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EFFECT OF SALINITY STRESS ON ANTIOXIDATIVE ENZYME ACTIVITIES IN TOMATO CULTURED *IN VITRO*

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Abstract

Under inappropriate environments, plants responses by changing their metabolisms to maintain homeostasis that acclimation abilities are different among species and varieties. Saline tolerance tomato is an alternative way to overcome saline soil condition of some areas in Thailand. This study aims to select one or some saline tolerance tomato varieties from mostly used commercial ones. Six tomato variety seeds (Pethlanna, Puangphaka, Seeda, Beefeater, Seeda chompoo and TE VF 1-3-4) were grown by tissue culture technique in MS medium and MS medium supplied with 0, 5, 10, 25 and 50 mM NaCl. The Puangphaka variety was selected since it could grow in all tests NaCl concentrations with best germination time compared to the others cultivar seeds and exhibited 80-90% growth compared to control group. The seedlings were further cultivated in the same medium for 7, 14 and 21 days before they were conducted to determine stem and root superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities as well as amount of chlorophyll. It was found that the SOD, CAT and GPx exhibited increase and decrease trends nearly the same pattern in salinity responses but with different activity levels. Inhibition of nutrient uptake could also be seen from the results. The maximum activities were 5, 0.18, 0.08, 2 and 3 U/mg protein for stem SOD, stem CAT, root CAT, stem GPx and root GPx, respectively. Furthermore, the chlorophyll A and B levels were decrease slightly except for the 21 days plants which presented considerable decrease.

Key words: Antioxidative enzyme, Salinity stress, Reactive oxygen species.

Introduction

Saline soil is a major world's agricultural problem affecting plant productivities in many territorial types especially the arid and semi-arid regions. Among various salts influencing soil salinity, NaCl is the most abundant and powerful one due to its ability to compete essential nutrients resulting to cause nutrient deficiency and certain toxicity symptoms to the plants (Azevedo *et al*., 2006; and Tester & Davenport, 2003). In addition, plants that are exposed to high salinity condition can also be stressed with reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$). These existed ROSs have much ability to harm plant tissues due to their highly reactive properties (McKersie & Leshem, 1994; and Implay, 2003). Naturally, some plants can develop several protective mechanisms that can effectively eliminate or reduce the ROSs at different stress-induced deterioration levels (Beak & Skinner, 2003) and the ability has been known to be varied in species and varieties. One free radical protective mechanisms is enzymatic antioxidant system that includes the superoxide dismutase (SOD: EC 1.15.1.1) found in various cell compartments. This enzyme catalyzes a conversion from two $O_2^{\bullet-}$ radicals to H_2O_2 and O_2 (Scandalios, 1993). In alternative way, several antioxidant enzymes can also eliminate the H_2O_2 such as catalases (CAT: EC 1.11.1.6) (Scandalios, 1993; Kono & Fridovich, 1983) and peroxidases (POX: EC 1.11.1.7) (De Gara *et al*., 2003; Jablonski & Anderson, 1982) by converting it to be water. Moreover, there remains some important enzyme systems that play the important role in ROS scavenging by working around ascorbate-glutathione cycle such as glutathione reductase (GR: EC 1.6.4.2), monodehydroascorbate reductase (MDHAR: EC 1.6.5.4) and dehydroascorbate reductase (DHAR: EC 1.8.5.1) (Candan & Tarhan, 2003; Yoshimura *et al*., 2000). In addition to be generated from saline soil exposure, the ROS can also be inevitably by-products from ordinary cellular metabolisms (Martinez *et al*., 2001) even under good regulation and under present of ROS removal systems (Mittler, 2002). Under harsh conditions, ROS production rate in plant tissues will overcome ROS scavenging rate and the oxidative stress symptom will be incarnated since the generated ROSs attack any vital biomolecules and disturb cellular metabolism which ultimately cause cell death (Sakihama *et al*., 2002). From the causes mentioned above, high salinity can decrease plant growth and affect crop yields. Thus, it can be assumed that plants which can grow and give satisfactory yield under high salinity conditions should (at least) produce significantly high antioxidative enzyme amounts or exhibit strong antioxidative activities. Tomato is one of Thailand's most significant crops which have been suffered from saline soil for decades. Although few saline soil resistant breeds are now commercially available, but newer breeds with some alternative traits are still in requesting by the farmers. In this research, selection was attempted to find out some significant varieties that exhibit satisfactory growth rate and specific antioxidative enzyme activities under high salinity conditions.

Material and Methods

Plant seeds and treating conditions: Seed surfaces of six tomato cultivars (Pethlanna, Puangphaka, Seeda, Beefeater, Seeda chompoo and TE VF 1-3-4) were sterilized with 95% ethanol and 15% chlorox solution for 10 min each, followed by 3 times thoroughly rinsing with distilled water. Then the seeds were cultured in MS medium (pH 5.7) (Murashige & Skoog, 1962)

supplementing with 0-100 mM NaCl under 25°C aseptic environment with 16 hours light time/day (200 lux at the specimen surfaces) for 21 days. The stem and root length together with overall fresh weight were measured in date 7, 14 and 21. Control groups were the seeds cultivated in the same circumstance except the NaCl supplementing step in MS medium were skipped.

Determination of seed germination rate and shoot/root growth: Germination rates of one plant's seeds under various salinity levels can also represent the saline resistant ability of such plant. To determine the germination rate, Seeds of six tomato cultivars (100 seeds per cultivar, 3 replicates) was carried out the germination test in MS medium (pH 5.7) with 0, 5, 10, 25, 50 and 100 mM NaCl and without NaCl (control) supplementations. Germination rate was determined by counting the number of germinated seeds along 20 days period at 24 h intervals. The seeds exhibiting at least 2 mm visibly protruded radicals were considered as germinated ones. After germination evaluating step, the germinated seeds were taken to be further incubated in the same media under 25°C aseptic environments with 16 hours light time/day (200 lux at the specimen surfaces) for 21 days and the levels of SOD and CAT were measured during this period. The stem and root lengths together with total fresh weight were measured in date 7, 14 and 21 of incubation.

Extraction of protein: To extract protein from the specimens, the stems and roots of test plants were homogenized in extraction buffer (50 mM potassium phosphate buffer, pH 7.0, 0.1 mM EDTA, 0.1% (v/v) Triton X-100 and 0.05 % (w/v) polyvinylpyrrolidone-40 (PVP-40). The homogenate was centrifuged at 12000 × g for 10 min. The supernatant fraction was collected and used as crude extract for protein content and enzyme activity determinations. All procedures were carried out at 4°C.

SOD assay: Superoxide dismutase activity was determined by measuring the ability to inhibit reduced-form of nitroblue tetrazolium chloride (NBT) generated by phytochemical constituent in plant samples according to the method modified from Giannopolitis & Ries, (1977). Briefly, the reaction mixture contained 1 ml of 50 mM phosphate buffer (pH 7.0), 100 µl of 0.1 M EDTA, 50 µl of 1.5 mM NBT and 500 µl enzyme extract was prepared and 1.5 ml of the mixture was separately poured into each test tube. Then 25 µl of 1.2 mM riboflavin was added to each tube. All tubes were shaken and illuminated with a pair of 20W fluorescent lamps. Then, the reaction was allowed to proceed for 15 min before the lamps were switched off. All tubes were covered with black cloth and absorbances of the reaction mixtures were read at 560 nm corresponding to photoreduction rate of NBT. The results were expressed as U/mg protein. The percentage of this inhibition is the basis on which the amount of activity is calculated as below:

$$\% \text{ Inhibition} = \frac{\text{Absorbance (reaction blank) - Absorbance (sample)}}{\text{Absorbance (reaction blank)}} \times 100$$

One unit of SOD was defined as the amount of enzyme that inhibits the rate of NBT reduction by 50%.

Catalase assay: The catalase activity determination method was adapted from Beers & Sizer (1952) that the principal is to measure amount of H_2O_2 decomposition. Briefly, a 1.5 ml reaction mixture contained 950 µl distilled water, 500 µl of 5.29 mM H_2O_2 and 50 µl of enzyme extract was prepared. The reaction was started by cell extract adding. The CAT activity was determined by taking the mixture to read the absorbance at 240 nm using a microtitre plate reader. One unit of enzyme was defined as the amount of enzyme required to hydrolyze 1 mmol of substrate per minute at 25°C.

Glutathione peroxidase (GPx) assay: GPx activity was indirectly determined by monitoring the amount of NADPH oxidation through a coupled reaction involved GR as described by Wendel (1981). Oxidized glutathione (GSSG), produced upon organic hydroperoxide reduction by GPx, is turned to its reduced state by GR and NADPH. Oxidation of NADPH to be $NADP^+$ is accompanied by absorbance decreasing at 340 nm. Decreasing rate of the absorbance is indirectly proportional to the sample's GPx activity.

Determination of protein: Protein content was determined by Bradford's method (Bradford, 1976). Briefly, appropriate volume (from 0-100 µl) of sample was aliquoted into a tube and the total volume was adjusted to 100 µl with distilled water. A 1 ml of Bradford working solution was added to each sample well. Then the mixture was thoroughly mixed by vortex mixer. After left for 2 min, the absorbance was read at 595 nm. The standard curve was established by replacing the sample portions in the tubes with proper serial dilutions of bovine serum albumin.

Chlorophyll content determination: Chlorophyll was extracted from fresh leaves (0.1 g) by grinding the sample with 5 ml absolute methanol in a mortar. The mixture was then centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and taken to read for absorbances at 666 and 653 nanometers. The amount of chlorophyll was calculated using the following formulae suggested by Lichtentaler & Wellburn (1985).

$$\text{Chlorophyll A} = 15.65\ A_{666} - 7.340\ A_{653}$$

$$\text{Chlorophyll B} = 27.05\ A_{653} - 11.21\ A_{666}$$

Statistical analysis: The experiments were three times repeated in order to check for reproducibility before going on next statistical analyzing step. Data were then subjected to analysis of variance (ANOVA) to assess significant mean differences among groups. The means with significant differences were then ranked using Duncan's Multiple Range Test (DMRT) at $p<0.05$. In all figures, the standard errors of each means were presented as error bars.

Results and Discussion

Germination analysis of six tomatoes cultivars: The seeds of six tomato cultivars were test for ability to survive in media with different salinities and results were illustrated in Fig. 1. In normal condition (0 mM NaCl supplementation), the seeds began to germinate on date 9, 5, 7, 14, 13 and 6 after cultivated for Pethlanna, Puangphaka, Seeda, Beefeater, Seeda chompoo and TE VF 1-3-4 cultivars, respectively. It can be obviously seen that the seeds from Puangphaka cultivar was the first one that began to germinate (5 days after cultivated). The germination rates were increase rapidly during date 5-7 and 7-8 after cultivated for Puangphaka and Seeda cultivars until reached around 80% and 90% germination rate, respectively. The germination of Pethlanna cultivar seeds started from date 8 after cultivated and continuously increased until reach the maximum rate (around 70%) at date 20 after cultivated while the TEVF 1-3-4 cultivar exhibited faster grew by starting on date 6 and reach the maximum rate over 80% on date 15 after cultivated. The Beefeater and Seeda chompoo cultivar seeds germinated in relatively too slow and too low rates (lower than 10% started at date 14 and around 15% started at date 13, respectively). For low concentration (5, 10 and 25mM) saline treating experiments, the seeds of Pethlanna, Seeda chompoo and TEVF 1-3-4 cultivars exhibited lesser germination rates than control (no NaCl supplementation). Only at 5 and 10 mM NaCl concentrations, the germination rates of Puangphaka and Seeda seeds were better than control while the rates were lesser for the other concentrations. Regardless relatively low germination time, the Beefeater seed gave higher germination rate than control at all test NaCl concentrations. Thus, the seed of Beefeater cultivar could be a saline resistant one. At 100 mM saline treating experiment, only the seeds of B and C cultivars could germinate at 100 mM saline concentration with the maximum germination rates of around 40% and 10%, respectively. While at 50 mM concentration, only the seeds of Puangphaka, Seeda, TEVF 1-3-4 and Seeda chompoo cultivars could germinate at the maximum germination rates of around 80%, 50%, 30% and 5%, respectively. Considering only the seed germination point of view, it could be said that the Puangphaka was salinity tolerant cultivar and thus was selected for further characterization (Fig. 1). In general, salinity can retard the germination rate due to high concentration salt decrease normal osmotic potential leading to diminish absorptions of water and nutrients plant required for germination. In addition, salts or ions can also be toxic to embryo and affect the seed germination. The experimental result corresponded to the work of Rahman *et al*. (2008) that delay rates of germination were directly related to the salt concentrations of the medium used or, in another word, the delay rates were directly related to amounts of available water absorbed by the seeds. Besides the salt concentration, the salt tolerance ability of a plant could be varied according to existing salt types and osmotic potential of the medium (Kayani & Rahman, 1988). Thus, further studies are needed to achieve inclusive information of a saline tolerance variety.

Analysis of seedling weight and length: Tomato varieties with salinity resistant ability should not only appear good seed germination rates in high salinity medium, but their seedlings should be able to survive and grow in such condition also. From the Fig. 2A, all treated shoots of Puangphaka seedlings were significantly shorter than the control group ($p<0.05$) and shoot lengths tended to decrease according to NaCl concentration as dose dependence manner especially at 50 and 100 mM NaCl concentrations. From the Fig. 2B, it could be seen that all root lengths of treated Puangphaka seedlings were significantly shorter than those were in control group ($p<0.05$) but all treated root lengths were not significantly different according to salt concentrations. However, all weights from treated shoot and root of Puangphaka seedlings were lower than those in control group and exhibited dose dependence especially the 50 and 100 mM NaCl concentrations (Fig. 3 A and B). It seemed that shorter of all shoot and root length from treated Puangphaka seedlings obviously depended on salt concentrations except the root length which seemed to be stable for all salt concentrations. The same root lengths with lower corresponding root weights can imply that either densities or diameter of root tissue decrease when exposes to higher salinity medium. In case of lower diameter, it is possible that higher salt concentration causes water intake harder for the root tissue and make the tissue wilt resulting to decrease the root weight. Indeed, thinner root appearance was observed in the experiments thus smaller root diameter should be the trend. Shorter shoot and root are a common salinity response from plants and is one of the most important agricultural indices for salinity stress tolerance suggested by many works (Munns, 2002 and Ruiz *et al*., 2005). Several workers had reported that the growth parameter significantly decreased with NaCl level elevating in culture circumstances. It probably caused by osmotic stress, toxicity of ion and nutrient imbalance (Bernstein, 1964). Similar results were obtained in Oak (Sehmer *et al*., 1995), sugar beet (Ghoulam *et al*., 2002), maize (Azevedo *et al*., 2006), sesame (Koca *et al*., 2007), rice (Kumar *et al*., 2008), soybean (Hamayun *et al*., 2010) and pepper (Chookhampaeng, 2011). It looks like that higher salinity condition can make the shoot and root length shorten. This get along with the plant size observed from culture bottles (Figs. 4-6).

Changes of antioxidant enzyme activities: All enzyme activity determinations were carried on Puangphaka cultivar as the reason mentioned before.

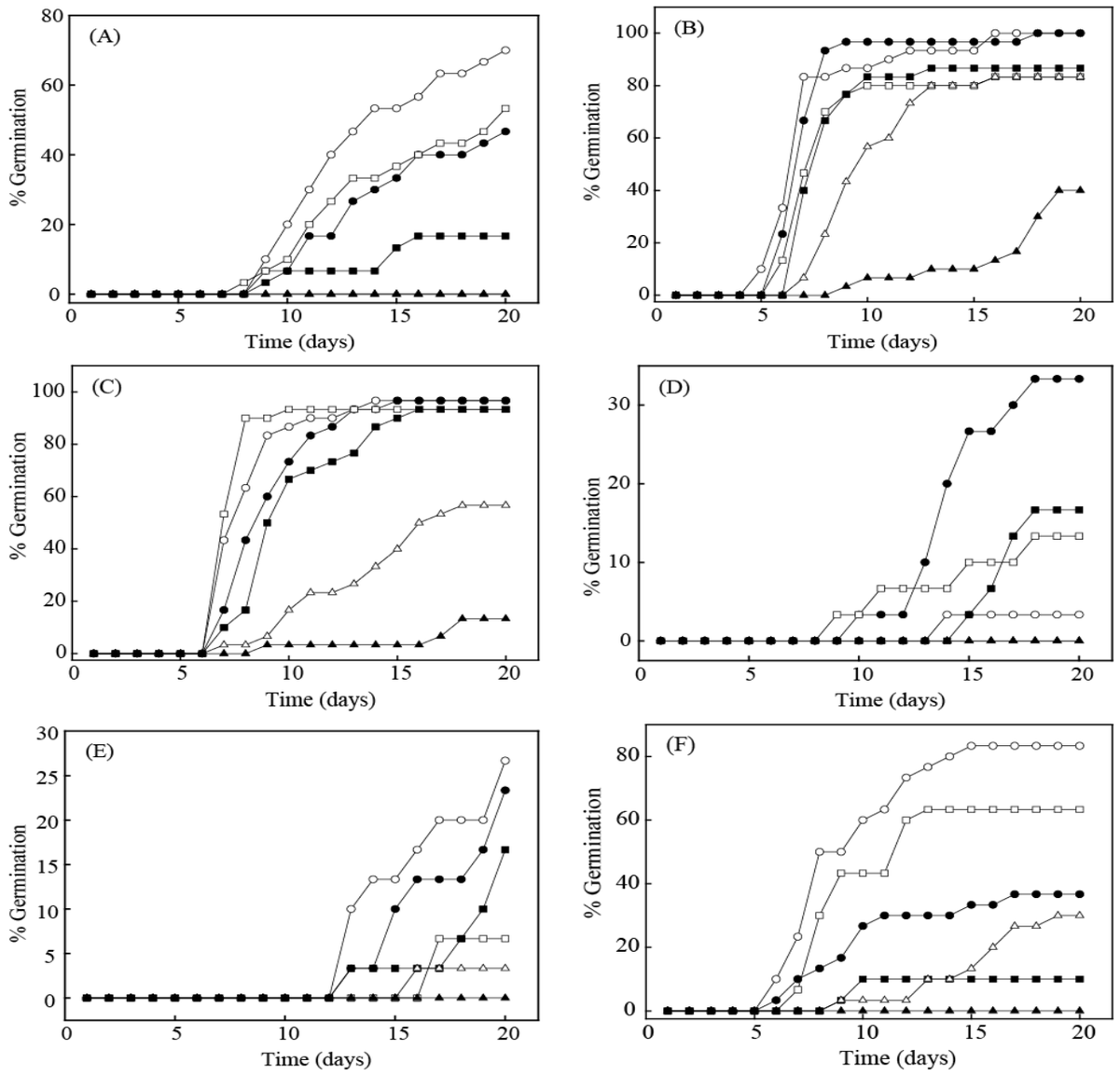

Fig. 1. Germination rate of tomato under (○) 0, (●) 5, (□) 10, (■) 25, (△) 50 and (▲) 100 mM NaCl for 20 days. (A) Pethlanna; (B) Puangphaka; (C) Seeda; (D) Beefeater; (E) Seeda chompoo; and (F) TE VF 1-3-4.

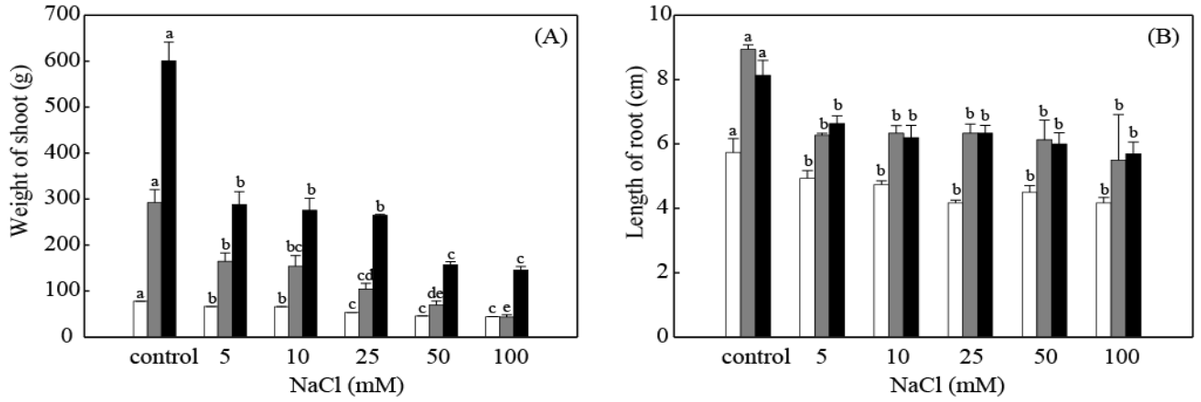

Fig. 2. Effects of NaCl stress on length of the shoot in cv. Puangphaka seedlings (white) 7, (gray) 14, and (black) 21 days. The values and standard errors (vertical bars) of three replicates are shown, value superscript letters are significantly different ($p<0.05$) using Duncan's multiple range test.

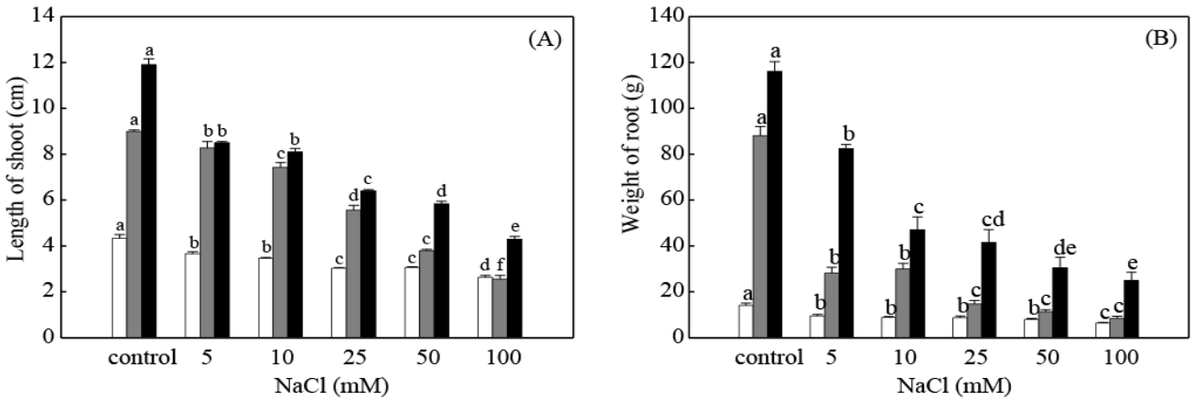

Fig. 3. Effects of NaCl stress on length of the root in cv. Puangphaka seedlings (white) 7, (gray) 14, and (black) 21 days. The values and standard errors (vertical bars) of three replicates are shown, value superscript letters are significantly different ($p<0.05$) using Duncan's multiple range test.

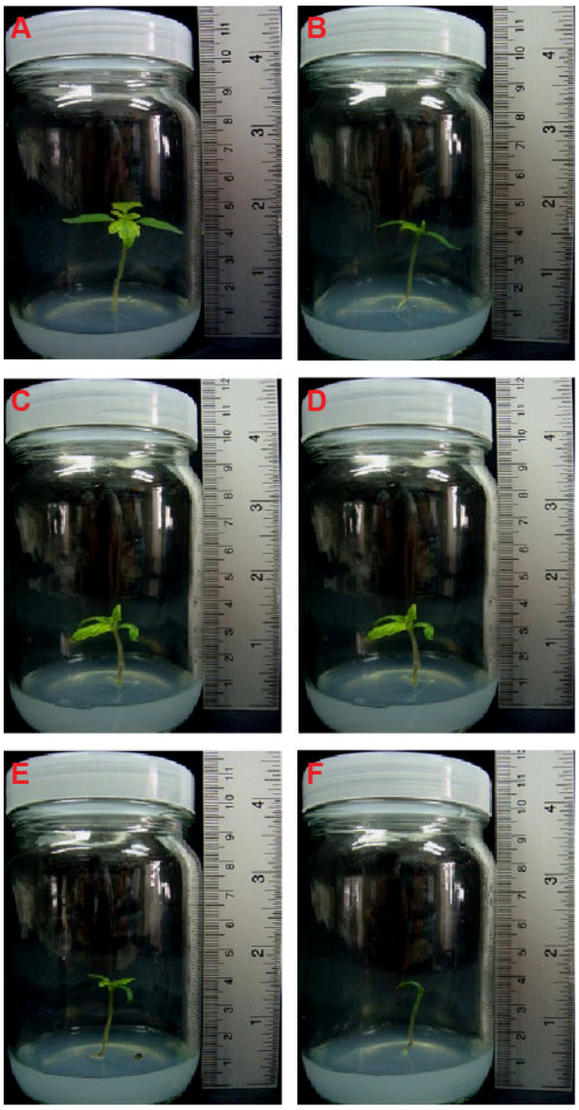

Fig. 4. Characteristics of cv. Puangphaka seedlings growth cultured on MS medium contain with various concentration of NaCl for 7 days. NaCl concentration: (A) 0 mM, (B) 5 mM, (C) 10 mM, (D) 25 mM, (E) 50 mM, (F) 100 mM.

Fig. 5. Characteristics of cv. Puangphaka seedlings growth cultured on MS medium contain with various concentration of NaCl for 14 days. NaCl concentration: (A) 0 mM, (B) 5 mM, (C) 10 mM, (D) 25 mM, (E) 50 mM, (F) 100 mM.

Fig. 6. Characteristics of cv. Puangphaka seedlings growth cultured on MS medium contain with various concentration of NaCl for 21 days. NaCl concentration: (A) 0 mM, (B) 5 mM, (C) 10 mM, (D) 25 mM, (E) 50 mM, (F) 100 mM.

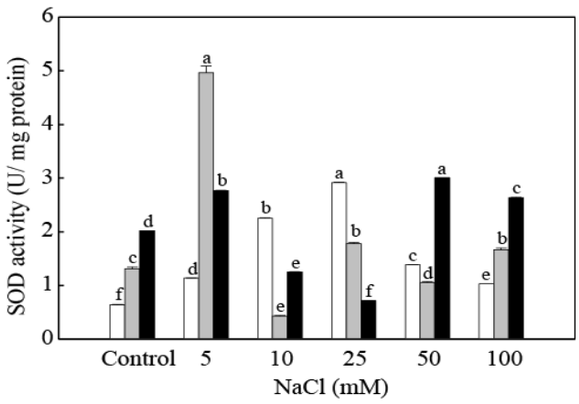

Fig. 7. Effects of salt stress on SOD activity in stems of Puangphaka at 7 days (white), 14 days (gray), and 21 days (black). Each value represents the mean of three replications and vertical bars indicate ± SE. Different letters indicate significant differences according to Duncan's multiple range test ($p<0.05$).

Superoxide dismutase (SOD): It was found that only stem SOD activities could be detected, no significant SOD activity were observed in root. The SOD determination method used in this study is based on totally level of free radical scavenged by the SOD and may not suitable to investigate root SOD since it is unable to separate diminished activity from H_2O_2 generated by CuZnSOD and increased activity from H_2O_2 removal of MnSOD in the root (Rubio *et al*., 2004). The stem SOD activities tended to be increased at low, dropped at medium and turned back again at high salt concentration (Fig. 7). This phenomenon can be simply explained by enzyme optimum that the salt concentrations within activity-decrease range may be out of enzyme's optimal margins. Alternatively, the phenomenon can also be explained in more sophisticate way. Firstly, from the work of Keles & Oncel (2000), salinity stress could increase SOD production in plant. This is common and may possibly be a reason for increase of SOD activities in the experiment. Secondly, decrease of SOD activities can be explained by the work of Khodary (2004) that NaCl salinity could inhibit nitrogen, phosphorus and potassium uptakes. Decrease in nitrogen uptake can certainly affect peptide synthesis and cause enzyme amount inevitable decrease. In addition, the decrease can also be from excess of NaCl in medium solution disturbs vital biomolecule productions and activities (Mittler, 2000) thus deteriorate the metabolism of SOD. Finally, acclimation of plant's metabolism may be a reason for turning back of the enzyme amount afterwards. It can be noted that no turning back present at highest NaCl concentration (100 mM) and this were consensus in stem CAT, root CAT, and stem GPx patterns. However, all these assumptions should be proved by future researches. The highest SOD activities in this study was 5 U/mg protein at 14 days under 5 mM NaCl condition (Fig. 7) which miserably far from what achieved by Rahman & Mackay (2004) that the four test-tomato (TM0126, VF134-1-2, Kyokko & Ratan) gave around 900-950 U/mg protein level at 12 days water stress. It implies that the tomato plant can produce or have much more SOD activities than that occurred in this experiment and it should be limited by entire environments, not the plant itself. In this study, the highest surplus of SOD activity change could be seen in 14 days plants between 5 and 10 mMNaCl concentration (~4.5 U/mg protein) followed by the 21 days (~2.5 U/mg protein) and 7 days (~1.5 U/mg protein), respectively. This can also be explained that the enzyme systems in 7 days plants were too young to respond the salinity stress well but the system in 14 days plants were, thus, the different can be obviously seen. The 21 days plants were mature enough to develop proper enzyme system as well as non-enzymatic antioxidative processes (Koca *et al*., 2007).

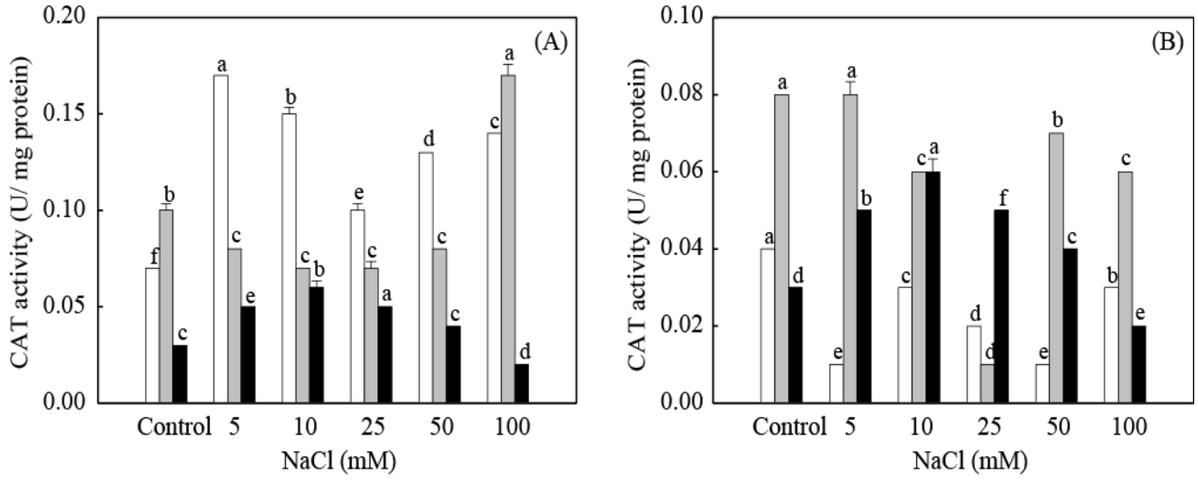

Fig. 8. Effects of salt stress on CAT activity in stems (A), and roots (B) of Puangphaka at 7 days (white), 14 days (gray), and 21 days (black). Each value represents the mean of three replications and vertical bars indicate ± SE. Different letters indicate significant differences according to Duncan's multiple range test ($p<0.05$).

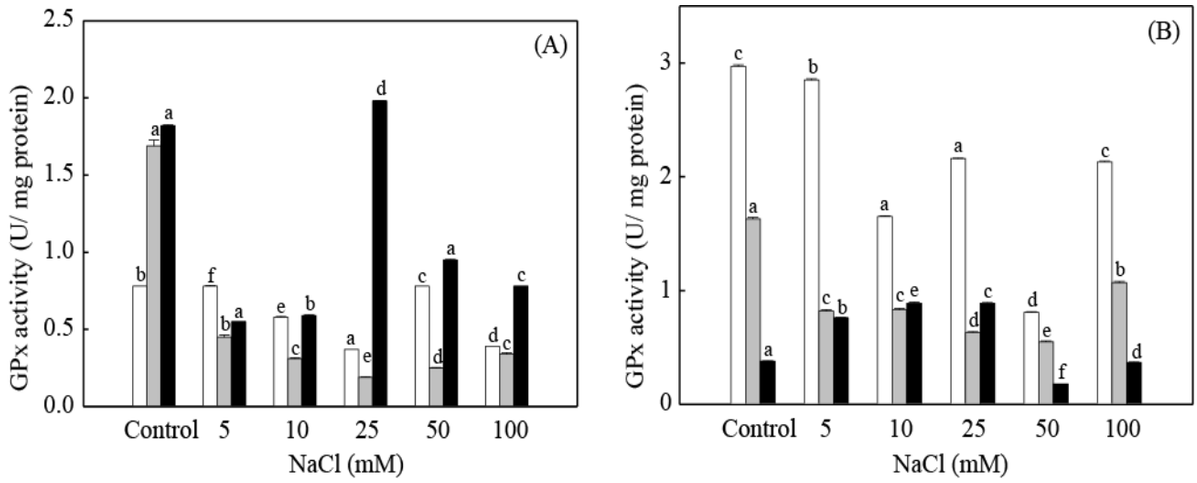

Fig. 9. Effects of salt stress on GPx activity in stems (A), and roots (B) of Puangphaka at 7 days (white), 14 days (gray), and 21 days (black). Each value represents the mean of three replications and vertical bars indicate ± SE. Different letters indicate significant differences according to Duncan's multiple range test ($p<0.05$).

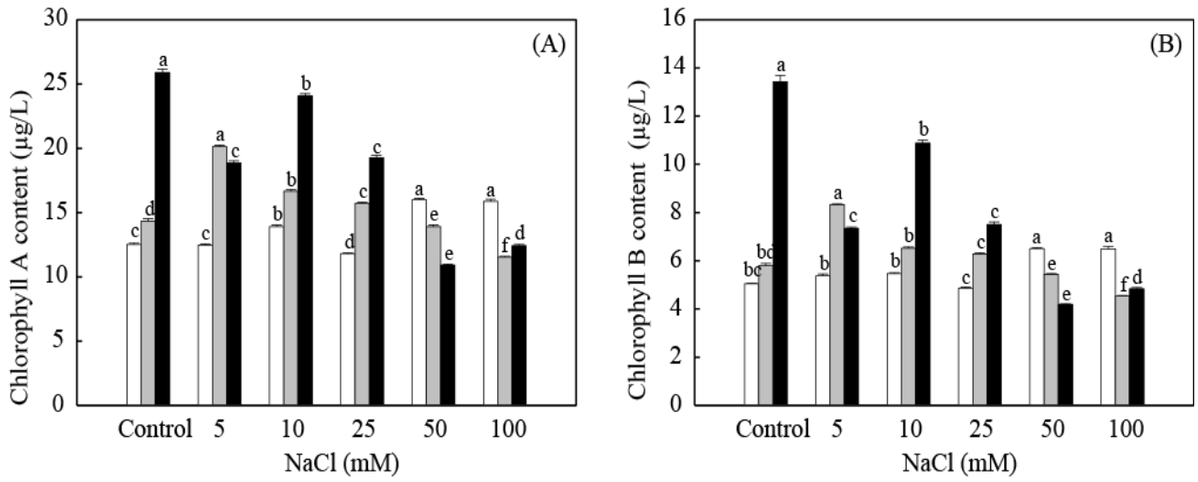

Fig. 10. The effect of NaCl treatments (0, 5, 10, 25, 50 and 100 mM) on Chlorophyll content in leaves of *L. esculentum* cv. Puangphaka on (white) 7, (gray) 14, and (black) 21 days after treatment. Data represents the average of three replicates. Vertical bars indicate ±S.E. Different letters indicate significant differences according to Duncan's multiple range test ($p<0.05$).

Fig. 11. Characteristics of leaves in cv. Puangphaka seedlings growth cultured on MS medium contain with various concentration of NaCl for 21 days. NaCl concentration: (A) 0 mM, (B) 5 mM, (C) 10 mM, (D) 25 mM, (E) 50 mM, (F) 100 mM.

Catalase (CAT): CAT, which is involved in the degradation of hydrogen peroxide into water and oxygen, is the most effective antioxidant enzymes in preventing oxidative damage. Both stem CAT and root CAT activity profiles against different NaCl concentrations in Fig. 8 exhibited the same trend as what SOD was and the assumptions of changed patterns can be explained in the same way. Increased CAT activities under salinity stress in *Cassia angustifolia* L. (Agarwal & Pandey 2004), maize (Azevedo *et al*., 2006), *Sesamum indicum* (Koca *et al*., 2007) and wheat (Hameed *et al*., 2008) were similar to our finding and depend on salt tolerance potential of plant's varieties. The changes in CAT activity depend on the species, the development and metabolic state of the plant, as well as on the duration and intensity of the stress (Willekens *et al*., 1995; Chaparzadeh *et al*., 2004).

Glutathione peroxidase (GPx): The levels of GPx activity in cv. Puangphaka at various NaCl concentrations are shown in Fig. 9. The GPx are key enzymes of the ascorbate-glutathione cycle, the cycle may be a potential mechanism of tomato adaptation to salinity. It was reported that in the stress conditions of intense light, high temperature, flood and salinity, free radicals and reactive oxygen molecules are also formed. Therefore, a scavenging system should be very active. Our results showed that cv. Puangphaka possessed an effective superoxide dismutase/ascorbate-glutathione cycle under high salinity. The major function of GPx in plants appears to be the scavenging of phospholipid hydroperoxides and thereby the protection of cell membranes from peroxidative damage (Gueta-Dahan *et al*., 1997). The expression of many GPx is enhanced in response to abiotic and biotic stresses, including salinity, heavy metal toxicity and infection with bacterial or viral pathogens (Avsian-Kretchmer *et al*., 2004). But this study the GPX decreased with higher salt concentration. This occurs may be due to H_2O_2 was eliminated by increasing the activity of CAT in the previous experiments.

Analysis of chlorophyll content: In the overall view, chlorophyll chlorophyll A and B were slightly increase by plant age (7, 14 and 21 days) but decrease along increase of salt concentrations (Fig. 10). The decrease may possibly be the result from inhibiting of nutrient (s) uptake or insufficiency of the essential nutrient(s) while the re-increase may possibly be the result from plant acclimation. Furthermore, the colors of leaves have changed in higher salt concentration which is obviously on 21 days after salt treatment. However, with high salt concentrations, the nitrogen deficiency could be observed from the Puangphaka plants as leaf stem necrosis present (Figs. 10-11). This can affect both enzyme and chlorophyll synthesis that agrees with the antioxidative enzyme activity results (Figs. 7-9) and decrease of chlorophyll in 21 days plants (Fig. 10). Moreover, according to Cha-Um & Kirdmanee (2009), salinity decreased the total chlorophyll concentration of two maize varieties. Reduction in chlorophyll concentrations is probably due to the inhibitory effect of the accumulated ions (Ali, 2004). This may possibly because total chlorophyll and its components depend on the biological process and development stages of the plant. Similar findings had been reported. These results correspond to the works of Petolino & Leone (1980) for *Phaseolus vulgaris*, sunflower, flax (*Linum usitatissimum*) and peanut (Hajar *et al*., 1993) which obtained similar findings. Many scientists have suggested a positive correlation between decrease in chlorophyll content and salt-induced weakening of protein-pigment-lipid complex (Strogonov *et al*., 1970) or increased chlorophyllase (EC: 3.1.1.14) enzyme activity (Stivsev *et al*., 1973).

Conclusion

The overall results indicate at least 3 points. 1) the saline (NaCl) stress can inhibit seed germination and seedling growth of all six tomato cultivars but with different levels 2) low, medium and high salt concentrations can incrase, decrease and re-inbound all test antioxidative enzyme activities, respectively and 3) the Puangphaka cultivar exhibited good growth in all test salt-concentrations with reasonable responses of antioxidative enzyme activities. Thus, it can be concluded that the Puangphaka is proven as a potential saline tolerant variety achieving the goal. Further studies should be stressed on gene expression levels of each enzyme to actually confirm their existences.

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