Research article

The Biochemical Substances in Plants on Salt Affected Area in Northeast Thailand, Bamnet Narong District, Chaiyaphum Province, Thailand

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Abstract This study was aimed to examine biochemical substances and their relationships in plants growing on salt affected area. The study took place in a very high salinity in Bamnetnarong District, Chaiyaphum Province between the rainy season (August 2011) and the dry season (April 2012). Two groups of plants were analyzed; halophytes and salt tolerant species. The halophytes consisted of *Azima sarmentosa*, *Maytenus mekongensis*, and *Pluchea indica* whereas the salt tolerance plant is *Combretum quadrangulare*. The results of the biochemical substances analyzes and showed that in the dry season all the plants produced more the contents of proline, hydrogen peroxide, peroxidase activity and malondiadehyde, and the percentage of electrolyte leakage than in the rainy season. Additionally, in both two seasons, the plants in the halophyte group produced substances less than in the salt tolerance plant. The best self-adjustment mechanism was discovered in *A. sarmentosa*, which produced a small and stable quantity of substances even though the electrical conductivity rose up.

Keywords important substances, halophytes, salt-tolerant species, salt affected area

INTRODUCTION

Salt stress leads to a series of morphological, physiological, biochemical and molecule changes that adversely affect plant growth and productivity. In glycophytes, plant growth and development are generally limited by salinity. The most of the word's crop species are glycophytes, and they do not grow under high soil salinity (Abbaspour, 2012). Salt tolerance is complex genetically and physiologically. Tolerance often shows the characteristics of a multigenic trait, with quantitative trait loci (QTLs) associated with tolerance identified in barley, citrus, rice, and tomato and with ion trans-port under saline conditions in barley, citrus and rice (Flowers, 2004).

When plants are subjected to salt stresses, active oxygen species (AOS) are generated in response to stress condition. AOS include superoxide (O_2^-), hydroxyl radicals (OH^-), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are metabolic by products of plant cell (Abbaspour, 2012). It is already known that these cytotoxic active oxygen species, which are also generated during metabolic processes in the mitochondria and peroxisomes (Khan and Panda, 2008).

Under stresses, plants possess several antioxidant enzyme systems to protect their cell form the negative effects of AOS, such as peroxidase (POX), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), and ascorbate peroxidase (Sevengor et al., 2011). Peroxidases

catalyze the subsequent breakdown of H_2O_2 water and oxygen Catalase is involved in scavenging H_2O_2 generated during the photorespiration and β-oxidative of fatty acids. Super oxide dismutase can convert O_2^- to H_2O_2 and then H_2O_2 is removed by ascorbate peroxidase and glutathione reductase in the ascorbate-glutathione cycle. (Lu et al., 2006). Moreover, plants accumulate compatible osmolytes such as proline, glycine betaine and sugar alcohols, when they are exposed to drought or salinity stress (Yoshiba et al., 1997). Verdoy et al. (2006) considered in higher plant, proline is synthesized form both glutamic acid and ornithine. Nanjo et al. (1999) reported to many organisms, including higher plants, accumulate free proline in response to osmotic stress.

Salt tolerance is a complex phenomenon, but the specific mechanisms of salt tolerance in each plants species, can grow in high soil salinity in Northeast Thailand is poorly defined. The detrimental effects of high saline soil salinity on biochemical substances such as the contents of proline, hydrogen peroxide, peroxidase activity and malondiadehyde, and the percentage of electrolyte leakage in both season (rainy and dry season) and their relationship in each plant grown were determined.

METHODOLOGY

Study Area

The study took place in Bamnetnarong District, Chaiyaphum Province between the rainy season (August 2011) and the dry season (April 2012).

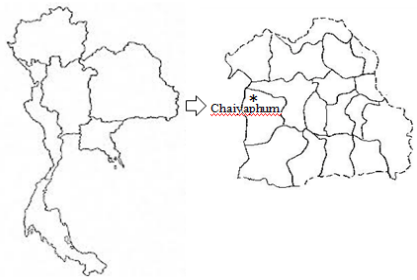

Fig. 1 The study area in Bamnetnarong District, Chaiyaphum Province between the rainy season (August 2011) and the dry season (April 2012)

Plant and soil samples

Plants and soil samples were collected by stratified sampling method between the dry season (April 2012) and the rainy season (August 2011). Two groups of dominate plants in the site study such as the 4 halophytic plants (namely, *Azima sarmentosa*, *Maytenus mekongensis*, *Pluchea indica*) and the salt tolerance plant (*namely, Combretum quadrangulare*) were selected.

Soil was sampled form root zone of each plant at width of 20 cm and at the depth of 15 cm Electrical conductivity (EC) and soil pH were determined in 1:5 (soil to water solution) by using a conductivity meter and a pH meter, respectively.

Electrolyte leakage (EL) was determined according to the method of Dionisio-Sese and Tobita (1998). The 0.1 g of fresh leaf samples was cut into 5 mm length and placed in test tubes containing 10 ml of redistilled water. The tubes were incubated in a water bath at 32 °C for 2 h and the initial electrical conductivity of the medium (EC_1). The samples were autoclaved at 121 °C for 20 min to release all the electrolytes and the final electrical conductivity (EC_2) measured. The percentage of EL was calculated by using the formula: EL (%) = (EC_1/EC_2) x100

Malondialdehyde (MDA) content was measured according to the method of Stewart and Bewley (1980). The 0.1 g of fresh leaf samples was homogenized under ice-cold (liquid nitrogen) was extracted with 50 mM phosphate buffer (pH 7.0) and centrifuged at 12,000 rpm at 4 °C for 30 min. As much as 1 ml of supernatant was then vortexed with 1 ml of 0.5% (w/v) thiobarbituric acid

solution containing 20% (w/v) trichloroacetic acid. The mixture was heated at 95°C for 25 min. The sample was cooled on ice for 10 min and centrifugated at 10,000 x g for 10 min. After subtracting the non-specific absorbance at 532 and 600 nm, the MDA content was determined by its extinction coefficient of 155 $mM^{-1}cm^{-1}$ and the concentration was express as mM g^{-1} FW.

Proline content was determined according to the procedures of Bates et al. (1973). Leaf sample 0.1 g was homogenized in 5 ml of 3% (w/v) sulfosalicylic acid and the homogrnate was filtrated through Whatman No.2. One ml of the filtrate was pipette laced in a 10 ml test tube and reacted with 1 ml of acid ninhydrin mixture and placed in a water bath at 100 °C for 1 h. The reaction was stopped on ice bath. The mixture was extracted with 2 ml. of toluene and stirred for 15 sec. The toluene phase containing the chromophores was aspired, warmed to room temperature, and the absorbance was read at 520 nm. The proline content was determined using from a standard curve and expressed as ug g^{-1}FW.

Hydrogen peroxide content in the leaves was determined according to the methods of Sergiev et al. (1997). Leaf tissues (0.5 g) were homogenized with 5 ml of 0.1% (w/v) trichloroacetic acid (TCA) on an ice bath. The homogenate was centrifuged at 12,000 x g for 15 min and 0.5 ml of the supernatant was transferred to a 15 ml test tube. The supernatant was added with 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0), 1 ml of 1 M KI. The absorbance of supernatant was measured at 390 nm. The content of H_2O_2 was determined using a standard curve and the concentration was expressed as μmole g^{-1} FW.

Peroxidase enzyme activity was determined using the guaiacol oxidation method (Chance and Maehly, 1955) in a 3 ml reaction mixture containing 10 mM phosphate buffer (pH 6.4), 8 mM guaiacol, 100-200 μl crude extract and 2.75 mM H_2O_2. The increase in absorbance was recorded at 470 nm within 30s (linear phase) after H_2O_2 was added. One unit of peroxidase activity was expressed as $\Delta A470\ min^{-1}$ mg $protein^{-1}$

The protein was extracted by the method of Bradford (1976) using the Bio-Rad assay kit (Bio-Rad Laboratories, USA) with bovine serum albumin (BSA) as standard.

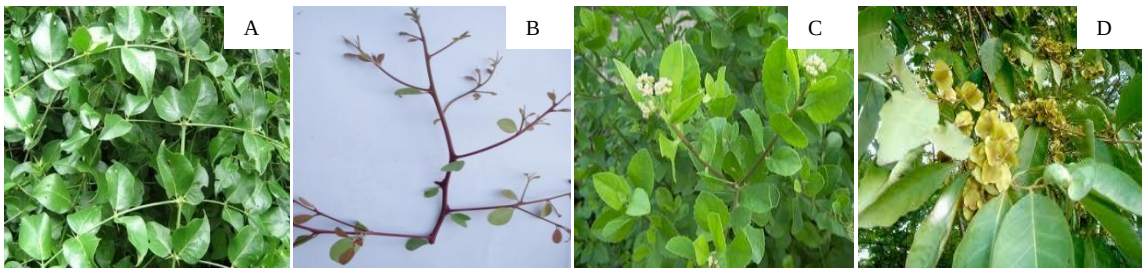

Fig. 2 Leaf of dominant plants in Bamnetnarong District, Chaiyaphum Province
Azima sarmentosa (A) *Maytenus mekongensis* (B), *Pluchea indica* (C), *Combretum quadrangulare* (D)

RESULTS AND DISCUSSION

Electrical conductivity and pH of soil

The EC in the rainy season and the dry season in the halophytes were ranged from 1-22 and 36-147 dS/m respectively, while in salt tolerance plant was ranged from 0.4-3.0 and 28-76 dS/m (Fig.1 A-C). During the rainy season the electrical conductivity was lower than in the dry season. In the rainy season, the rain will wash off salt of the surface. Soil pH in the rainy season and the dry season in the halophytes were ranged from 5.35-8.38 and 4.56-8.11, respectively, while in salt tolerance plant was ranged from 6.00-7.61 and 5.96-6.82 (data not shown).

Effect of soil salinity on electrolyte leakage

The effect of soil salinity on membrane integrity was monitor means of EL test in fresh samples. The amount of EL out of the cells was assessed indirect by conductometric measurements. EL of

the halophytes in the rainy season and in the dry season was in the range of 11-36% and 24-55%, respectively, whereas EL of salt tolerance plant (*C. quadrangulare*) was in the range of 15-24% and 21-45%, respectively (Fig. 3A-C). In both season, EL increased with increasing soil salinity level. Similar changes in the level of lipid peroxidation and electrolyte leakage in response to NaCl stress have been reported by several authors. Dhindsa et al. (1981) reported that there are similar changes in the level of lipid peroxidation and in electrolyte leakage in tobacco leaves. Lutts et al. (1996) also found a significant positive correlation between MDA and EL.

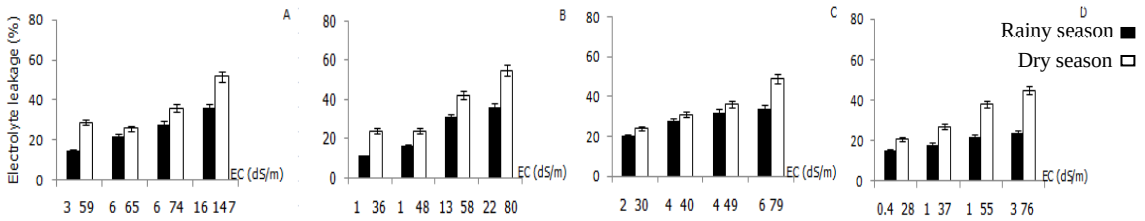

Fig. 3 The effect of soil salinity on electrolyte leakage in leaves of 4 plants
Azima sarmentosa (A), *Maytenus mekongensis* (B), *Pluchea indica* (C), *Combretum quadrangulare* (D), *Vertical bars represent mean + S.E. (n = 5)*

Effect of soil salinity on lipid peroxidation

A product of lipid peroxidation in the leaves of 4 plants was assessed as the content of MDA. In the rainy season, the content of MDA in the halophytes ranging from 0.3-4 mM/gFWx10^{-3} and in the salt tolerance group produced MDA ranging from 1-5 mM/gFWx10^{-3} is more than in the halophyte group. The dry season in the salt tolerance group produced MDA more than in the halophyte group. In the dry season salt tolerance group produced MDA more than the rainy season. Due to the higher electrical conductivity of the soil affect the oxidation increases as shown in Fig. 4.

These result is support by previous reports such as by Khan and Panda (2006) studied the effect of NaCl stress (50, 100 and 150 mmol l^{-1}). They found that the salt-tolerant Lunishree showed higher MDA than the salt-sensitive Begunbitchi. Indicating a higher rate of MDA in Begunbitchi due to salt stress MDA with the increase in salt stress.

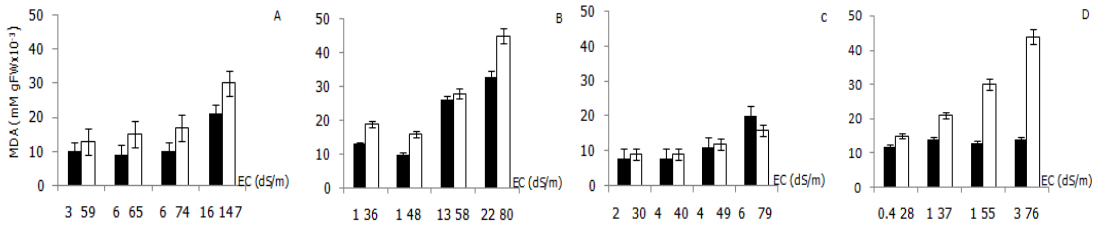

Fig. 4 The effect of soil salinity on malondialdehyde content (mM gFWx10^{-3}) in leaves of 4 plants
Azima sarmentosa (A), *Maytenus mekongensis* (B), *Pluchea indica* (C), *Combretum quadrangulare* (D), *Vertical bars represent mean + S.E. (n = 5)*

Effect of soil salinity on proline

Proline is an osmoprotectant that has been show to accumulate in plants in response to salt stress. Earlier studies form this showed that there is accumulation of proline in 4 plant species. In concurrence with this (Fig. 5), our results show that in the rainy season, proline content in the halophyte group and in the salt tolerance group were similar ranging from 1-15 µg g^{-1} FW. Whereas, in the dry season, the salt tolerance plant accumulate proline more than in the halophyte group. The results are in accordance to other findings such as by Lin and Kao (1996) studied the effect of NaCl stress (0, 50 and 150 mM) on root rice accumulation of proline when the concentration of the solution increases.

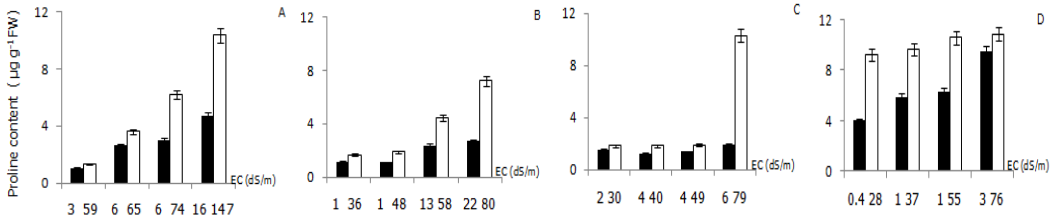

Fig. 5 The effect of soil salinity on proline content (µg/gFW) in leaves of 4 plants
Azima sarmentosa (A), *Maytenus mekongensis* (B), *Pluchea indica* (C), *Combretum quadrangulare* (D), *Vertical bars represent mean + S.E. (n = 5)*

Effect of soil salinity on peroxidase enzyme activity

The present results on increase in the peroxidase enzyme activity are shown in Fig. 6. Peroxidase enzyme activity in the rainy season plants in the halophyte group with peroxidase enzyme activity ranged from 0.1-1Δ470 min mg g^{-1} protein. Plants in the salt tolerance group ranged from 0.1-4 Δ470 min mg g^{-1} protein. In dry season plants in the halophyte group with peroxidase enzyme activity ranged from 0.1-4 Δ470 min mg g^{-1} protein and salt tolerance plants ranged from 0.1-4 Δ470 min mg g^{-1} protein. When plants are under stress to reduce stress by creating antioxidants, antioxidant enzymes to prevent damage from ROS to the plant cell such as catalase, glutathione reductase, superoxide dismutase and ascorbate peroxidase when compared with the controlled plant.

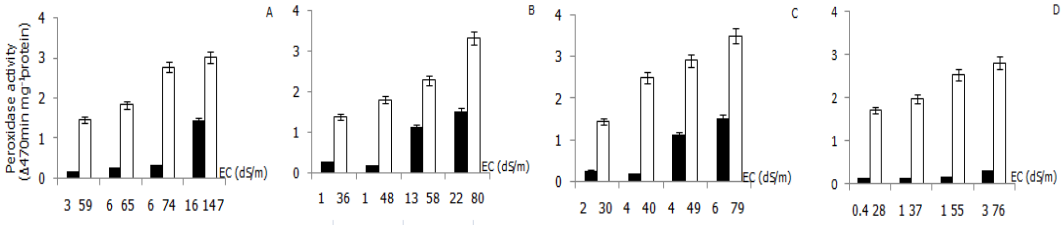

Fig. 6 The effect of soil salinity on peroxidase enzyme activity (Δ470min mg^{-1}protein) in leaves of 4 plants
Azima sarmentosa (A), *Maytenus mekongensis* (B), *Pluchea indica* (C), *Combretum quadrangulare* (D), *Vertical bars represent mean + S.E. (n = 5)*

Effect of soil salinity on hydrogen peroxide

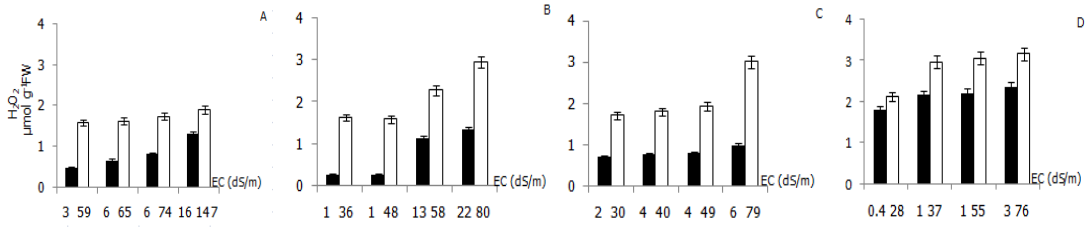

Fig. 7 The effect of soil salinity on hydrogen peroxide content (µmol g^{-1}FW) in leaves of 4 plants
Azima sarmentosa (A), *Maytenus mekongensis* (B), *Pluchea indica* (C), *Combretum quadrangulare* (D), *Vertical bars represent mean + S.E. (n = 5)*

Many authors has been reported that reactive oxygen species, including superoxide and hydrogen peroxide, are elevated with increased salinity, due to the imbalance in the production and destruction of reactive oxygen species (Uchida et al., 2002; Vaidyanathan et al., 2003) Fig. 7 show that the hydrogen peroxide level was increased with increasing salinity in this similar in 4 plant species. This result of hydrogen peroxide in the dry season than in the rainy season and halophytes

group increased hydrogen peroxide than the salt tolerant species of soil salinity was increased. This result supported the previous report of Vaidyanathan et al. (2003), who studied the effect of NaCl stress (100-300 mM) on two rice cultivars differing in salt tolerance. They found that the salt-tolerant Pokkali showed higher activity of catalase and lower levels of H_2O_2 than the salt-sensitive Pusa Basmati 1.

CONCLUSION

In summary, with increasing soil salinity, all plants showed a remarkable increase in the percentage of EL, the contents of proline, MDA, hydrogen peroxide, and peroxidase enzyme activity. The ability of salt tolerance in each plant depend on it's ability to produce biochemical substances, helps that it can be grow and survive in different soil salinity level. The results showed that *A. sarmentosa* is the best one that can be grown on high soil salinity upto 147 dS/m, while the other plants can grow in soil salinity not over than 102 dS/m. One of the characteristics of thick and glossy leaves of *A. sarmentosa* can be reduce water loss and maintain water status. In the future *A. sarmentosa* may be used to improve saline soil structure and used as a renewable energy such as wood for firewood.

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