Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 6 (5): 788-794, 2007
ISSN 1682-3974

Screening Some Tomato Commercial Cultivars from Thailand for Salinity Tolerance

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Abstract: Hydroponically-grown seedlings of thirteen commercial tomato cultivars and breeding lines were exposed to 0 (control) and 200 mM NaCl (salt stress) for ten days. Salt tolerance was evaluated based on visual appearance of plant damage and the plants were assigned the scale from 1 (most tolerant) to 4 (most sensitive). The salinity scales ranged from 1.00-3.75. All genotypes responded to salt by an accumulation of Na^+, reduction in K^+, Ca^{2+}, N and shoot/root dried weight and a small increase in P. Salinity tolerance scales, ion concentration and shoot/root dried weight differed greatly among tomato genotypes. Highest correlation was found between salinity tolerance scale classes and the reduction in root dried weight, followed by the reduction in shoot dried weight, Na^+ concentration, Ca^{2+}/Na^+ and K^+/Na^+ ratios. The amount of N and P content did not correlate with salinity scale classes. In general, Na^+ concentration and the reduction in root/shoot dried weight were the most reliable parameters useful for screening salt tolerance of tomato at the vegetative stage.

Key words: *Lycopersicon esculentum*, *Lycopersicon pimpinellifolium*, NaCl, salt tolerance, ionic regulation, Na, K^+/Na^+, Ca^{2+}/Na^+

INTRODUCTION

Soil salinity is one of the most important abiotic stress factors that limits crop productivity worldwide. In northeastern Thailand, saline soils cover the area or 2.8 million hectares and another 3.0 million hectares are at risk of becoming saline (Anonymous, 2001). Reducing the spread of salinization by better irrigation and farm management practices, and increasing the salt tolerance of crops are hence important global issues (Munns, 2002). Adverse effects of salinity on plant growth are mainly due to metabolic imbalance caused by ion toxicity, osmotic stress and nutritional deficiency which may also lead to oxidative stress (Zhu, 2002).

Most grain crops and vegetables are glycophytes and are highly or moderately sensitive to saline soils having Electrical Conductivity (EC_e) as low as 3 dS m^{-1} (Francois and Maas, 1994). For tomato, yields are reduced when plants are grown with a nutrient solution of 2.5 dS m^{-1} or higher and above 3 dS m^{-1} an increase of 1 dS m^{-1} results in a yield reduction of about 9-10% (Cuartero and Frenández-Muñoz, 1999). Genetic variability among different varieties within a crop species is a valuable tool for screening and breeding for enhanced salt tolerance (Flowers and Yeo, 1995). Several characteristics have been shown to be related to salt tolerance in tomato at the vegetative stage i.e. root/shoot/leaf dry weight, leaf Na^+ content, leaf Na^+/K^+, succulence, water use efficiency and stem growth and at the reproductive stage i.e. the number of flowers, fruit set, fruit number and yield (Asíns *et al*., 1993; Cruz and Cuartero, 1990; Tal and Shannon, 1983; Wilson and Shannon, 1995). During the vegetative growth, high heritabilities have been found for stem growth, stress symptoms in leaves and plant dried weight (Asíns *et al*., 1993; Foolad, 1996). It is suggested that these characteristics can be introduced into breeding program to obtain elite salt-tolerant genotypes.

The main objective of this study was to determine screening parameters of thirteen commercial tomato cultivars, whose salt tolerance status is unknown, by relating the stress symptoms and leaf injury with Na^+ accumulation, Na^+/K^+, Na^+/Ca^{2+}, N and P content and plant dried weight.

MATERIALS AND METHODS

Thirteen tomato genotypes (Table 1) were used as plant materials. Cultivars No. 1-12 are commercial cultivars

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Table 1: Salinity tolerance scale classes of tomato genotypes based on the shoot appearance. Shoot Na^+, K^+, Ca^+ concentrations and K^+/Na^+, Ca^+/Na^+ ratios of the genotypes grown under saline condition for 10 days

Genotypes	Scale (1-4)[a]	Na^+(mmol g^{-1} DW)	Ca^+ (mmol g^{-1} DW)	K^+ (mmol g^{-1} DW)	K^+/Na^+	Ca^+/Na^+
Sida	1.25ab	1.00ab	0.46e	1.47a-c	1.48a-c	0.46e
Perfect89	2.75c-d	1.32b-e	0.44c-e	1.52bc	1.12a	0.33b-c
Extra390	2.75cd	1.46de	0.48e	1.83d	1.28ab	0.34b-c
KKU40	2.25b-c	1.34c-e	0.43c-e	1.57bc	1.19a	0.32bc
HW96	1.00a	1.08a-c	0.41a-e	1.99de	1.84c	0.38c-e
CH154	3.75d	1.62e	0.29a	2.10e	1.30a-c	0.18a
KKU Sida hybrid	2.75cd	1.35c-e	0.33ab	1.85d	1.39a-c	0.25ab
KKU Sida Somtum hybrid	2.25bc	1.39c-e	0.28a	1.62c	1.26ab	0.21ab
KKU Sida Huai-sai	1.25ab	0.94a	0.34a-c	1.39ab	1.76 bc	0.42de
Red sweet	2.50c	1.30b-e	0.31a	1.93de	1.48a-c	0.24a-c
CLn 463 A×Ace 55VF	2.00a-c	1.15a-d	0.30ab	1.25a	1.10a	0.27ab
CLn 1463 A×SK 8-1	1.75a-c	1.21a-d	0.36a-d	1.41a	1.14a-c	0.30a-e
Moneymaker	2.50ab	1.24ab	0.45e	1.91a-c	1.53a-c	0.36e

[a]Increasing salinity tolerance scale classes from 1-4 indicates increases in salt damages. Means in the same column followed by different letter(s) differ significantly at $p<0.05$

or hybrids. Seeds of cultivars No. 1-10 were obtained from the Department of Horticultural Science, Faculty of Agriculture, Khon Kaen University. Seeds of hybrids No. 11 and 12 were obtained from Srisaket Horticultural Station, Srisaket, Thailand. Seeds of commercial cultivar, Moneymaker (No. 13), was kindly provided by Professor Tony Chen, Department of Horticulture, Oregon State University. All genotypes used are *Lycopersicon esculentum* Mill., except for cultivar No. 5, HW96, which is *Lycopersicon pimpinellifolium* (L.) Mill. The plants were grown in a culture room at Mahasarakham University, Thailand, during May to June, 2006 under artificial light source (400 µmol m^{-1} sec^{-1}, 16 h photoperiod) with approximate temperature range of 23-27°C and 60-89% relative humidity. Seeds were germinated and seedlings were grown in water for 7 days, tomato seedlings at the second-true leaf stage were transferred to 25-l plastic containers containing half-Hoagland solution (thirteen plants, one for each genotype per container). Nutrient solution was renewed at weekly interval throughout the growing period and the level of the culture solution was maintained by periodic additions of the solution. The experiment was carried out with four replications. Plants were grown under non-saline conditions for 19 days. When the plants were 26 days old, at the fourth-true leaf stage, salt treatment was initiated by adding NaCl to the culture solution at the concentration of 25 mM. The concentration of NaCl was gradually elevated at 25 mM increments every other day and on day 40, final NaCl concentration (200 mM) had been reached. The plants were then grown for 10 days under 200 mM salt stress condition. A set of control plants were simultaneously grown in non-salinized solution throughout the experimental period for comparison. On day 50, the plants were evaluated for their salt tolerance by examining the visual appearance. Individual plant was rated for severity of salt susceptibility by the 1-4 scale (Fig. 1). The scale used was 1, normal green plants with turgid, expanded leaf lamina; 2, plants smaller than control plants, leaves are green, slightly wilt with the edge of the lamina slightly curved downward; 3, older leaves completely wilt, younger leaves partly wilt; 4, all leaves completely wilt (Fig. 1). The tolerance scale for each genotype was the average of the scales obtained from four replications.

After visual evaluation of salinity damage, the plants were harvested and separated into shoots and roots, dried at 65°C for 48 h and weighed. Dried whole-plant materials were used for the determination of Na^+, K^+, Ca^{2+}, N and P contents. Plant samples were digested with wet oxidation method using nitric and perchloric acids. Phosphorus content was measured with spectrophotometer (Boeco, Model S-20, Germany) and the contents of Na^+, K^+ and Ca^{2+} were measured with the flame photometer (Sherwood, M410, Scientific Limited). Nitrogen was digested with the semi-micro Kjeldahl digestion procedure (Bremner, 1960) and analyzed with flow injection analyzer (Tecator, FIA series 5012).

The data are presented with the respective standard errors of means and the least significant difference ($LSD_{0.05}$) between treatments, derived from an analysis of variance.

RESULTS

The tomato genotypes responded differently to the salinity stress as judged from the visual appearance; however, most genotypes are moderately tolerant and rated in the scale classes 1 to 3 (Fig. 1). Of the thirteen genotypes screened, four (30.77 %) were slightly affected and fell between scale classes 1.00 to 1.75, including HW96, Sida, KKU Sida Huai-sai, Cln 1463A×SK8-1 (Table 1). For the most tolerant genotype HW96 (scale = 1), the plants were able to effectively tolerate the 200 mM NaCl treatment, remained unaffected and appeared as healthy as the control plants. The remaining

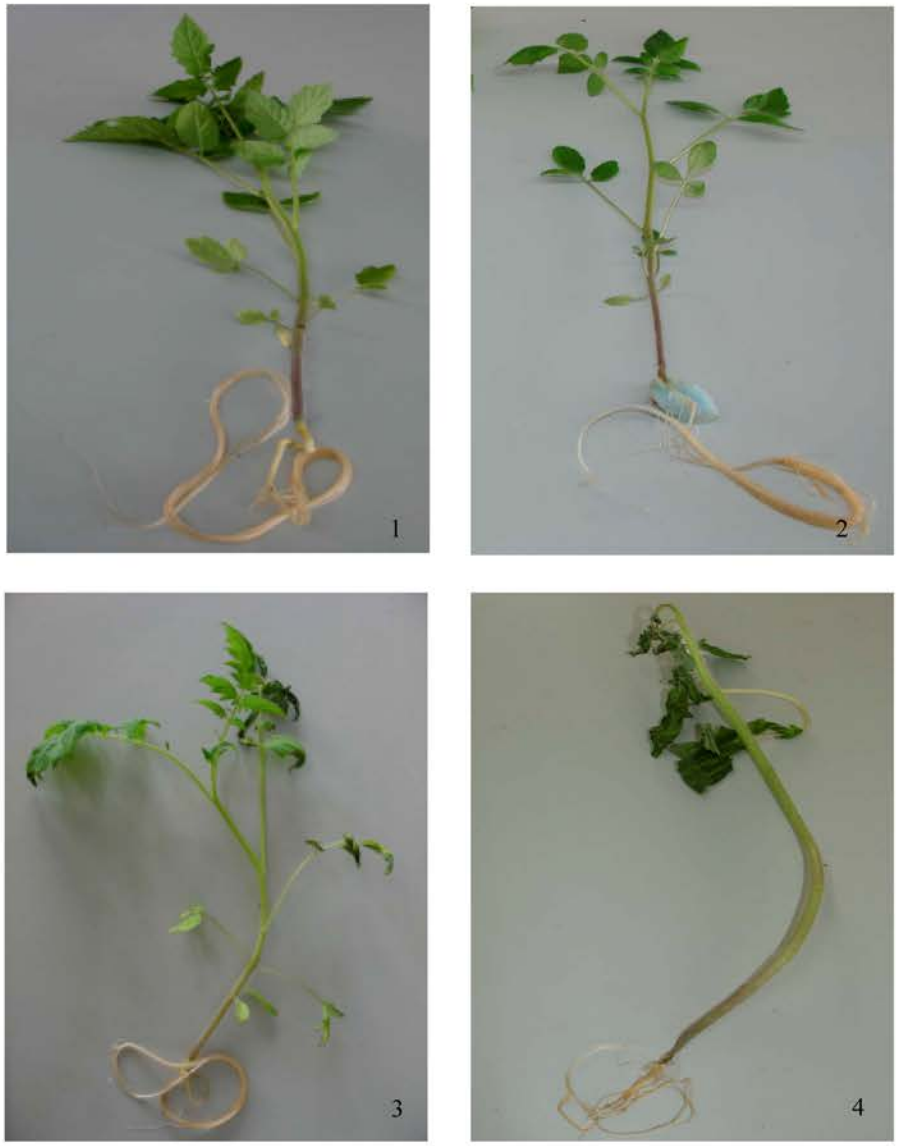

Fig. 1: The salinity scale classes used in the experiment. (1) normal green plants with turgid expanded leaf lamina; (2) plants smaller than controlled plants, leaves are green with the edge of the lamina slightly curved downward; (3) older leaves completely wilt, younger leaves partly wilt and (4) all leaves completely wilt

three genotypes were only mildly affected, some plants were almost normal, while others were slightly smaller than normal and showed some degrees of wilting in the older leaves. Eight (61.54%) genotypes, namely, CLn463A x Ace55VF, KKU40, KKU Sida Somtum hybrid, red sweet, Moneymaker, perfect89, extra390 and KKU Sida hybrid were moderately damaged and obtained the tolerance scales between 2.00 to 2.75. Plants in this group showed varying degrees of wilting of older leaves and reduction in stem diameter and leaf area compared with the set of control plants. The most sensitive genotype CH154, which was rated 3.75, suffered from severe wilting of both younger and older leaves.

In comparison with the control plants, tissues of salt-treated plants accumulated more Na^+ but less K^+ and Ca^{2+} (data not shown), resulting in lowered K^+/Na^+ and Ca^{2+}/Na^+ ratios. Tomato genotypes showed great differences in Na^+ concentration under the 200 mM NaCl stress. It has been ranged from 0.940 to 1.620 mmol g^{-1} dry weight (Table 1). There was a highly significant

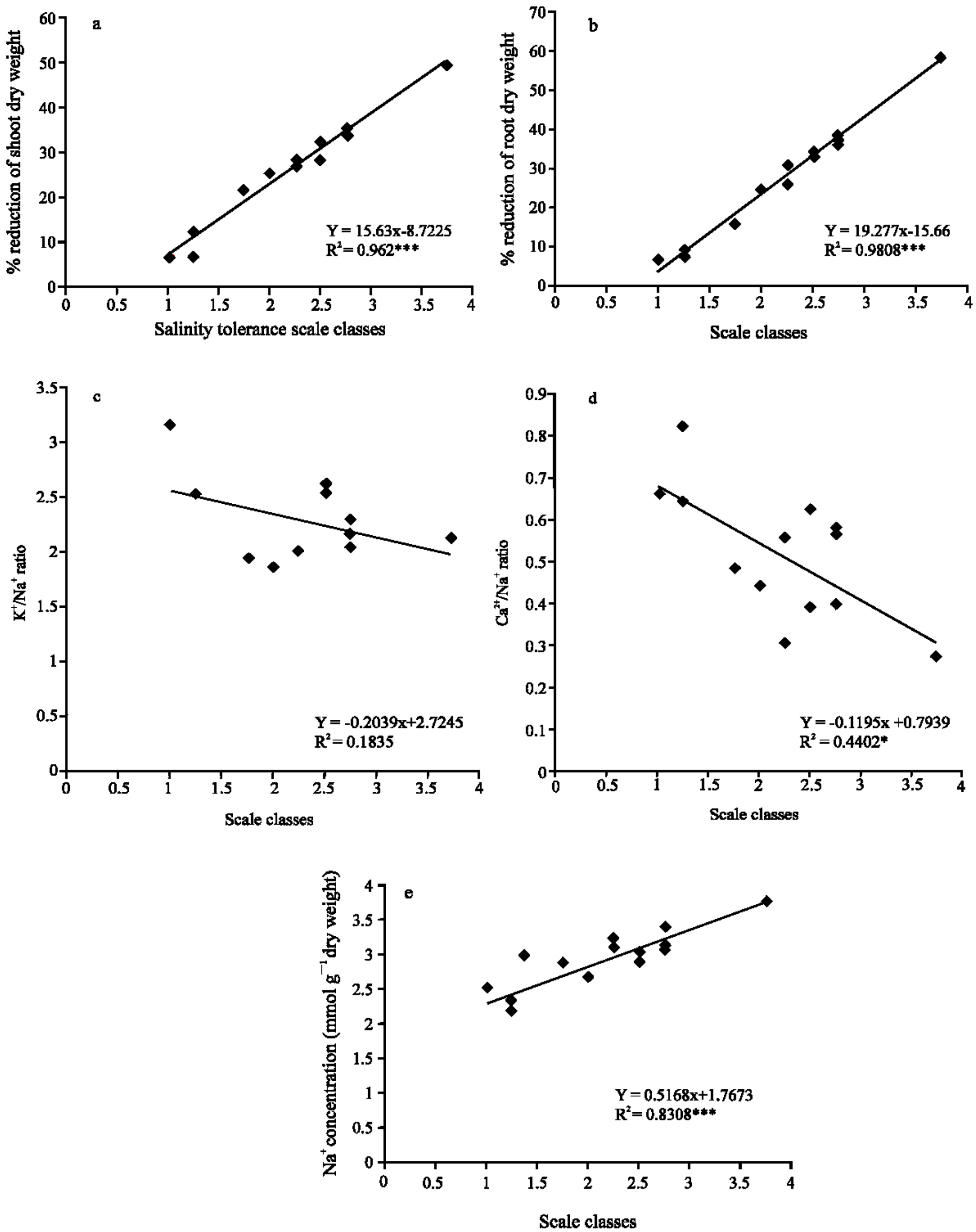

Fig. 2: Relation between the salinity tolerance scale classes and (a) percent reduction of shoot DW, (b) percent reduction of root DW, (c) K^+ /Na^+ ratios, (d) Ca^{2+}/Na^+ ratios and (e) Na^+ concentration of the tomato genotypes grown under saline condition. n = 13, *p = 0.05, **p = 0.01 and ***p = 0.001. Increasing scale classes from 1-4 indicates increases in salt damages

positive correlation (r^2 = 0.8308***) between the scale classes and Na^+ concentrations (Fig. 2e). Genotypes with higher Na^+ concentrations are included in the higher scale classes showing more injury and those with lower Na^+ concentration were in the lower scale classes with less damage. The genotypes in scale classes 1 and 2 are considered having a better Na^+ exclusion mechanism than the genotypes in scale classes 3 and 4, respectively. Different genotypes showed less variation in the amounts of K^+ and Ca^{2+} under 200 mM NaCl stress. Genotypes with higher K^+/Na^+ ratios tended to be rated in the lower scale classes showing less salt damage and those with lower K^+/Na^+ in the higher scale classes (Table 1). However, the K^+/Na^+ ratios showed no significantly correlation (r^2 = 0.1835) with the salinity scale classes (Fig. 2c). The Ca^{2+}/Na^+ ratios, on the other hand, showed significant negative correlation (r^2 = 0.4402*) with the salinity scale classes (Fig. 2d). Genotypes with higher Ca^{2+}/Na^+ ratios are included in lower scale classes with less salinity damage. In our study, there was higher correlation between Ca^{2+}/Na^+ ratios and scale classes (r^2 = 0.4402*) than the correlation between K^+/Na^+ ratios and scale classes (r^2 = 0.1835). Under the 200 mM NaCl stress, the genotypes with higher K^+ concentrations had lower Ca^{2+} concentrations. In all genotypes studied, nitrogen content in salt-treated plants declined compared with the control plants, whereas phosphorous content raised slightly (data not shown). However, no significant correlations were found between either N (r^2 = 0.005) or P (r^2 = 0.039) content in the salt stressed plants with the scale classes.

Tomato genotypes grown under 200 mM NaCl stress showed great variations in shoot and root DW and percent reduction in shoot and root DW compared with the controls (Table 2). Shoot and root DW of the most tolerant genotype, HW96 (scale class 1.00) showed only 6.01 and 7.32% reduction from the control plants. The reduction in shoot and root DW of the two genotypes with scale class values of 1.25 were 5.74 and 9.47% for KKU Sida Huai-sai and 12.39 and 6.44% for Sida. In contrast, the most sensitive genotype, CH154 (scale class 3.75) suffered from 48.61 and 58.09% reduction in shoot and root DW, respectively. Highly significant correlations were found between percent reduction in shoot DW with the scale classes (r^2 = 0.962***, Fig. 2a) and root DW with the scale classes (r^2 = 0.9808***, Fig. 2b). These indicated that plant shoot and root growth was highly dependent on salt tolerance at the growth stage reached in this study.

Table 2: Salinity tolerance scale class, shoot DW, percent reduction in shoot DW, root DW and percent reduction in root DW of tomato genotypes grown under saline condition for 10 days

Genotypes	Scale (1-4)[a]	Shoot DW (g/plant)	% reduction in shoot DW	Root DW (g/plant)	%reduction in root DW
Sida	1.25ab	3.25c-e	12.39	0.98cd	6.44
Perfect89	2.75cd	2.45b	32.04	0.67b	38.30
Extra390	2.75cd	2.33 b	34.94	0.67b	37.03
KKU40	2.25cbc	2.73bc	28.32	0.73b	31.28
HW96	1.00a	3.48e	6.01	1.01d	7.32
CH154	3.75d	1.90a	48.61	0.44a	58.09
KKU Sida hybrid	2.75cd	2.41ab	35.17	0.69b	36.11
KKU Sida Somtum hybrid	2.25bc	2.65b	26.57	0.78bc	25.29
KKU Sida Huai-sai	1.25ab	3.33de	5.74	0.95cd	9.47
Red sweet	2.50c	2.59b	27.38	0.69b	34.44
CLn 463 A×Ace 55VF	2.00a-c	2.75bc	25.20	0.81b-d	24.23
Cln 1463 A×SK 8-1	1.75a-c	2.83b-d	21.40	0.86b-d	15.68
Moneymaker	2.50b	2.55c-h	32.09	0.72b	33.33

[a]Increasing scale classes from 1-4 indicates increases in salt damages. Means in the same column followed by different letter(s) differ significantly at $p<0.05$

DISCUSSION

Accumulation of harmful Na^+ and Cl^- and retardation in the uptake of macronutrients especially, K^+ and Ca^+, are regarded as one of the most important consequences of salinity stress causing a reduction in plant growth and salt injury leading to plant death. Although, cultivated tomato is generally classified as being moderately salt-sensitive, different genotypes of tomato displayed widely different degrees of salinity tolerance (Alian *et al*., 2000; Dasgan *et al*., 2002; Juan *et al*., 2005). Under our experimental conditions using hydroponic culture containing 200 mM NaCl, thirteen tomato genotypes were classified into salinity tolerance scale classes from 1 to 4 according to increasing degrees of leaf wilting which appeared first in the older leaves. In contrast, in the screening of 55 commercial cultivars and breeding lines grown in potting medium to which 200 mM NaCl was added, Dasgan *et al*. (2002) have determined five scale classes and the wilting symptoms appeared first in the younger leaves.

The thirteen genotypes may be divided into three groups according to the degree of severity of plant damage and reduction in plant growth. The first, most tolerant group included those being scaled 1.00 and 1.25 (HW96, Sida and KKU Sida Huai-sai). Salt-treated plants in this group were only slightly affected showing no signs of wilting. The reduction in root growth was lower than 10% and that of shoot was between 5.74-12.39%. Sodium ion concentration and Ca^{2+}/Na^+ ratios of these four genotypes did not differ significantly. The second, moderately tolerant group, comprising nine genotypes (CLn1463 A×SK 8-1, CLn 463×A Ace 55VF, KKU40, KKU Sida Somtum Hybrid, Moneymaker, Red sweet, perfect89, extra390 and KKU Sida Hybrid) obtained the scales ranging from 1.75-2.75. These plants survived the period of 10 day salt stress but showed varying degrees of wilting of older leaves. The reduction in shoot and root dried weight ranged from 21.4-35.17% and 15.68-38.30%, respectively. The most sensitive group was represented by CH154 (scored 3.75). The growth of this genotype was severely inhibited, with 48.61 and 58.09% reduction in shoot and root dried weight, respectively. After ten days of exposure to NaCl, young as well as old leaves completely wilted. Salt stressed plants of CH154 accumulated the highest amount of sodium ion and showed lowest Ca^{2+}/Na^{+} ratio.

The most tolerant variety, HW96, belongs to *L. pimpinellifolium* which is well-documented for its high level of salinity tolerance compared to *L. esculentum* (Bolarín *et al.*, 2001). *L. pimpinellifolium* has been effectively used as donors in conventional breeding programs to improve salt tolerance (Monforte *et al.*, 1997). Information on salt tolerance of tomato grown in Thailand are seriously lacking. Most studies on tomato improvement in Thailand have been concentrated on yield, fruit quality and disease resistance. In this study, we found that most of the popular commercial cultivars or hybrids tested fell in the scale classes ranging from 1.25-2.75 and are therefore moderately tolerant to salinity.

In order to determine the relationship between salt tolerance, as indicated by scale classes and plant biomass production, we calculated both the correlation coefficients between scale classes and the shoot/root DW of salt stressed plants and between scale classes and percent reduction in shoot/root DW compared with the control plants. We found higher correlation between scale classes and percent reduction in DW than between scale classes and absolute values of DW. Strong significant correlation between tolerance scale classes and percent reduction in shoot and root DW indicated that growth of tomato plants at this stage of development is highly dependent on salt tolerance. In contrast, Dasgan *et al.* (2002) working with 55 tomato genotypes found no relation between shoot-root DW and scale classes. This lack of correlation may be due to large differences in growth potential of different genotypes. In an investigation of the relationships between tolerance and growth in five species of *Lycopersicon*, it was found that if growth of salinity-treated plants was measured in absolute term, two cultivars of *L. esculentum* performed as well as *L. pimpinellifolium* and better than *L. pennellii* and *L. cheesmanii* and *L. peruvianum* showed the poorest tolerance. In relative terms, *L. pimpinellifolium* was tolerant than *L. esculentum* and *L. peruvianum* was the most sensitive and *L. cheesmanii* was little more tolerant than *L. peruvianum* (Cuartero *et al.*, 1992). Therefore, it would be useful to grow a set of controlled non-salinized plants and the growth reduction used as the screening parameter.

It is evident from the results that NaCl caused distortions in the ion contents by raising Na^{+} and diminishing those of K^{+} and Ca^{2+} compared with the control plants grown in non-saline solutions. Significant correlations were found between salt damage as indicated by tolerance scale classes and Na^{+} concentration (r^2 = 0.8308***), K^{+}/Na^{+} (r^2 = 0.1835) and Ca^{2+}/Na^{+} (r^2 = 0.4402*) ratios. Similar values were reported by Dasgan *et al.* (2002) i.e., r^2 = 0.763, 0.396 and 0.498 for correlations between tolerance scale classes and Na^{+} concentration, K^{+}/Na^{+} and Ca^{2+}/Na^{+} ratios, respectively. Moreover, we found the similar trend in the relationship between these three ionic parameters and total plant dry weight. The correlations between total plant DW and Na^{+} concentration, K^{+}/Na^{+} and Ca^{2+}/Na^{+} were 0.8438, 0.3322 and 0.5103, respectively. Therefore, these ionic composition parameters are extremely reliable as indicators for screening salt tolerance reflecting both the degree of damage due to toxic Na^{+} and the growth reduction. These parameters have been used to evaluate salt tolerance in different cultivars of several cultivated crops. In a field evaluation using 23 rice genotypes, Asch *et al.* (2000) found that more tolerant genotypes had higher K^{+}/Na^{+}, measured in young leaves at late vegetative stage, than less tolerant and sensitive ones. Moreover, strong correlations were found between leaf K^{+}/Na^{+} and salinity-induced grain yield reduction. Under salt stressed greenhouse condition test of eight wheat genotypes, the tolerant genotypes had higher shoot K^{+}/Na^{+} than the sensitive ones and strong correlation was found between shoot K^{+}/Na^{+} and dry matter in plants treated with 150 mM NaCl (Houshmand *et al.*, 2005). However, no relationship was observed between salt tolerance level and Ca^{2+}/Na^{+} ratio due to supplementation of Ca^{2+} in the culture solution. The results from this preliminary study provided useful information for future work on development of salinity-tolerant tomato in Thailand.

ACKNOWLEDGMENTS

This study was financially supported by Thai Government's University Staff Development Program to Mahasarakham University. We are indebted to Prof. Dr. Tony Chen for the supply of tomato seeds cultivar Moneymaker. We also acknowledge Assoc. Prof. Dr. Sucheela Tachawongsatien from Faculty of Agriculture, Khon Kaen university for kindly providing tomato seeds.

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