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Growth and physiological responses of cucumber (*Cucumis sativus* L.) to sodium chloride stress under solid hydroponics

George Mbira Kere [1, 2] *, Qingwei Guo[1], Jinfeng Chen[1]

[1]State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing, 210095 China

[2] University of Kabianga, Kericho, Kenya.

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Abstract: NaCl induced salinity stress has been studied for several years. However, development of salt tolerant cucumber has remained elusive partly due to lack of suitable screening system. Four cucumber genotypes (11411S and 11432S as salt tolerant whereas 11439S and HH1-8-57 as salt sensitive) were subjected to two levels of salinity (0 and 80 mM NaCl) under greenhouse conditions to determine the effect of salinity on growth, leaf gas exchange characteristics and cell membrane stability. The pre-germinated seeds of each genotype were sown in 1.6 L plastic pots filled vermiculite and peat in the ratio 2:1 (v/v). The pots were arranged in randomized complete block design with 15 seedlings per treatment replicated three times. The data on growth, survival, gas exchange characteristics was taken at the end of 21 days from the start of the experiment. Two cucumber genotypes, 11411S (salt tolerant) and 11439S (salt sensitive) were selected for the germination experiment. The germination was assessed at 5 salinity levels (0, 40, 60, 80,100 mM NaCl) and replicated five times. The germination percentage (GP), mean germination rate (MR) and mean germination time (MT) was recorded every 24 hours for 8 days. The data collected was subjected to standard analysis of variance using SAS statistical software and means separated by Dancun's multiple range tests at $P < 0.05$. The results indicated that salt stress significantly reduced growth and survival rates of all cucumber genotypes but reduction in survival rate was severe in salt sensitive genotypes. Salinity significantly reduced photosynthetic rate, stomatal conductance, carboxylation efficiency, water use efficiency and transpiration rate of all genotypes but the decrease was higher in salt sensitive than tolerant cucumber genotypes. salinity did not affect the germination percentage but increased mean germination time especially of the salt sensitive genotype. We conclude that survival rate, photosynthetic rate and cell membrane stability can be used to assess salt tolerance in cucumber.

***Corresponding authors:** Mbira George Kere**:** keregeorge@yahoo.com

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1. Introduction

Salt stress is a major abiotic factor limiting crop productivity and growth (Degl'Innocent et al., 2009; Tiwari et al., 2010). An estimated 830 million hectares of the global land area and 20% of irrigated land are salt affected (FAO, 2009). Detrimental effect of salinity is widely documented as osmotic stress, interruption of metabolic activities, nutrient imbalance and oxidative stress such as lipid peroxidation, membrane destruction and pigment co-oxidation (Abd El Samad and Shaddad, 1997).

Salinity induced plant growth inhibition is usually attributed to reduced photosynthetic capacity (He et al., 2009; Huang et al., 2011). Photosynthetic responses of plants to salinity have often resulted to contradictory conclusions. Previous studies reported decreased stomatal conductance and inter-cellular carbon dioxide concentration in melon (*Cucumis melo*) and tomato (Colla et al., 2006; He et al., 2009). On the contrary, high salinity reduced stomatal conductance and increased inter-cellular carbon dioxide of cucumber (Zhou et al., 2009). These conflicting results may be due to differences in salt levels, genotypes and species of plants used, length of salt stress and other interacting factors (Munns and Tester, 2008). Moreover, most of the findings were obtained from liquid hydroponics where salinity levels remained almost constant and transpiration rate is highly reduced (Tavakkoli et al., 2010). Little information is available on the effect salinity on

growth and photosynthesis of cucumber genotypes with varying salt tolerance.

Cucumber is moderately sensitive to salinity stress (Maas and Hoffman, 1977). However, there exists a wide variation among cucumber genotypes in response to salt stress (Tiwari et al., 2010). Recently, Malik et al. (2010) evaluated 29 different cucumber genotypes for salt tolerance under *in vitro* conditions and reported that 11411S, 11432S, HH1-8-57 and 11439S as tolerant, moderately tolerant, sensitive and most sensitive, respectively. It is prudent to confirm salt tolerance and sensitivity of cucumber by comparing *in vitro* and pot experiments under greenhouse conditions.

Germination is an important phase in the life cycle of a plant. Salinity has been shown to reduce germination of many plant species (Ashraf and Foolad, 2007; Seckin et al., 2010). Salinity, moisture content and seed genetic architecture negatively affect seed germination (Seckin et al., 2010). While low germination due to salinity may be alleviated by sowing at higher seed rates, poor uniformity in emergence interferes with planting calendar and ultimate yield reduction. Salinity adversely affect seed germination percentage, mean germination time and germination rate. Salinity tolerance varies with plant developmental stage (Queseda et al., 2002; Munns and Tester, 2008). The objective of the germination experiment was to compare the salinity response at germination and seedling stages.

Development of cucumber cultivars tolerant to salt stress has not been successful. There is need to develop an efficient and accurate selection criteria for salt tolerance in cucumber. A clear understanding of the mechanisms of tolerance against salt stress in cucumber may contribute significantly to improvement of the crop salt tolerant traits. The objective of this study was to determine the effect of salinity on growth and photosynthetic responses of four cucumber genotypes grown under greenhouse conditions to evaluate their salinity tolerance.

2. Materials and Methods

2.1 Plant materials and treatments

The experiment was conducted in a glasshouse at Nanjing Agricultural University. Seeds were sterilized in NaOCl and germinated in darkness for 24 hours. Seeds were sown in plastic pots of 1.6 L filled with vermiculite and peat in the ratio 2:1 (w/w).

The seedlings were watered with full strength Hoagland solution every other day at to field capacity (irrigation varied from 300-500 mL according to weather conditions and growth stage). The mean temperature and relative humidity during the study was 31.5°C and 60%, respectively.

The electrical conductivity and pH of irrigation solutions are summarized in the Table 1. Complete cucumber nutrient solution was prepared as described by (Guo, 2004). Briefly, analytical grade compounds were dissolved in distilled water to prepare desired volumes of stock basic solutions as shown in Table 1. Four categories of stock solutions: A, B, C and D were prepared as shown in Table 2. The composition of the basic nutrient solution of cucumber was equivalent to 14.0 mM NO_3–N, 1.0 mM NH_4–N, 1.5 mM S, 1.5 mM P, 6.0 mM K, 5.0 mM Ca, 1.5 mM Mg, 1.0 mM Na, 1.0 mMCl, 20 µM Fe, 9 µM Mn, 0.3 µM Cu, 1.6 µM Zn, 20µM B and 0.3 µMo.

Four cucumber genotypes, 11411S (tolerant), 11432S (moderately tolerant), HH1-8-57 (sensitive) and 11439S (sensitive) with respect to salt stress were subjected to two levels of salinity (0 and 80 mM NaCl). The experiment was arranged in randomized complete block design replicated 3 times and each treatment had 15 plants. Salinity treatment commenced at 10 days after germination when the seedlings were at second true-leaf stage. To avoid salt stress shock, the salinity was applied at an incremental rate of 20 mM NaCl until 80 mM NaCl was attained. The plants were exposed to salt stress for twenty days.

Table 1 . Electrical conductivity and pH of irrigation water solution

Treatment	Solution EC (dS/m)	pH
Control (0 mM NaCl)	2.09	7.8
NaCl (80 mM NaCl)	10.04	7.9

Table 2. Complete basic nutrient of cucumber (Guo, 2004)

Compound	Concentration (mg/L)	Remark
$Ca(NO_3)_2.4H_20$	945	Solution A
KNO_3	607	
$NH_4H_2PO_4$ (MAP)	115	Solution B
$MgSO_4.7H_2O$	493	
H_3BO_3	2.86	Solution C
$MnSO_4.4H_2O$	2.13	
$ZnSO_4.7H_20$	0.22	
$CuSO_4.5H_2O$	0.08	
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.02	
Na_2Fe-EDTA	30	Solution D

Table 3 Effect of salt stress on growth of cucumber genotypes (*Cucumis sativus* L.) under greenhouse conditions.

Genotypes	Salt level (mM NaCl)	Height (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Root/ shoot ratio
11439S	0	136.3±15.0a	63.8±7.7b	8.5±0.5bc	5.2±0.1a	0.36±0.04bc	0.07±.01b
	80	97.06±2.31bc	38.18±5.39c	4.74±0.89d	3.6±0.69bc	0.21±0.05d	0.06±0.01bc
HH1-8-57	0	106.33±17.00bc	72.09±7.95b	9.85±0.71bc	5.07±0.0a	0.27±0.04cd	0.05±0.01c
	80	78.33±6.84c	37.58±2.55c	7.01±1.35c	3.42±0.31bc	0.31±0.01cd	0.09±0.01a
11432S	0	96.00±13.59c	65.9±6.29b	12.15±2.0b	4.98±0.62b	0.23±0.03cd	0.05±0.01c
	80	63.33±12.33c	36.72±3.64c	5.67±0.49d	3.26±0.52c	0.20±0.04d	0.06±0.01bc
11411S	0	128.67±12.1a	89.65±9.84a	16.93±2.79a	8.52±1.44a	0.86±0.17a	0.10±0.01a
	80	108.00±8.74ab	69.28±12.91b	7.98±0.86bc	5.53±0.61ab	0.48±0.05b	0.09±0.00a

Values followed with same letter within the same column are significantly different at $P < 0.05$.

2.2 Plant growth and survival rate

Two plants per treatment in each replicate were randomly selected and their heights measured using a ruler. At the end of the experiment, plants were carefully washed off the rooting media and rinsed with distilled water and blotted using tissue paper. The plant samples were divided into roots and shoots immediately weighed to determine fresh weight. The samples were dried in a forced draft oven at 70 ºC for 72 hours and dry weights determined. Survival rate was estimated by dividing the final number of living plants by initial seedling number at the start of treatment.

2.3 Cell membrane stability

Cell membrane stability was indirectly assessed by measuring electrolyte leakage of leaves stressed and unstressed cucumber genotypes. Leaves were harvested and cut into discs and rinsed with deionized water to remove surface electrolytes. 20 leaf discs from two plants per treatment per replicate were then put in test tubes filled with 10 ml of deionized water and left to stand at room temperature (25ºC) for 3 hours. The electrical conductivity of the bathing solution was determined (C1). The samples were then heated at 80 ºC for 2 hours, cooled to room temperature and final electrical conductivity (C2) of the bathing solution measured. Relative ion leakage was expressed as a percentage of the total conductivity after heating at 80ºC (relative ion leakage % =C1/C2 × 100 (Lutts et al, 1995).

2.4. Gas exchange characteristics

Net photosynthetic rate (P_n), stomatal conductance (G_s), inter-cellular carbon dioxide concentration (C_i) and transpiration rate (T_r) were determined at 21 days after the start of salt treatment using portable photosynthesis system (LI-6400, LI-COR, Lincoln, NE, USA). The data was taken from fully expanded leaves of 3 different plants per treatment. The leaf chamber area was 6 cm^2. Light intensity was 800 µ mole $m^{-2}s^{-1}$. Carboxylation efficiency (CE) was estimated using the formula, CE= (Net photosynthetic rate/internal carbon dioxide concentration) $molm^{-2}s^{-1.}$ Water use efficiency (WUE) was estimated using the formula WUE= (P_n / T_r).

2.5. Plant pigment determination

Leaf samples were collected, washed with distilled water and blotted dry. Leaf discs without midrib were punched and pigments extracted with 95% ethanol for 72 h in darkness at room temperature. 3 ml of the extract was transferred into cuvette cup and absorbance measured at 664, 649 and 470. Chlorophyll content was estimated according to (Litchtenthaler and Bushmann; 2001).

2.6. Mineral distribution

The shoot samples were finely ground. 15 mL of HNO_3-H_2O_2 (l) (5:1) was added to 0.5 g of the powdered samples and boiled for 1 hour at 95°C to extract cations. The resulting solutions were filtered using Whatman filter paper. The concentration of $Na^+$and K^+ in the digest was analyzed using Atomic Spectrophotometer.

2.7. Germination experiment

Two cucumber genotypes, 11411S (salt tolerant) and 11439S (salt sensitive) were selected for the germination experiment. The germination was assessed at 5 salinity levels (0, 40, 60, 80,100 mM NaCl) and replicated five times. 125 healthy seeds of each genotype were selected, sterilized and rinsed

thoroughly with distilled water. The seeds were then soaked in warm distilled water for 8 hours. Germination treatment was conducted in petri dishes (9.0 cm) lined with filter paper moistened with 5 mL of selected salt treatment. For each treatment level, 25 seeds of each genotype were evenly placed and incubated in a growth chamber at 28ºC in darkness. The number of seeds whose radical length was half the seed length counted after 24 hours interval. The procedure was repeated every 24 hours until the eighth day after the onset of treatment. The germination parameters were calculated using the following formula:

Germination percentage (GP) = 100 x (n/N)

Mean germination time (MT) = $\sum$ (ni x ti/Ni)

Mean germination rate (MR) = 1/MT

Where, ni = number of seeds germinated at i^{th} time; ti days from start of experiment; n = number of seeds sprouted at the end of the experiment; N = total number of seeds sown

2.8. Statistical Analysis

The data were subjected to analysis of variance using PROC ANOVA of SAS statistical software and means separated by Duncan's Multiple Range Test at $P < 0.05$. Correlation analysis of physiological and biochemical traits was conducted using PROC CORR procedure of SAS statistical software.

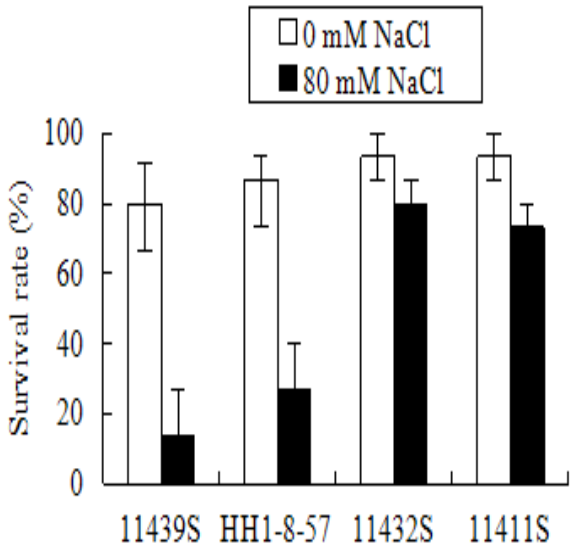

Fig. 1. Survival rate of four cucumber genotypes subjected to salt stress at 0 and 80 mM NaCl levels at 21 days post treatment. The white and black bars denote control and salt stress conditions, respectively. Values are means (n=3) ±SE.

3. Results

3.1 Growth and survival rate

Salt stress significantly ($P < 0.05$) reduced growth parameters of all the genotypes (Table 3). The genotypes significantly ($P < 0.05$) responded differently to salt stress at vegetative stage. Plant height of salt stressed 11439S, HH1-8-57, 11432S and 11411S was 71.1, 73.7, 66 and 91% of the corresponding height under unstressed conditions. Genotypic variation on root length as a result of salt stress was not observed. There was no significant difference on survival rate of the cucumber genotypes under unstressed conditions (Fig. 1). Salt stress significantly ($P< 0.001$) reduced survival rate of cucumber genotypes especially the salt sensitive genotypes (11439S and HH1-8-57).

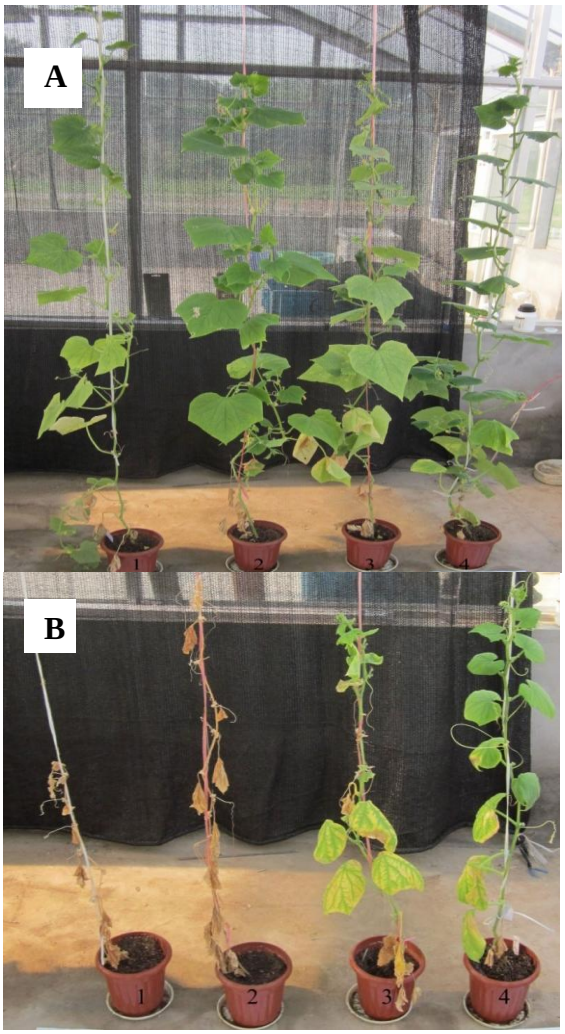

Fig. 1 Responses of four cucumber genotypes (1, 2, 3 and 4 pots contain HH1-8-57, 11439S, 11411S and 11432S, respectively) to NaCl salinity stress after 21 days. A **and** B **represents unstressed and stressed (80 mM NaCl), respectively.**

Figures 2A and 2B show growth responses of the four cucumber genotypes at end of the experiment. Under unstressed conditions, the growth of the four genotypes was similar while unstressed conditions resulted to significant yellowing and deaths of the cucumber seedlings. The salt sensitive genotypes exhibited severe negative salt stress response than the relatively salt tolerant ones.

3.2 Cell membrane permeability

Salt stress significantly *(P* < 0.05) increased ion leakage (IL) in all genotypes (Table 4). Salt treatment significantly *(P* < 0.05) induced higher ion leakage in salt sensitive genotypes (HH1-8-57 and 11439S) than tolerant ones (11432S and 11411S).

3.3 Gas exchange characteristics

The effect of salinity on gas exchange characteristics of cucumber genotypes with varying salinity tolerance is illustrated in Fig. 3. Salinity significantly (*P*< 0.0001) reduced photosynthetic rate of all genotypes with greatest inhibition exhibited by the salt sensitive one (Fig. 3A). The Pn of salt sensitive genotypes (11439S and HH1-8-57) were highly reduced than the tolerant ones (11432S and 11411S) (Fig.2-3A). The tolerant genotypes had similar net photosynthetic rate under stressed and non-stressed conditions (Fig. 3A). Similarly, salinity reduced gs of all genotypes (Fig. 3B). Genotypes 11439S and HH1-8-57 registered lowest stomatal conductance under salt stress (Fig. 2-3B). Salt stress did not significantly affected Ci in all the genotypes (Fig. 3C).

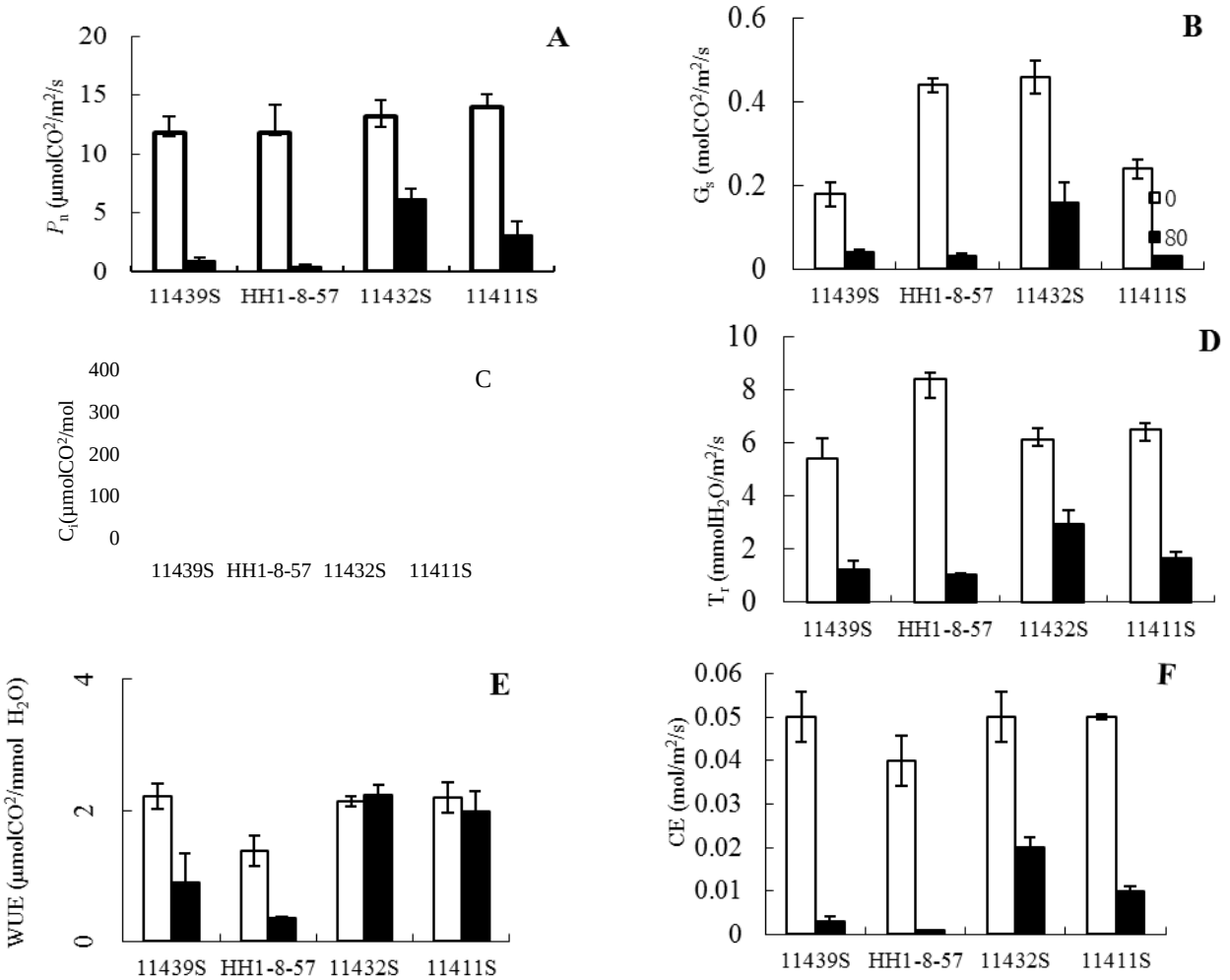

Fig. 3. Effect of salt stress on (A**) photosynthetic rate (P_n), (**B**) stomatal conductance (G_s), (**C**) internal carbon dioxide concentration (C_i), (**D**) transpiration rate (E), (**E**) water use efficiency (WUE), (**F**) carboxylation efficiency (CE) of four cucumber genotypes subjected to salt stress for 21-days.** Values are means ±SEM (n = 3).

Table 4. Effect of salt stress on ion leakage (%) from leaves of different cucumber genotypes.

Genotypes	Ion leakage (%)	
	0 (mM NaCl	80 mM NaCl
11439S	27.06 ± 0.87 a	78.57±4.00 a
HH1-8-57	27.06± 2.25 a	81.62±2.15 a
11432S	31.56±3.34 ab	61.09±3.75 b
11411S	26.67±2.89 ab	60.67±3.49 b

Values followed by same letters within the column are not significantly different ($P < 0.05$).

Generally, salinity tended to increase Ci of the sensitive cucumber genotypes. Salt stress significantly ($P < 0.0001$) reduced transpiration rate of all the cucumber genotypes. As expected salinity significantly reduced transpiration rate of all genotypes with the salt sensitive genotypes, 11439S and HH1-8-57 registering higher decrease than salt tolerant 11411S and 11439S (Fig. 3D). A similar trend was observed in water use efficiency (WUE) where salinity induced higher reduction in salt sensitive genotypes than salt tolerant in all the genotype Fig. 2E). Carboxylation efficiency (CE) as measured as a ration between net photosynthetic rate was depressed by salinity. CE reduction among the genotypes was in the decreasing order 1-8-57>11439S>11411S>11432S (Fig. 3F).

3.4 Leaf pigment and mineral distribution

Salt stress significantly ($P < 0.05$) reduced total chlorophyll content of all cucumber genotypes. Salt stress reduced chlorophyll content by 69.57, 77.4, 27.55 and 36.50% in 11439S, HH1-8-57, 11432S and 11411S, respectively (Table 5). There was significant ($P < 0.05$) interaction between genotype and salt stress. Salt stress markedly increased shoot sodium content of all cucumber genotypes (Table 5). In contrast, there was remarkable reduction in shoot potassium content of all the genotypes by high salinity stress at varying degrees. Na^+/ K^+ of all the genotypes increased but at a higher extent in salt sensitive genotypes.

Pn strong had significant positive relationships with Gs, E, WUE, CE and Su while its relationship with Chl was moderately positive (Table 6). However, a strong negative correlation was observed between IL and all the parameters except Ci where a weak negative correlation was observed (Table 6).

3.5 Germination response of cucumber genotypes to NaCl salinity stress

Salinity level did not affect GP while it increased MT and consequently reduced MR of both genotypes (Table 7).11411S had significantly higher GP and MR but lower MT than 11439S. At 100 mM NaCl salinity level, the MT of 11411S and 11439S increased by 13 and 30%, respectively. Genotype and salinity interactions significantly influenced MT and MR. (Table 7). At 0 mM NaCl, MT was higher in 11411S than 11439S. Increasing salinity level increased MT of 11411S progressively while that of 11439S increased between 0-60 mM NaCl and suddenly declined at increased 80 mM NaCl while MR decreased. Strangely, at 80 mM NaCl 11439S had better MT and MR than either control or highest salinity level.

3.6 Germination response of cucumber genotypes to NaCl salinity stress

Salinity level did not affect GP while it increased MT and consequently reduced MR of both genotypes (Table 7).11411S had significantly higher GP and MR but lower MT than 11439S.

Table 5. Shoot concentration Na^+, K^+, Na^+/K^+ and chlorophyll content of four cucumber genotypes under 80 mM NaCl stress for 21 days.

Cultivar	Treatment (mM NaCl)	Na+ (mg g^{-1} DW)	K^+ (mg g^{-1} DW)	$Na^{+/} K^+$	Total chlorophyll content (mg g^{-1} FW)
11439S	0	5.47±0.83bc	126.86±15.94ab	0.043±0.02	1.38±0.05ab
	80	66.50±4.21a	43.27±1.67d	1.545±0.16a	0.42 ± 0.03c
HH1-8-57	0	4.44±0.25c	123.88±10.73ab	0.036±0.02cd	1.61±0.03a
	80	74.92±6.48a	54.66±9.14d	1.374±0.09a	0.38±0.05c
11432S	0	6.50±0.27b	148.48±9.41a	0.044±0.01c	1.67±0.06a
	80	49.08±1.14ab	65.38±7.49c	0.782±0.10b	0.94±0.12b
11411S	0	6.95±0.85b	159.52±14.22a	0.043±0.02c	1.36±0.07ab
	80	61.56±6.18a	80.52±3.47c	0.764±0.12b	0.87±0.10b

DW: Dry weight; FW: Fresh weight. Means followed by same letter within the column are not significantly different at $P < 0.05$.Values are means ±SEM (n = 3).

Table 6. Correlation analysis of gas exchange characteristics, chlorophyll content, electrolyte and survival of cucumber

	P_n	G_s	C_i	E	WUE	CE	Chl	IL	Su
P_n	1								
G_s	0.71	1							
C_i	-0.36	-0.17	1						
E	0.85	0.89	-0.2	1					
WUE	0.67	0.18	-0.63	0.28	1				
CE	0.97	0.62	-0.48	0.80	0.70	1			
Chl	0.48	0.28	-0.34	0.47	0.34	0.54	1		
IL	-0.90	-0.64	-0.39	-0.79	-0.68	-0.86	-0.5	1	
Su	0.73	0.57	-0.63	0.61	0.74	0.74	0.31	-0.77	1

The acronyms represent: photosynthetic rate (Pn),stomatal conductance (gs), internal carbon dioxide concentration (Ci), water use efficiency (WUE), Transpiration rate (E), Carboxylation efficiency (CE), chlorophyll content (Chl), electrolyte leakage (IL) and survival (su). Values in grey and white are significant and not significant, respectively at $P < 0.05$.

At 100 mM NaCl salinity level, the MT of 11411S and 11439S increased by 13 and 30%, respectively. Genotype and salinity interactions significantly influenced MT and MR. (Table 7). At 0 mM NaCl, MT was higher in 11411S than 11439S. Increasing salinity level increased MT of 11411S progressively while that of 11439S increased between 0-60 mM NaCl and suddenly declined at increased 80 mM NaCl while MR decreased. Strangely, at 80 mM NaCl 11439S had better MT and MR than either control or highest salinity level.

4. Discussion

Growth and survival performance has been used to assess the salt tolerance of many plants (Kchau et al., 2010; Mori et al., 2011). In this study, root/ shoot ratio increased under salt stress suggesting shoots suffered salinity stress than the roots. At elevated salt level, a general growth reduction was noted in all cucumber genotypes indicating response to the initial osmotic effect of salinity stress (Furtana and Tipirdamaz, 2010). In this study, salinity stress symptoms such as leaf yellowing and plant withering became quite vivid on the third week after treatment initiation especially in the salt sensitive genotypes (11439S and HH1-8-57). Our observation agrees with that of (Furtana and Tipirdamaz, 2010) which showed that salt specific effects takes a long time to be physically visualized. Thus, greenhouse pot based system screening should be conducted for at least three weeks to distinguish between osmotic and ionic stress tolerance of cucumber.

Salinity induced growth inhibition and death of plants is linked to reduced photosynthesis (He et al, .2009). Salinity induced reduction in photosynthetic rate is due to stomatal, non-stomatal or a combination of both (Zhang et al., 2009). The plant growth and survival response to salinity depend on the genotype, salinity pressure, duration, and interaction from other abiotic stresses. In this study, we selected four cucumber genotypes with already confirmed variation in salinity tolerance under *in vitro* conditions (Malik et al., 2010).

Table 7. Effect of salinity on germination percentage (GP), mean germination time (MT), mean germination rate (MR) of cucumber seeds under salinity.

Genotype	Salt level (mM NaCl)	GP (%)	MT (days)	MR (days^{-})
11411S	0	100 ±0.00	2.01±0.01 a	O.50±0.00 c
	40	100 ±0.00	2.01±0.01a	0.50±0.00 c
	60	100 ±0.00	2.04±0.00 a	0.49±0.00 c
	80	100 ±0.00	2.09±0.01 a	0.48±0.00 c
	100	100 ±0.00	2.29±0.11 ab	0.44±0.02 c
11439S	0	97.33 ±2.67	2.64±0.20 c	0.37±0.00 a
	40	98.67 ±1.33	3.04±0.00 c	0.33±0.01 a
	60	97.33 ±2.67	3.08±0.08 c	0.32±0.01 a
	80	94.66±1.33	2.31±0.01 b	0.43±0.00 b
	100	97.67 ±1.33	3.33±0.05c	0.30±0.01 a

Salinity reduced growth of all the genotypes as indicated by reduced dry weight. The reduction in growth was due to reduced photosynthetic rate. The genotypic variation in photosynthetic rate explains the better growth and survival of the relatively tolerant genotypes, 11411S and 11432S.

Photosynthetic rate in plants depends on carbon dioxide conductance and assimilation. Since salinity reduced gs but not C_i in the current study, it can be suggested that reduced P_n is due to both stomatal and non-stomatal factors (Stepien and Johnson, 2009; Degl'Innocenti et al., 2009; Pompelli et al., 2010; Silva et al., 2010). The effect of salinity on net CO_2 assimilation appeared to depend on the relative salt tolerance of the cucumber genotypes. The reduction in CO_2 assimilation was relatively higher in the salt sensitive genotypes (HH1-8-57 and 11439S) than the salt tolerant one (11411S and 11432S). Similar observations were made in olive subjected to salt stress (Chartzoulakis et al., 2002). Previous reports indicate that high salinity decreased net photosynthetic rate, stomatal conductance and intercellular CO_2 in water melon, *Citrullus lanatus* (Colla et al., 2006) and tomato, *Solanum lycopersicum* (He et al., 2009).

Although the Ci was not significantly different under control and stressed conditions in all the genotypes, the study showed a general increase of C_i in the salt sensitive genotypes. Similar observations had been made in *Citrus grandis* (Li et al., 2010) and *Cucumis sativus* (Zhou et al., 2009; Huang et al., 2011). This suggests that salinity level at 80 mM NaCl is above tolerance limit of the cucumber genotypes employed in this study. The high Ci may be associated with damage of photosynthetic apparatus and cell membrane as indicated by high electrolyte leakage.

High WUE is important in salt tolerance since it decreases uptake of salt and alleviate water deficiency caused by salinity (Karaba et al, 2007). High WUE in relatively tolerant genotypes, 11432S and 11411S is a sign of sustained photosynthetic rate under high salinity stress (He et al., 2009). Transpiration rate in stressed plants reduced despite high soil moisture in the medium (visual observation). This may be attributed to low osmotic potential due to high salinity as plants respond by reducing stomatal opening to avoid further water loss and reduced ion uptake from the root medium. Dong et al. (2010) reported higher water content in pots of cotton (*Gossypium hirsutum*) plants irrigated at 300 mM NaCl than 100 mM NaCl but the latter had higher osmotic potential. The high significant positive correlation coefficient (r = 0.89) between stomatal conductance and transpiration rate further supports this observation. Studies conducted by Pompelli et al. (2010) parallel our present findings. It is therefore conceivable that reduced stomatal conductance and transpiration in the present study may be due to low osmotic potential occasioned by high salinity.

Salinity like other abiotic stresses causes higher electrolyte leakage by displacing Ca^{2+} ions from the plasmalemma. Consequently, cell membrane is damaged and increased efflux of electrolytes within the plant tissues (Hassegawa et al., 2000). In the present study, salt stress increased electrolyte leakage of cucumber leaves in all genotypes albeit at varying degrees. Under salt stress, ion leakage was relatively higher in salt sensitive genotypes (11439S and HH1-8-57) than tolerant one (11411S and 11432S). This shows that the salt tolerant genotypes had lower membrane stability hence could tolerate the NaCl induced stress. Similar findings had been reported in cucumber, *Cucumis sativus* (Tiwari et al., 2010; Furtana and Tipirdamaz, 2010), wild barley, *Hordeum spontaneum* (Vsotkaya et al, 2010), Arabidopsis, *Arabidopsis thaliana* (Wang et al., 2010), wild potato species, *Solanum acaule, solanum stoloniferum and solanum bulbosum* (Daneshmand et al., 2010) and muskmelon, *Cucumis melo* (Welbaurn and Bradford, 1990). Significant negative correlation observed between the electrolyte leakage and all the physiological parameters suggest that the cell membrane is the main target of salt stress damage in cucumber. Thus, genotypes with relatively lower electrolyte leakage under salinity stress may be considered salt tolerant.

In our study, it is interesting to note that 11432S that was previously categorized as moderately salt tolerant under *in vitro* conditions had a parallel salt tolerance to the salt tolerant, 11411S.This observation may be attributed to gradual addition of salt in the root medium, which might have induced salt tolerance in 11432S. The gradual increase of salt levels under pot experiment probably enabled the cucumber genotypes to acclimatize to the stress as opposed to the suddenly imposed high salinity under *in vitro* conditions. Furthermore, salt sensitive genotypes 11439S and HH1-8-57 tolerated salt stress of 80 mM NaCl for about 20 days after start of salt treatment contrary to the previous *in vitro* screening.

Reduction in total leaf chlorophyll content in salt stressed plants is a general phenomenon (Agong et al., 2003; Tiwari et al., 2010; Amirjani, 2011). Previous

studies attributed the reduction in chlorophyll content under salt stress to pigment degradation or inhibition of its biosynthesis (Garcia-Sanchez et al. 2002). In our study, genotypes with the lower reduction in chlorophyll content also registered least electrolyte leakage. Significant negative correlation between electrolyte leakage and chlorophyll content may partially explain chlorophyll degradation through cell membrane damage. Increased shoot sodium content with concomitant reduction in potassium content for plants growing in saline medium is well known. Accumulation of high sodium levels in plant tissue results in ionic imbalance and adverse effects of plant metabolic processes (Tester and Davenport, 2003).

Relatively lower Na^+/K^+ in the salt tolerant 11411S and 11432S at high salinity level suggests that sustained potassium uptake under stress conditions may be one of the mechanism of salt tolerance in cucumber. Plants maintain high Na^+/K^+ through various strategies such as Na^+ exclusion, vacuolar compartmentation and reduced salt induced K^+ leakage from the cells (Yamaguchi and Blumwald, 2005). Increased Na^+ content in the shoots under salinity stress in our study indicates that salt tolerance at least for these cucumber genotypes is not due to Na^+ ion exclusion. It appears that the two salt tolerant cucumber genotypes possessed other mechanisms of reducing K^+ leakage under salinity challenge.

Yamaguchi and Blumwald (2005) pointed out that ion exclusion works only for mild salinity for short time. The salinity at 80 mM NaCl for about three weeks showed that the ion exclusion mechanism cannot explain salt tolerance in cucumber. This may indicate that the salt tolerant cucumber genotypes employs other mechanisms like tissue tolerance where excess Na^+ is compartmentalized in the in the cell vacuoles (Munns and Tester, 2008). The deleterious effect of Cl^-component in cucumber needs further investigation. At 80 mM NaCl, the Na^+/ K^+ of both 11411S and 11432S was less than one suggesting better potassium selectivity and hence greater survival ability. Our finding is in line with previous reports that showed that salt tolerance of glycophytes is attributed to the plants ability to retain K^+ (Munns and Tester, 2008).

Finally, it is important to note that 11432S that was previously categorized as moderately salt tolerant under *in vitro* conditions had similar salt tolerance as the salt tolerant, 11411S. Also, salt sensitive genotypes 11439S and HH1-8-57 survived salt stress of 80 mM NaCl for about 20 days after start of salt treatment contrary to the previous *in vitro* screening. The gradual increase of salt levels under pot experiment probably enabled the cucumber genotypes to acclimatize to the stress as opposed to the suddenly imposed high salinity under *in vitro* conditions. Our study differs with previous solution culture studies where salt level was relatively uniform contrary to pot mix conditions in a greenhouse environment, where the air humidity is likely to be lower than the solution culture medium. Therefore, g_s might generally be lower in *in vitro* studies and the stomata might have a better and faster regulatory mechanism. The pot experiment simulated soil conditions as it allowed gradual acclimatization by cucumber plants to salinity stress. Thus, results from various screening systems need cautious interpretation.

This study revealed that up to 100 mM NaCl salinity did not have significant effect on final germination of either cucumber genotypes selected. This contradicts reports by Chang et al. (2010), which showed that salinity reduced GP. We attribute this to higher salinity of 200 mM NaCl used in their study. However, higher salinity delayed germination especially for the salt sensitive, 11439S. Similar observations were made in spinach (Turkhan et al., 2010) and melon (Chartloulakis and Laoupassaki, 1997). Tukahan et al. (2010) suggested that reduced germination time may contribute salt tolerance in later stages. Shorter germination time under saline conditions would be a desirable trait in cucumber as it allows faster and uniform crop establishment upon transplanting. At 80 mM NaCl, the MR of 11439s improved while its seedling survival was highly reduced indicating that the salinity tolerance at germination and seedling stages is under different genetic mechanisms. However, the rate of germination of 11439S was higher at 80 mM NaCl compared to non-stressed condition needs further studies. DeRose-Wilson and Gaut (2011) also reported similar observations in Arabidopsis. Although both MT and MR had significant genotypic variation, salinity and genotypic interactions complicates the conclusive reason for delayed germination in the two cucumber genotypes. MT appeared more responsive than GP hence may serve as a potential selection indicator for salinity tolerance in cucumber during germination phase especially for large scale screening.

5. Conclusion

In vitro selection of salt tolerance in cucumber is useful for large scale screening, but may only reflect part of the story as it does not reveal the full interaction of intact plants. However, re-evaluation of

selected genotypes in pot grown plants in greenhouse conditions validates the preliminary results. Salinity reduced survival, gas exchange, chlorophyll content, K^+, while it increased Na^+, Na^+/ K^+, Na^+ and electrolyte leakage. These changes were related to salinity tolerance of the genotypes. Thus survival rate, stomatal conductance, Na^+/ K^+ ratio and cell membrane permeability indices can be reliably used to assess salt tolerance in cucumber under natural greenhouse conditions. Based on our conditions and parameters we can confirm the genotypes 114112S and 11432S as being salt tolerant while 11439S and HH1-8-57 as salt sensitive. The salinity tolerance of the genotypes, 114112S and 11432S may be attributed to enhanced K^+ retention and sustained photosynthetic activities under NaCl stress.

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