68 and 1.02 mM NaCl concentrations for 28 days.

NaCl (M) (mg)	Fresh weight (mg)	Dry weight	Leaf area (mm^2)	*LAR* ($mm^2\ mg^{-1}$)
0.00	852	120a	1341a	11.2a
0.34	866	116a	1210a	10.4a
0.68	724	112a	832b	7.4b
1.02	691	79b	706b	8.9b
LSD-test	NS	*	**	**

Different letters in each column show significant difference at *P*≤*0.01* and *P*≤*0.05* using the Least Significant Different test (LSD test).
NS Not significant

as leaf area reduction. High salt treatment (0.68-1.02 M NaCl) strongly reduced leaf expansion as defined by leaf area and *LAR*. Young tissue of leaves, shoots, and roots exhibited greater damage symptoms under salt stress condition than those mature parts.

Salt ions associated with saline soil attract water, raising the suction (osmotic potential) of the water head in the soil, thereby reducing the water potential of the plant. The root zone of plants growing under salt stress condition is the first attachment before defense responsive mechanisms. The limitation of available water in the soil directly affects plant-water relationship, shown as low water potential (Ψ_w), low solution potential (Ψ_s) but high pressure potential (Ψ_p). The Ψ_w of leaves mainly limits the efficiency of water use, because the leaves need to develop more negative water potential to maintain a downhill gradient of water potential between the soil and the leaves.[2] Moreover, salts are harmful to most plant organelles, cells, tissues and organs because they induce negative water potential in the soil, disturb cell membrane permeability, inhibit nutrient uptake and induce anoxia or oxidative stress.[4,16] The biochemical and physiological responses in term of the photosynthetic capability of the plant under salt stress condition have been previously reported. Firstly, the degradation of photoreceptor pigments, such as chlorophyll *a*, chlorophyll *b* and carotenoid is a simple method for analyzing plant responses under salt stress condition at the light reaction.[17] Secondly, the low efficiency of water use under salt stress condition directly affects the reduction in stomatal conductance in the dark reaction. The stomatal conductance is a critical point for protecting against water loss and altering the CO_2 fixation rate during photosynthesis.[2] The reduction in CO_2 concentration in the leaf tissues of salt stressed plants consistently result in the low photosynthetic rate, as well as the reduction of Rubisco enzyme content and activity.[18] These physiological responses mainly cause reduction in sugar and starch biosynthesis, which in turns decrease biomass production. Salt sensitive species are susceptible to injury by salt stress, resulting in the reduction in the leaf expansion and chlorophyll synthesis, prior to plant mortality. Moreover, the plants cultivated under salt stress condition show damage symptoms such as wilting, chlorosis, necrosis, burn and senescence, causing low growth and low productivity in many crop species.[3]

Salt tolerance screening and salt-tolerant ability assay

The survival percentage of Thai neem seedlings decreased significantly with increasing NaCl concentration in the culture medium. The NaCl concentration of 0.34 M was the 50% lethal dose (LD_{50}) of Thai neem seedlings (Fig 5). This concentration was used in the salt-tolerance screening program. *In vitro* seedlings from 660 clones were screened for salt tolerant clones by adjusted the culture medium to 0.34 M NaCl. Nine clones of Thai neem showed dramatically higher salt tolerance than other clones. The salt tolerant and salt sensitive clones were chosen to assay salt tolerant ability. The salt tolerant ability is indicated by the salt tolerance index (*STI*), which is the ratio of net photosynthetic rate (*NPR*) under salt stress condition (1.02 M NaCl) relative to control (0 M NaCl) condition. Nine salt tolerant clones and one salt sensitive clone were propagated using *in vitro* culture techniques, treated with salt stress conditions (0.34 M NaCl) and the *NPR* were then measured. The *NPR* of the salt tolerant clones decreased slightly with increasing NaCl concentrations, while *NPR* of the salt sensitive clone decreased strongly (Fig 6). Thus, the *NPR* of salt tolerant clones demonstrated better growth than those salt sensitive plantlets under salt stress conditions.

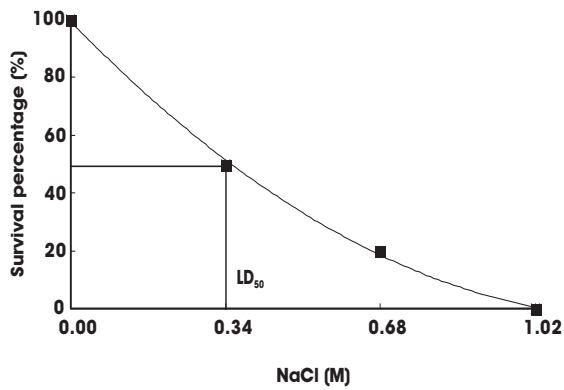

Fig 5. The survival percentage of Thai neem seedlings cultured *in vitro* on Murashige and Skoog medium supplemented with 0, 0.34, 0.68 and 1.02 mM NaCl concentrations for 28 days. Lethal dose 50 (LD_{50}) represents the concentration of NaCl at 0.34 M that reduced the survival percentage for 50%.

Therefore, the *STI* of salt tolerant clones was significantly expressed higher than those salt sensitive clones by a factor of 1.3-1.5 times (Fig 7).

Salt tolerance screening has been widely investigated by field trials or hydroponic systems. Some errors may have occurred due to the uncontrolled environmental conditions, causing incorrect data measurement.[19] *In-vitro* culture has been used in screening programs of many crop species, such as rice,[5] barley,[6] tomato,[7] tobacco[8] and mulberry.[9] However, conventional *in vitro* culture generally uses sugar as the carbon source (photomixotrophic system), which differs from natural conditions. The modification of *in vitro* plantlets by photoautotrophic system (CO_2 as a carbon source) could be regarded using the whole plant in *ex vitro* conditions.[10] The plantlets in this system should express realistic phenotypes in term of anatomical, morphological, and physiological characteristics. The photoautotrophic system has been successfully applied in measurement of salt stress responses in *Albizzia lebbek*[20] and salt tolerance screening of one hundred forest tree species.[21] This method should represent a better system for salt tolerant screening and for testing salt tolerant ability than the conventional method. The physiological traits as net photosynthetic rate (*NPR*) reduction and chlorophyll degradation have been used as the alternative ways to assay the salt tolerant ability.[21,22] In addition, the salt tolerant clones of Thai neem described here should be useful for future planting as a field trial in the saline soil area.

Genetic variation within species offers a valuable tool for salt-tolerant screening program. Normally, the salt tolerant plants have high capacity to accommodate extreme salinity because of vary special anatomical and morphological adaptations or avoidance mechanisms.[16] The defense mechanisms used by salt tolerant or halophyte species include ion homeostasis, ion transport, osmoregulation, detoxification and repair stress damage and hormonal and signal transduction systems.[23-25] The toxic ions in salinity soil, typically Na^+ and Cl^-, can move passively into root cells, and alternatively, enter the cells through ion channels. The halophyte species have low affinity ion channels and transporter at high external toxic ions.[24] In addition, these ions are generally secreted into the vacuole by H^+-ATPase ion pumping, and plant cells synthesized the osmotic solutes as amino acids, sugars and proteins for osmotic adjustment or cell stabilization.[23] Moreover, the toxic ions imposed on plants by salinity may create the oxidative stress, causing accumulation of toxic compounds, perturbance in cellular metabolisms and nutritional disorders.[24] The salt tolerant plants may also reduce the unwanted compounds by detoxification and repair stress damage. Abscisic acid (ABA) is well known as a hormonal signal for programmed cell death or senescence in plants, and is normally synthesized in high levels upon salt stress.[25] The salt tolerant clones should also express defensive gene(s) in response to salt stress at higher levels than those salt sensitive clones. The evidence of defense mechanisms in salt tolerant clones of Thai neem will be further investigated.

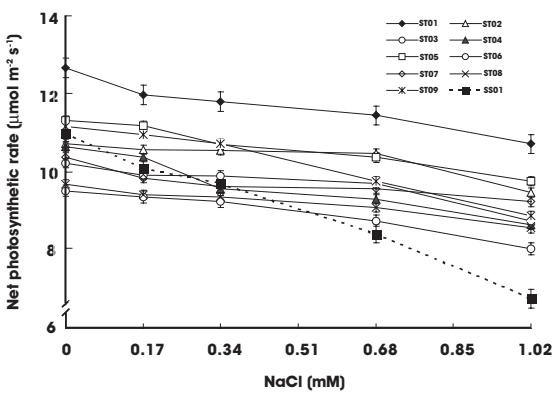

Fig 6. Net photosynthetic rate responses of nine salt tolerant clones (ST01-ST09) and one salt sensitive clone (SS01) to salt stress cultured *in vitro* under photoautotrophic condition. Error bars represent ± SE.

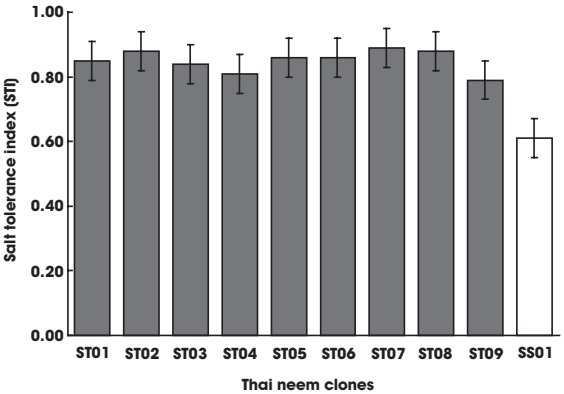

Fig 7. Salt tolerance index (*STI* = *NPR* at 1.02 M NaCl / *NPR* at 0 M NaCl) of nine salt *t*olerant clones (ST01-ST09) and one salt sensitive (SS01) clone evaluates from *NPR* reduction of Thai neem cultured *in vitro* under salt-stress condition. Error bars represent ± SE.

Conclusion

Water use efficiency and chlorophyll concentration in the leaf tissues of Thai neem seedlings decreased significantly under salt stress conditions. Limitation in water use efficiency and degradation of chlorophyll in leaf tissues of plantlets cultured under salt stress

conditions directly reduced the net photosynthetic rate, resulting in the low growth efficiency and low survival percentage. The 50% lethal dose of NaCl was used in the salt-tolerance screening program. Clones of Thai neem which survived under salt stress condition were classified to be salt tolerant, while non-surviving clones were classified to be salt sensitive. The plantlets of salt tolerant clones showed significantly better salt tolerant ability than those salt sensitive clones, as determined by the salt tolerance index (*STI*).

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