

Full Length Research Paper

Biochemical/physiological characterization and evaluation of *in vitro* salt tolerance in cucumber

Ahmed Abbas Malik, Wei-Guan Li, Li-Na Lou, Jia-Hua Weng and Jin-Feng Chen*

State Key Laboratory for Crop Genetics and Germplasm Enhancement, Key Laboratory of Southern Vegetable Genetic Improvement, Nanjing Agricultural University, China.

Accepted 26 April, 2010

The difference in biochemical and physiological parameters of selected tolerant, medium tolerant and sensitive genotypes of cucumber (*Cucumis sativus* L.) derived from *in vitro* screening was investigated in order to put forward the relative tolerance or sensitivity of the genotypes and to identify parameters that can be used as index for *in vitro* evaluation of salt tolerance in cucumber. On the basis of our comparative analysis, the salt tolerant genotype (Hazerd) successfully tolerated highest salinity level (120 mM) by accumulating significantly higher levels of free proline and exhibited higher antioxidant enzyme (superoxide dismutase (SOD) and peroxidase (POD)) activities than the moderately tolerant (Poinsett 97 and Pingwang) and sensitive genotypes (HH1-8-57 and L6). The tolerant genotype (Hazerd) showed less vulnerability against high salinity by showing low lipid peroxidation and electrolyte leakage with slight reduction in photosynthetic pigment. Furthermore it seems that higher salinity tolerance in the tolerant genotype also correlated to limited translocation of Na⁺ ions to leaves resulting in the maintenance of high K⁺/Na⁺ ratio. Soluble sugars and protein showed decreased with increasing salinity in all the genotypes tested irrespective of their tolerance level. Taken together, our data partly explain the mechanism use to avoid salt stress by cucumber plants, when excessive in the culture medium.

Key words: *Cucumis sativus* L., salinity, sodium chloride.

INTRODUCTION

Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality. Management of water and land can be successful in reclamation of salt affected soil (Brestein, 1975), but the most economical and effective means is to plant crops that can establish and be productive on such soils. Therefore, impact of salinity on plant can also be managed through biological manipulating the plant (Rains, 1981). Identification of plant genotypes capable of increased tolerance to salt and incorporation of these desirable traits into economically

useful crop plants may reduce the effect of salinity on productivity.

Cucumber (*Cucumis sativus* L.) is one of the main crops widely grown all over the world. Cucumber is moderately sensitive to salinity (Ayers and Westcot, 1985; Dorota, 1997). Salt stress in cucumber involves both osmotic stress, by limiting absorption of water from soil, and ionic stress, resulting from high concentrations of potentially toxic salt ions within plant cells (Savvas et al., 2005). The synthesis and accumulation of compatible solutes is a ubiquitous mechanism for osmotic adjustment in plants (Trajkova et al., 2006). Among the antioxidative defense system in cucumber, antioxidant enzymes play an important role in scavenging ROS through series of complex reactions. These reactions include the dismutation of superoxide anion (O₂⁻) to hydrogen peroxide (H₂O₂) by superoxide dismutase (SOD) and detoxification of H₂O₂ by various enzymes like peroxidase (POD) and Catalase (CAT) (Zhou et al., 2003; Zhu et al., 2008). Measurement of cell membrane stability has been widely used to

*Corresponding author. E-mail: jfchen@njau.edu.cn. Tel/Fax: +86-025-84396279.

Abbreviations: SOD, Superoxide dismutase; POD, peroxidase; CAT, catalase; MS, Murashige and Skoog; BAP, benzyl amino purine; TI, tolerance index, DW, dry weight; FW, fresh weight; MDA, malondialdehyde; Chl, chlorophyll; EDTA, ethylenediaminetetraacetic acid; PVP, polyvinylpyrrolidone.

differentiate stress-tolerant and susceptible cultivars (Alpaslan and Gunes, 2001; Zhang et al., 2001).

To ensure future productivity of the agricultural regions, there is a need to select and characterize salt-tolerant plants. In order to improve salt tolerance through breeding, genetic variability for the trait is required. Although response of cucumber plants to salinity has been discussed earlier, variation in salt tolerance if any among different cultivar of cucumber plants has not been worked out in detail. Biochemical and physiological criteria are able to supply more objective information than agronomic parameters or visual assessment when evaluating for component traits of complex characters (Yeo, 1994). In spite of numerous published researches, no well-defined indicators are available to facilitate the improvement of salinity tolerance in cucumber. There is therefore a need to determine the underlying biochemical/physiological mechanisms of salinity tolerance so as to provide breeders with appropriate and standard indicators to introduce genetic or environmental improvement to salt tolerance in cucumber.

To evaluate salinity tolerance, a number of models for the response of plants to salinity have been defined. However, the evaluation of a large number of genotypes for salinity tolerance under *ex-vitro* conditions is rather difficult as it entails a large amount of resources and space. Similarly, the determination of absolute salt tolerance under *ex-vitro* conditions also poses difficulties because of the complex interactions existing between the plant and different soil components as well as seasonal fluctuations. A number of researchers have suggested that screening for salt tolerance could be more effective if the assessment was undertaken under controlled environmental conditions and using biochemical and physiological markers/traits rather than selecting for yield and yield components under saline soil conditions (Shannon and Noble, 1990; Flowers and Yeo, 1995). *In vitro* culture is an ideal system for evaluating saline tolerant plants as it can be carried out under controlled conditions with limited space and time (Ghosal and Bajaj, 1984; Munns et al., 2000). Axillary bud/shoot apex culture has been found to be an effective method for isolating salt-tolerant genotypes from a large population within a short period of time (Martinez et al., 1996; Cano et al., 1998). *In vitro* techniques have been employed with success in several other crops (Mungala et al., 2008; Vijayan et al., 2003; Erturk et al., 2007).

It should be mentioned that in our previous *in vitro* screening experiment, 31 cucumber ecotypes were used comprising of wild, commercial cultivars (greenhouse and open filed type) and inbreed lines from China, USA, Korea, India etc (data not presented). The genotypes were grouped as tolerant, medium tolerant, sensitive and highly sensitive on the basis of survival and growth (fresh shoot weight and dry shoot weight). The only genotype which survived the highest sodium chloride (NaCl) level (120 mM) successfully was Hazerd. The genotypes selected for the present investigation is the representative of each salinity group from the previous screening

experiment which shows highly consistent results in all the replication throughout the experimental period, except highly sensitive genotypes which are unable to grow on the lowest salinity level. The present study was conducted to evaluate the biochemical/ physiological performances of selected tolerant, medium and salt sensitive cucumber genotypes derived from *in vitro* screening to put forward their tolerance or sensitivity, and also to identify parameters that can be used as index for *in vitro* salt tolerance in cucumber.

MATERIALS AND METHODS

Plant materials and salinity treatments

The experimental materials consisted of five cucumber genotypes including one salt tolerant "Hazerd" (USA), two medium tolerant "Poinsett 97" (USA), "Pingwang" (China), salt sensitive inbred lines HH1-8-57 (China) and L6 (south china) derived from *in vitro* screening (data not presented). All the genotypes used in the present experiment were open pollinated. Hazerd is a commercial cultivar mostly grown in green house or under plastic tunnel while Pingwang and Poinsette 97 are cultivated under both green house and open field condition. The HH1-8-57 is an introgression line derived from cross between *Cucumis hystrix* chakr. ($2n = 24$) and commercial cucumber cultivar Beijing jietou ($2n = 14$), whereas L6 is a parthenocarpic cucumber from south China. All the seed materials were obtained from the State Key Laboratory for Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, P.R China. Based on the results of previous *in vitro* screening experiment, the salinity was induced in the medium by the addition of various concentration of NaCl viz. 40, 80 and 120, 150 mM and using *in vitro* grown excised axillary shoot tips as explants in this study.

Shoot multiplication and selection procedure

Axillary shoot tips explants of different cucumber genotypes developed from seeds were used in the present study. Seeds were soaked in tap water for 15 min. The seeds were surface sterilized with 70% alcohol for 30 s and then kept in 0.1% mercuric chloride solution (w/v) for 5 min. Finally the seeds were rinsed four times in sterile distilled water to remove the sterilant. The seeds were then air dried in a laminar flow hood and subsequently germinated in darkness for 48 h on sterile moist cotton. Shoot tips each originating from a different seed and consisting of an apical bud with one adjacent leaf pair were excised and used as explants. The excised explants were inoculated on MS medium (Murashige and Skoog, 1962) supplemented with 0.5 mg l^{-1} benzyl amino purine (BAP) concentrations. At the end of the second subculture, single-node shoots were excised from the proliferating cultures and subjected to four different NaCl concentrations in Murashige and Skoog medium supplemented with 0.2 mg l^{-1} BAP concentrations. The pH of all the media was adjusted to 5.8 before autoclaving at 121°C for 20 min. The cultures were kept at $25 \pm 2^\circ\text{C}$ with a 16 h photoperiod under diffused cool-white fluorescent lamps ($80 \mu \text{ mol m}^{-2} \cdot \text{s}^{-1}$).

Subsequent to the 20 days salinity treatments, explants were removed from the media washed with sterile water and evaluated for their response to salinity. Youngest fully expanded leaves were harvested, immediately freeze dried in liquid nitrogen and then subsequently stored at -70°C till further analysis.

Tolerance index (TI)

It was used to summarize the general effect of 4 different NaCl con-

centrations on different cucumber genotypes and to compare the cucumber genotypes on the basis of reactions to salt treatment, eliminating growth differences according to genotypes. The dry weight (DW) of the plants cultured on various concentrations of NaCl was measured after drying the samples at 70°C for 72 h. The tolerance index was determined as (LaRosa et al., 1989) $[FW \text{ or DW on NaCl medium } (T_x) / FW \text{ or DW on NaCl free medium } (T_o)] \times 100$.

$$T_i = (T_x/T_o)100$$

Where FW = Fresh weight; DW = dry weight.

Cell membrane damage

Malondialdehyde (MDA) activity was determined to indicate the level of lipid peroxidation as described by Zhao (2000). Electrolyte leakage was measured using an electrical conductivity meter as described by Liu et al. (1985).

Soluble sugar and proline content

Soluble sugars were determined by the anthrone method (Spiro, 1966), a calibration curve with D-glucose was done as a standard. Free proline content was determined according to Bates et al. (1973), proline concentration ($\mu\text{g g}^{-1}$ FW) was determined from a standard curve.

Chlorophyll pigments determination

0.2 g of leaf samples from each group were homogenized with 80% acetone (v/v) and then the homogenate was filtered through filter paper. Absorbency of the resulting solution was read at 663 and 645nm for chlorophyll a (Chl-a) and chlorophyll b (Chl-b), respectively (Arnon, 1949).

Enzyme assay and protein determination

Frozen leaf segments (0.2 g) were homogenized in 0.1M Tris HCl buffer, pH 7.5, 0.5 mM ethylenediaminetetraacetic acid (EDTA) and 1% polyvinylpyrrolidone (PVP), at 4°C. The homogenate was centrifuged at 15,000 g for 20 min at 4°C and the supernatant was immediately used for enzyme assays.

An aliquot of the extract was used to determine protein content by the method of Bradford (1976) utilizing bovine serum albumin as the standard. Total SOD activity was assayed by monitoring the inhibition of photochemical reduction of 50% nitro blue tetrazolium according to the method of Giannopolitis and Ries (1977). SOD activity values are given in units per gram of protein (Martinez et al., 2001).

CAT activity was done according to Cakmak and Marschner (1992). The reaction mixture in a total volume of 2 ml contained 25 mM sodium phosphate buffer (pH 7.0), 10 mM H₂O₂. The reaction was initiated by the addition of 0.1 ml of enzyme extract and activity was determined by measuring the initial rate of disappearance of H₂O₂ at 240 nm for 40 s. Peroxidase activity was determined using the guaiacol oxidation method (Kochba et al., 1977) in a 3 ml reaction mixture containing 100 mM phosphate buffer (pH 6.0), 8 mM guaiacol, 0.1 ml enzyme extract and 2.75 mM H₂O₂. The increase in absorbance was recorded at 470 nm for 40 s within 3 min after enzyme extract was added. A unit of peroxidase and catalase activity was expressed as the change in absorbance per minute and specific activity as enzyme units per gram soluble protein.

Sodium and potassium analysis

The tissue concentrations of Na⁺ and K⁺ in leaf blades were measured on a dry weight basis (Thomas et al., 1967). The samples were ground to pass a 20 mesh sieve and digested with a mixture of H₂SO₄-H₂O₂ using microwave energy, modified technique of Lachica et al. (1973). Sodium and potassium content was measured from acid-digested samples using atomic absorption spectrophotometry (Perkin-Elmer 3100, Norwalk, CT, USA) and also expressed as relative values.

Statistical design and analysis

Flasks were placed in randomized complete block (RCB) design on shelf, 60 explants were used from each genotype in each treatment, and the experiment was repeated 2 times. All data were analyzed using statistical package for the social sciences (SPSS) software. When ANOVA showed significant treatment effects, Duncan's multiple range tests were applied to compare the means at P < 0.05 (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Analysis of variance

In the present study, salt stress treatments of 0, 40, 80 and 120 mM were used. Analysis of variance (Table 3 and 4) revealed significant differences amongst genotypes and genotype \times salt stress level interaction for all the physiological and biochemical parameters, indicating the existence of genetic variability amongst the genotypes and differential response of the genotypes to different salt stress levels.

Tolerance index

On the basis of survival and growth performances at specific salt level, the genotypes were categorized as tolerant, medium tolerant and sensitive. All the tested genotypes survived the lowest level (NaCl 40 mM), but neither survived the highest level (NaCl 150 mM) of induced salinity. The tolerant genotype Hazerd exhibited 100% survival and growth on the medium containing 120mM NaCl, whereas the genotype Poinsett 97 and Pingwang successfully tolerated 80mM and thus ranked as the medium tolerant genotypes. The sensitive genotypes (L6 and HH1-8-57) endured only the lower salinity level (40 mM NaCl). The effect of NaCl on the FW and DW shoot growth weight of *in vitro* plantlets is presented in Table 1. The genotype Hazerd showed reduction in fresh shoot weight of 8, 16, 30% and dry shoot weight of 11, 14 and 36% at 40, 80 and 120 mM, compared to the control, respectively. A significant loss of the fresh shoot growth (55 and 38%) and dry shoot weight (53 and 43%) was observed in the salt sensitive genotypes (L6 and HH1-8-57) at their highest surviving salinity level (40 mM NaCl) than the plants grown on the control medium, respectively.

In order to judge the tolerance of plants to salinity, growth

Table 1. Effect of NaCl on shoot growth (tolerance index), MDA content, electrolyte leakage, soluble protein and percent soluble sugar of cucumber genotypes differing in salinity tolerance.

Genotype	Salt stress	Tolerance index (FW)	Tolerance index (DW)	MDA content ($\mu\text{mol g}^{-1}$ FW)	Electrolyte leakage (%)	Soluble protein (mg g^{-1})	Percent soluble Sugar (%)
Hazerd	Control	100a	100a	3.04cd	21.19g	387.88cd	0.35ab
	40 mM	91.95a	89.4b	3.06cd	39.81f	395.75bc	0.37a
	80 mM	83.91b	85.9b	3.24bcd	54.77e	347.94f	0.23e
	120 mM	68.38cd	63.9d	3.7bc	66.07cd	325.77g	0.14fg
Pingwang	Control	100a	100a	3.29bcd	21.73g	401.29b	0.317bcd
	40 mM	78.82b	77.5c	2.69d	67.74cd	246.08i	0.29cd
	80 mM	54.49ef	51.7ef	3.89b	66.29cd	197l	0.14fg
Poinsett 97	Control	100a	100a	3.01cd	20.68g	497.61a	0.33abc
	40 mM	69.73c	70.1d	3.51bc	63.97d	367.34e	0.32bc
	80 mM	52.75e	48.9f	4.86a	83.77a	280.73h	0.13fg
HH1-8-57	Control	100a	100a	3.47bc	25.1g	363.88e	0.31bcd
	40 mM	44.51g	47.2f	3.95b	79.21ab	213.11k	0.11g
L6	Control	100a	100a	2.69d	28.49g	379.61d	0.27d
	40 mM	61.59de	56.7e	3.72bc	73.86bc	233.2 j	0.17f

Values in each column followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range tests.

and survival of the plant is measured because this is the culmination of many biochemical and physiological mechanisms occurring within plants. Analysis of the plant growth showed variability in salt responses within cucumber genotypes, depending upon the salinity level. Inhibition of growth by salt stress has been universally observed even in tolerant plant species (Jones et al., 1989; Mittler et al., 2001). Although growth is the visible indicator of plant performance under stress, it is considered to result from the sum of the adaptive mechanisms that are adopted by a given species. The cucumber is considered as moderately salt sensitive crop (Alan, 1994), but in recent studies a significant tolerance was found in genotype Hazerd after screening from a large stock of cucumber cultivars and highly inbred lines (data not shown). Genotypic differences to salinity have also been reported in sunflower (Wahid et al., 1999). Similarly, growth rate was less affected in salt tolerant sugar beet and moderately salt tolerant cotton (Greenway and Munns, 1980).

Cell membrane damage

Effect of salt stress on the plant tissues were determined by measuring the MDA content (Table 1), which is the product of lipid peroxidation. The membrane damage is indirectly assessed by the conductivity of solute leakage from the cells (Table 1). The electrolyte leakage and MDA content of all the genotypes is correlated with increasing salinity in the medium. The percent solute leakage and MDA content was significantly higher in the salt sensitive genotypes (L6 and HH1-8-57) as compared to medium (Poinsett 97 and Pingwang) and tolerant

genotypes (Hazerd) at varying level of salinity. The difference in MDA content between the salt treated and the control plantlets of the tolerant genotype (Hazerd) was not significant at different NaCl level. As peroxidation of membrane lipids and electrolyte leakage is an indication of membrane damage and leakage under the salt stress conditions (Katsuhara et al., 2005), growth inhibition in salt sensitive and medium tolerant genotypes under salinity is in good correlation with increased lipid peroxidation levels. Low level of electrolyte leakage and lipid peroxidation may be one of the reasons for the observed tolerance in the tolerant genotype (Hazerd) exposed to high level of salinity. Similarly, a lower level of lipid peroxidation in the leaves of salt tolerant tomato (Shalata and Tal, 1998) and cotton (Meloni et al., 2003) were recorded under salt stress.

Soluble protein

Soluble protein content decreased significantly with increasing salinity in the medium except tolerant genotype (Table 1), which showed increase protein content at lower salinity level (NaCl 40mM), and then slightly decreased at higher salinity levels (80 and 120 mM NaCl). The differences in total soluble protein content among the sensitive, medium and salt tolerant genotypes were significant. The increase in soluble protein at low salinity and decreases at high salinity has already been observed in mulberry cultivars (Agastian et al., 2000). It could be predicted that plants under stress would have a powerful protein turnover machinery to degrade stress-damaged and environmentally regulated proteins (Abdel et al., 2003).

Table 2. Effect of NaCl on proline, chlorophyll pigment, potassium, sodium and potassium/sodium ratio of cucumber genotypes differing in salinity tolerance.

Genotype	Salinity level (NaCl)	Proline ($\mu\text{g g}^{-1}$ FW)	Chlorophyll Pigment (mg g^{-1} FW)	Potassium (mg g^{-1} DW)	Sodium (mg g^{-1} DW)	Sodium/potassium ratio
Hazerd	Control	22.27h	7.82a	30.64a	1.03h	29.72a
	40mM	88.26c	6.95b	22.39cd	8.82g	2.68c
	80mM	118.89b	5.63d	14.4ef	14.54e	0.97c
	120mM	137.51a	3.53g	9.45fg	20.18c	0.50c
Pingwang	Control	35.86g	6.73c	24.24bc	0.95 h	25.59ab
	40mM	69.83d	3.91f	15.11ef	11.94 f	1.28c
	80mM	82c	2.9h	9.2fg	36.01 b	0.255c
Poinsett 97	Control	24.75h	6.06d	26.08abc	0.98 h	26.6a
	40mM	59.41e	4.17e	17.11de	9.91 g	1.72c
	80mM	84.26c	3.64g	8.21 g	40.27a	0.2c
HH1-8-57	Control	25.26h	3.79f	28.59 ab	1.05 h	27.15a
	40mM	47.11f	2.77h	11.08fg	17.37d	0.64c
L6	Control	27.21h	4.11e	24.97 abc	1.12 h	22.30b
	40mM	50.13f	2.81h	10.77 fg	18.56cd	0.58c

Values in each column followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range tests.

Soluble sugars

In general, percent soluble sugars decreased in response to the salinity stress (Table 1). However, percent soluble sugars in the leaves of genotype Hazerd (tolerant) gradually increased 40 mM NaCl, and decreased steadily on higher NaCl levels. Soluble sugars contents in the leaves of other genotypes showed a decreasing pattern with increasing salinity; more prominent decrease was recorded in the salt sensitive than medium salt tolerant genotypes. The decrease in sugars accumulation due to salt treatment might be associated with salinity induced decrease in pigment content which impaired metabolic activities in plants (Upadhaya et al., 1981). In *Lens culinaris* (Ashraf and Waheed, 1993) and sunflower (Ashraf and Tufail, 1995), it has been observed that the salt stress resulted in decrease percent soluble sugars, but decrease was significantly less in tolerant accessions than the non tolerant ones.

Free proline content

Proline content increased significantly in the leaves of all the genotypes as the salt concentration increased (Table 2). The most tolerant genotype Hazerd accumulated 6 folds proline, while the medium tolerant genotypes Pingwang and Poinsett 97 accumulated 2.4 and 3.5 folds proline as compared to the control, respectively. Lower but significant proline accumulation was noted in the sensitive genotypes, that is HH1-8-57 (1.88 fold) and L6

(1.9 folds) at their highest survival salinity level (40 mM NaCl). Proline plays an adaptive role in mediating osmotic adjustment and protecting the sub-cellular structures in stressed plants. In many studies a positive correlation between the accumulation of proline and stress tolerance in plants has been found (Lutts et al., 1996; Kumar et al., 2003). Higher proline content in genotype Hazerd might be the one of the reason for higher salt tolerance when compared to other genotypes (medium tolerant and sensitive).

Chlorophyll pigment

Chlorophyll pigment reduction was observed with increasing salt concentration in all the genotypes, but the reduction is more pronounced in the salt sensitive genotypes than the medium and tolerant genotypes (Table 2). As compared to control, 42% decrease in chlorophyll pigment in the tolerant genotype Hazerd was noted at 120 mM NaCl level, while the medium tolerant genotypes Pingwang and Poinsett 97 showed 39 and 60% loss of chlorophyll pigment at their higher salinity level (80mM NaCl), respectively. Whereas 32 and 27% decrease in chlorophyll pigment was recorded in the salt treated (40mM NaCl) and the control plantlets of sensitive genotypes HH1-8-57 and L6, respectively. Parida and Das (2005) suggested that decrease in chlorophyll content in response to salt stress is a general phenomenon. Chen and Yu (2007) also observed a significant decrease in chlorophyll content at high NaCl level.

Table 3. Analysis of variance for physiological and biochemical parameters.

Source of variation	df	Tol. index (FW)	Tol. index (DW)	MDA content ($\mu\text{mol g}^{-1}$ FW)	Electrolyte leakage (%)	Soluble protein (mg g^{-1})	Percent soluble sugar	Proline ($\mu\text{g g}^{-1}$ FW)
Treatment	19	3325.93**	3298.38**	5.67**	1912.30**	58649.52**	0.038**	3524.61**
Replication	1	122352.35**	119093.56**	231.55**	50791.27**	2150413.39**	1.165**	76169.25**
Genotype	4	3090.23**	2948.23**	3.75**	683.22**	69636.87**	0.038**	7138.85**
NaCl	3	14061.97**	14312.58**	14.05**	5124.60**	220332.57**	0.167**	3623.63**
Geno*NaCl	12	720.49**	661.55**	4.22**	1518.92**	14566.31**	0.005**	2295.11**
Error	20	8.51	6.80	0.06	9.49	19.87	0.000	9.57

df, Degrees of freedom; **significant at 0.05% level.

Table 4. Analysis of variance for physiological and biochemical parameters.

Source of variation	df	Chlorophyll pigment (mg g^{-1} FW)	SOD (U g^{-1} Protein)	POD ($\text{U g}^{-1} \text{min}^{-1}$ FW)	CAT ($\text{U g}^{-1} \text{min}^{-1}$ FW)	Potassium (mg g^{-1} DW)	Sodium (mg g^{-1} DW)	Sodium/potassium ratio
Treatment	19	13.336**	22074.54**	7198828.71**	1372.158**	229.05**	298.74**	264.07**
Replication	1	420.423**	828993.66**	283801039.80**	43027.040**	6362.50**	3320.77**	1976.83**
Genotype	4	24.836**	33525.91**	13529036.61**	1105.960**	129.45**	129.47**	7.65**
NaCl	3	46.391**	45936.89**	13434415.82**	6636.642**	1218.22**	633.21**	1650.52**
Geno*NaCl	12	1.239**	12291.83**	3529862.63**	144.769**	14.96**	271.55**	2.94**
Error	20	0.003	22.81	163.11	5.102	4.31	0.471	2.54

df degrees of freedom, **significant at 0.05% level.

Enzyme activity

In the present study, antioxidant enzyme activities changed significantly in response to the salinity stress. SOD and POD activities in the leaves of the tolerant genotype (Hazerd) increased with increasing salinity (NaCl: 40 and 80 mM) over the control plants, and then decrease slightly at higher salinity level (NaCl 120 mM) (Figure 1). Whereas, the medium tolerant genotypes also showed increasing trend with increasing salt concentration in the medium, but SOD and POD activities in the sensitive genotypes (HH1-8-57 and L6) decreases with increasing salinity as compared to the control. Unlike SOD and POD, CAT (Figure 1) showed a considerable decrease in its activity in response to the salt treatments in all the genotypes tested, irrespective of their tolerance level. At given concentration of NaCl, the decrease in the activity of the enzyme was more pronounced in sensitive genotypes than moderately tolerant and tolerant genotypes. SOD, POD and CAT were the main enzymes involved in the detoxification of the deleterious oxygen species (Mittova et al., 2003). In the present salt tolerance study, significantly higher SOD and POD activity found in the tolerant genotype (Hazerd) than the medium tolerant genotypes (Poinsett 97 and Pingwang) under increasing salinity stress signifies its relative tolerance to salinity, suggesting that the higher antioxidant enzymes activity

have a role in imparting tolerance to these genotypes against salt stress. It appears that the differences in SOD and POD enzyme activity have a direct relation to the sensitivity of the genotypes to salinity. These results showed that the salt-tolerant and medium tolerant plants have similar dismutating capacities of superoxide anion (Elkahoui et al., 2005). Similarly, CAT inhibition by salt stress was also observed in rye, Vigna and rice (Singha and Choudhuri, 1990; Hertwig et al., 1992).

Sodium and potassium ions concentration

Effect of salinity on Na^+ and K^+ concentrations of the plants is presented in Table 2; increased Na^+ contents were observed with increasing salinity in the nutrient medium. Accumulation of Na^+ was significantly higher in the salt sensitive genotypes than moderately salt tolerant and tolerant genotypes. While comparing with the control, the tolerant genotype Hazerd accumulated 19 fold more leaf Na^+ ions at 120 mM NaCl level, the medium tolerant genotypes Poinsett 97 and Pingwang accumulated 36 and 40 fold leaf Na^+ ions at 80 mM NaCl concentration and the salt sensitive genotypes HH1-8-57 and L6 accumulated 16 and 17 folds leaf Na^+ ion at 40 mM NaCl level in the medium. Elevated NaCl levels resulted in significant decreased of leaf K^+ in all the genotypes

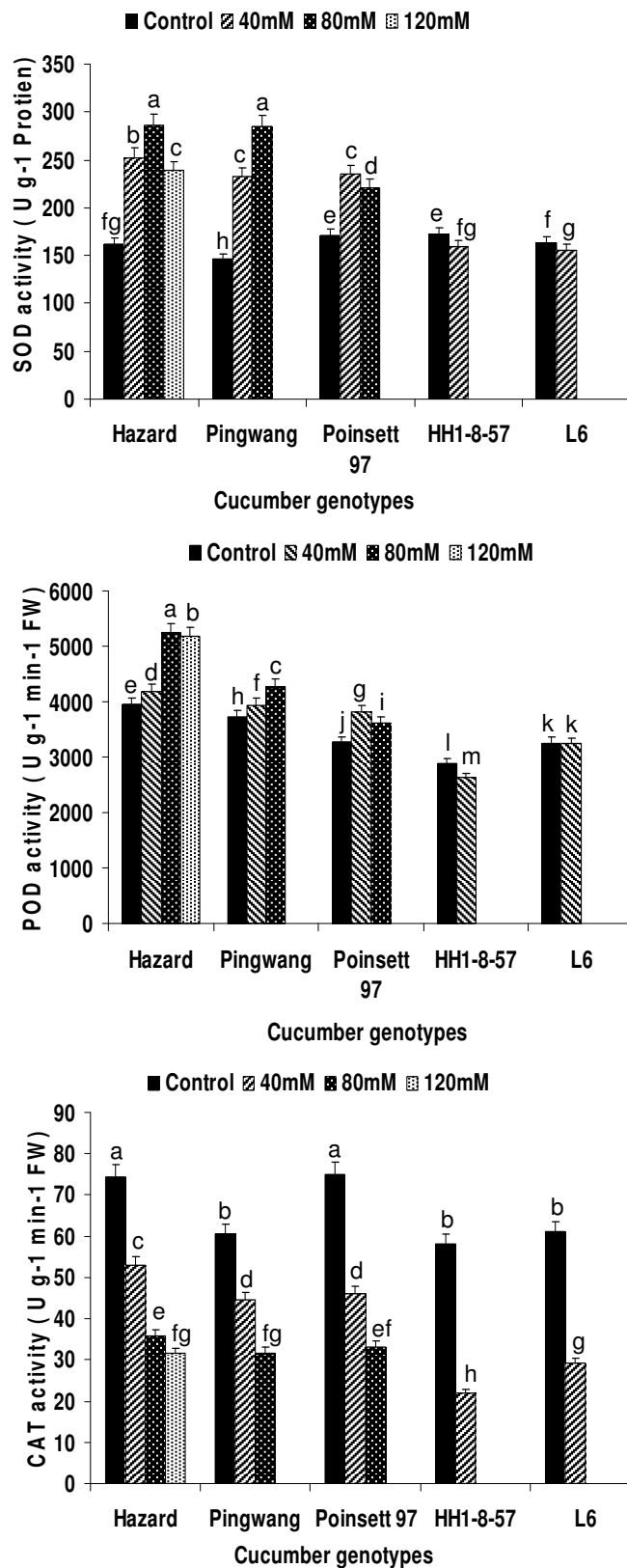


Figure 1. Effect of different levels of NaCl on SOD, POD and CAT activity in cucumber leaves. Values with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

(Table 2), although the drastic decrease of leaf K⁺ ion content was found in sensitive genotypes, and rather steady decline was determined in the moderately salt tolerant and tolerant genotypes.

Salt tolerance is the ability of the plants to limit the accumulation of excess ions in the leaves and thus, avoid toxic buildups and nutrient imbalances. In the present study, the distribution of ions in cucumber genotypes differing in salt suggested that the Na⁺ exclusion from leaf tissues appears to play an important role in the salt tolerance of cv. Hazerd (Salt tolerant) by keeping the optimal Na⁺/K⁺ ratio. High salt (Na⁺) uptake competes with the uptake of other nutrient ions, especially K⁺, leading to K⁺ deficiency. It is often found that many glycophytes exhibiting enhanced tolerance to salinity stress have a greater ability for sodium exclusion, maintaining high levels of K⁺/Na⁺ ratio (Zhu, 2001; Flowers and Hajibagheri, 2001).

Conclusion

In conclusion, this study showed that the difference of antioxidant enzyme, cell membrane permeability, soluble protein content, percent soluble sugar, chlorophyll pigment, high proline and potassium/sodium ratio in cucumber genotypes could be ascribed to the difference in mechanisms underlying oxidative stress injury and subsequent tolerance to salinity. It is thus apparent from the present investigation that no single parameter could be suggested as sole factor responsible for salinity stress tolerance of cucumber genotypes. A combination of characters contributes to salinity stress tolerance in cucumber genotypes. In future, these findings on biochemical and physiological indicators at the cellular level may serve as *in vitro* selection criteria for salt tolerance in cucumber. Besides cucumber being a moderately sensitive crop, considerable tolerance was found in one genotype (Hazard), which showed growth stimulation at the NaCl concentrations evaluated.

ACKNOWLEDGEMENTS

This research was partially supported by the Key Program (30830079) and the General Program 30671419 and 30700541 from the National Natural Science Foundation of China, National Basic Research Program of China (973 Program)(2009CB119000), the '863' Programs (2008AA10Z150, 2006AA10Z1A8, 2006AA100108), and the National Supporting Programs (2008BADB105, 2006BAD13B06, 2006BAD01A7-5-11) from the Ministry of Science and Technology of China.

REFERENCES

Abdel HA, Khedr MAA, Amal A, Abdel W, Quick WP, Abogadallah GM

- (2003). Proline induces the expression of salt stress responsive proteins and may improve the adaptation of *Pancreaticum maritimum* L to salt stress. *J. Exp. Bot.* 54(392): 2553-2562.
- Agastian P, Kingsley SJ, Vivekanandan M (2000). Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. *Photosynthetica*, 38: 287-290.
- Alan DB (1994). Soil salinity salt tolerance and growth potential of horticultural and landscaped plants. Cooperative Extension Service university of Wyoming Laramie. 294(5): 50.
- Alpaslan M, Gunes A (2001). Interactive effects of boron and salinity stress on the growth membrane permeability and mineral composition of tomato and cucumber plants. *Plant Soil*, 236: 123-128.
- Arnon (1949). Copper enzymes in isolated chloroplasts Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* 24: 1-10.
- Ashraf M, Tufail M (1995). Variation in salinity tolerance in sunflower (*Helianthus annuus* L.). *J. Agron. Crop. Sci.* 175: 351-362.
- Ashraf M, Waheed A (1993). Responses of some local/exotic accessions of lentil (*Lens culinaris* Medic) to salt stress. *J. Agron. Soil Sci.* 170(2): 103-112.
- Ayers RS, Westcot DW (1985). Water quality for Agriculture FAO Irrigation and Drainage. UN Rome. 29: 1.
- Bates LS, Wadern RP, Teare ID (1973). Rapid determination of free proline for water stress studies *Plant Soil*, 39: 205-207.
- Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* 72: 248-254.
- Brestein L (1975). Effect of salinity and sodicity on plant growth. *Annu. Rev. Phytopathol.* 13: 295-312.
- Cakmak I, Marschner H (1992). Magnesium deficiency and high light intensity enhance activities of superoxide dismutase ascorbate peroxidase and glutathione reductase in bean leaves. *Plant Physiol.* 98: 1222-1227.
- Cano AE, Perez-Alfocea, F, Moreno V, Caro M, Bolarin MC (1998). Evaluation of salt tolerance in cultivated and wild tomato species through *in vitro* shoot apex culture. *Plant Cell Tissue Organ Cult.* 53: 19-15.
- Chen XQ, Yu BJ (2007). Ionic effects of Na⁺ and Cl on photosynthesis in *Glycine max* seedlings under iso osmotic salt stress. *J. Plant Physiol. Mol. Biol.* 33(4): 294-300.
- Dorota Z (1997). Irrigating with high salinity water Bulletin 322 Agricultural and Biological Engineering Dep; Florida Cooperative Extension service Institute of Food and Agriculture Sciences University of Florida.
- Elkhoui S, Hernandez JA, Abdely C, Ghir R, Limam F (2005). Effects of salt on lipid peroxidation and antioxidant enzyme activities of *Catharanthus roseus* suspension cells. *Plant Sci.* 168: 607-613.
- Erturk U, Sivritepe N, Yerlikaya C, Bor M, Ozdemir F, Turkan I (2007) Responses of the cherry rootstock to salinity *in vitro*. *Biol. Plant*, 51(3): 597-600.
- Flowers TJ, Hajibagheri MA (2001). Salinity tolerance in *Hordeum vulgare*: ion concentrations in root cells of cultivars differing in salt tolerance. *Plant Soil*, 231: 1-9.
- Flowers TJ, Yeo AR (1995). Breeding for salinity resistance in crop plants: where next? *Aust. J. Plant Physiol.* 22: 875-884.
- Ghosal SS, Bajaj YPS (1984). Isolation of sodium chloride resistant cell lines in some grain legumes. *Indian J. Exp. Biol.* 22(4): 209-214.
- Giannopolitis CN, Ries SK (1977). Superoxide dismutase I Occurrence in higher plants. *Plant Physiol.* 59: 309-314.
- Greenway H, Munns R (1980). Mechanism of salt tolerance in non halophytes. *Annu. Rev. Plant Physiol.* 31: 149-190.
- Hertwig B, Streb P, Feierabend J (1992). Light dependence of catalase synthesis and degradation in leaves and the influence of interfering stress conditions. *Plant Physiol.* 100: 1547-1553.
- Jones RW, Pike LM, Yourman LF (1989). Salinity influences cucumber growth and yield. *Hort. Sci.* 114: 547-551.
- Katsuhara M, Otsuka T, Ezaki B (2005). Salt stress-induced lipid peroxidation is reduced by glutathione S-transferase but this reduction of lipid peroxidation is not enough for a recovery of root growth in *Arabidopsis*. *Plant Sci.* 169: 369-373.
- Kochba J, Lavee S, Roy SP (1977). Differences in peroxidase activity and isoenzymes in embryogenic and nonembryogenic *Shamouti*

- orange ovular callus lines. *Plant Cell Physiol.* 18: 463-467.
- Kumar SG, Reddy AM, Sudhakar C (2003). NaCl effects of proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. *Plant Sci.* 165(6): 1245-1251.
- Lachica M, Aguilar A, Yanez J (1973). Analisis foliar Metodos utilizados en la Estacion Experimental del Zaidin CSIC (II). *Anal. Edaf. Agrobiol.* 32: 1033-1047.
- LaRosa PC, Singh NK, Hasegawa PM, Bressan RA (1989). Stable NaCl tolerance of tobacco cells is associated with enhanced accumulation of osmotic. *Plant Physiol.* 91(5): 855-861.
- Liu HX, Zeng SX, Wang YR, Li P, Chen DF, Guo JY (1985). The effect of low temperature on superoxide dismutase in various organelles of cucumber seedling cotyledons with different cold tolerance. *Acta. Phytol. Physiol. Sin.* 1: 48-57.
- Lutts S, Kinet JM, Bouharmont J (1996). Effects of salt stress on growth mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L) cultivars differing in salinity tolerance. *Plant Growth Regul.* 19: 207-218.
- Martinez CA, Loureiro ME, Oliva MA, Maesri M (2001). Differential responses of superoxide dismutase in freezing resistant *Solanum curtilubum* and freezing sensitive *Solanum tuberosum* subjected to oxidative and water stress. *Plant Sci.* 160: 505-515.
- Martinez CA, Maesri M, Lani EG (1996). In vitro salt tolerance and proline accumulation in Andean potato (*Solanum* spp.) differing in frost resistance. *Plant Sci.* 116: 177-184.
- Meloni DA, Oliva MA, Martinez CA, Cambraia J (2003). Photosynthesis and activity of superoxide dismutase peroxidase and glutathione reductase in cotton under salt stress. *Environ. Exp. Bot.* 49: 6976.
- Mittler R, Merquiol E, Hallak HE, Rachmilevitch S, Kaplan A, Cohen M (2001). Living under a 'dormant' canopy: a molecular acclimation mechanism of the desert plant *Retama raetam*. *Plant J.* 25: 407-416.
- Mitova V, Tal M, Volokita M, Guy M (2003). Up regulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt induced oxidative stress in the wild salt tolerant tomato species *Lycopersicon pennellii*. *Plant Cell Environ.* 26: 845-856.
- Mungala AJ, Radhakrishnan T, Jayanti R, Dobarra (2008). In vitro Screening of 123 Indian Peanut Cultivars for Sodium Chloride Induced Salinity Tolerance. *World J. Agric. Sci.* 4(5): 574-582.
- Munns R, Hare RA, James RA, Rebetzke GJ (2000). Genetic variation for improving the salt tolerance of durum wheat. *Aust. J. Agric. Res.* 51: 69-74.
- Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15: 473-497.
- Parida AK, Das AB (2005). Salt tolerance and salinity effects on plants: a review. *Ecotoxicol. Environ. Saf.* 60: 324-349.
- Rains DW (1981). Salt tolerance: New Developments. In: J T Manassah, EJ Briskley (eds) *Advances in Food Producing System for Arid and Semiarid Lands*. Academic Press New York. pp. 431-456.
- Savvas D, Meleti G, Margariti S, Tsirogiannis I (2005). Modeling the relationship between water uptake by cucumber and NaCl accumulation in a closed hydroponic system. *Hort. Sci.* 40(3): 802-807.
- Shalata A, Tal M (1998). The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt tolerant relative *Lycopersicon pennellii*. *Physiol. Plant.* 104: 169-174.
- Shannon M, Noble C (1990). Genetic approaches for developing economic salt tolerant crops. In: Tanji K (Ed.), *Agricultural Salinity Assessment and Management*. ACSE Manuals and Reports on Engineering Practice No. 17, ASCE, New York. pp. 161-185.
- Singha S, Choudhuri MA (1990). Effect of salinity (NaCl) on H₂O₂ mechanism in *Vigna* and *Oryza* seedlings. *Biochem. Physiol. Pflanzen.* 186(1): 69-74.
- Spiro RG (1966). Analysis of sugars found in glycoproteins. *Method, Enzymol.* 8: 3-26.
- Steel RGD, Torrie JH (1980). *Principals and Procedures of Statistics. A Biometric Approach*. McGraw Hill New York.
- Thomas RL, Sheard RW, Moyer JR (1967). Comparison of conventional and automated procedures for nitrogen phosphorus and potassium analysis of plant material using a single digestion. *Agron. J.* 59: 240-243.
- Trajkova F, Papadantonakis N, Savvas D (2006). Comparative effects of NaCl and CaCl₂ salinity on cucumber grown in a closed hydroponics system. *Hort. Sci.* 41(2): 437-441.
- Upadhaya A, Reel S, Tikku A, Trivedi S, Sankhla N (1981). Effect of salt on early seedling growth and metabolic pattern in moong. *Indian Soc. Desert Technol.* 6: 124-127.
- Vijayan K, Chakraborti SP, Ghosh PD (2003). In vitro screening of mulberry (*Morus* spp.) for salinity tolerance. *Plant Cell Rep.* 22: 350-357.
- Wahid A, Masood I, Javed IUH (1999). Phenotypic flexibility as marker of sodium chloride tolerance in sunflower genotypes. *Environ. Exp. Bot.* 42: 85-94.
- Yeo AR (1994). Physiological criteria in screening and breeding. In: Yeo AR, Flowers TJ (eds) *Soil Mineral Stresses Approaches to Crop Improvement*, SpringerVerlag Berlin, pp. 37-60.
- Zhang EP, Zhang SH, Shi LT, Pang GA, Ma DH (2001) Effects of NaCl stress on the membrane lipid peroxidation in cotyledon of cucumber seedling. *J. Shenyang Agric Univ.* 32: 446-448.
- Zhao SHJ (2000). Detection of chlorophyll pigment. In: Zou Y (ed) *Manual of Plant Physiology Experiment*, Chinese Agriculture Press Beijing. pp. 72-75.
- Zhou YH, Yu JQ, Qian QQ, Huang LF (2003) Effects of chilling and low light on cucumber seedlings growth and their antioxidative enzyme activities. *China J. Appl. Ecol.* 14(6): 921-924.
- Zhu J, Bie Z, Yana Li (2008). Physiological and growth responses of two different salt sensitive cucumber cultivars to NaCl stress. *Soil Sci. Plant Nutr.* 54: 400-407.
- Zhu JK (2001). Plant salt tolerance. *Trends Plant Sci.* 6: 66-71.