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ASSESSMENT OF GENOTYPIC VARIATION IN SALT TOLERANCE OF PEPPER (*Capsicum annuum* L.) CULTIVARS USING GAS EXCHANGE CHARACTERISTIC, GROWTH PARAMETERS AND CHLOROPHYLL CONTENT

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ABSTRACT. *In this study, the relationship of Leaf gas exchange parameters with salt tolerance was investigated in 26 pepper (Capsicum annuum L.) cultivars. The seedlings were exposed to salt stress treatment (100 mM NaCl) under the greenhouse conditions and the nutrient solution without NaCl was used as a control. Stress tolerance index in related to gas exchange parameters such as assimilation rate, stomata conductance, water vapor conductance, intercellular* CO_2 *concentration, transpiration rate, photosynthetic water use efficiency, mesophyll conductance, chlorophyll content, and growth parameters, relative leaf water content (RLWC) were measured after two weeks treatment. The results showed that under salt stress all characteristics were decreased, except to photosynthetic water use efficiency and chlorophyll a/ chlorophyll b (Chl. a/Chl. b). The salt stress responses were completely dependent to pepper cultivars, except intercellular* CO_2 *concentration, mesophyll conductance and Chl. a/Chl. b ratio. The stress tolerance index was significantly correlated with growth parameters, assimilation rate, chlorophyll content and RLWC. Under salt stress Paramo cultivar showed superiority than others cultivars in the most studied characteristics. Overall, the gas exchange parameters, chlorophyll content and RLWC could be useful techniques for screening salt tolerance cultivars in pepper cultivars.*

KEY WORDS: *assimilation rate, chlorophyll,*
stomatal conductance, transpiration

INTRODUCTION

Salt stress is one of the most widespread environmental hazards worldwide crop production, especially in arid and semi-arid regions, where land degradation, water deficit and population growth are already dominant concerns (Eisa et al. 2012). Salinity stress disturbs some physiological and biochemical processes, especially the photosynthesis (Netondo et al. 2004). In fact, the most sensitive process that affect by salt stress, is photosynthesis (Munns et al. 2006). Previous studies showed that a significant decrease in net CO_2 assimilation rate correlated with salt stress-induced decline in crop productivity (Netondo et al. 2004, Chaum & Kirdmanee 2009, Colla et al. 2010). This decrease in photosynthetic rate depends on photosynthesizing tissue, chlorophyll content, stomata conductance and no stomatal factors (Siddiqi et al. 2009). Lichtenthaler et al. (2005) reported that salt stress decreased biosynthesis of chlorophyll and efficiency of photosynthesis, because salt stress decline stomata conductance, which reflects to reduce carbon uptake and metabolism, inhibition of photochemical capacity or a combination of all these factors (Kaouther et al. 2012). Therefore, photosynthetic parameters are used as useful selection criteria to evaluate salt tolerance in different crops (Ulfat et al. 2007, Siddiqi et al. 2009).

The water uptake which ultimately affects the relative leaf water content (RLWC), is also responded by salt stress (Ziaf et al. 2009). It has been reported in several crops that RLWC reduces, when salinity increases (Rodriguez et al. 2005, Yokas et al. 2008, Ziaf et al. 2009). The decreased RLWC indirectly limits the photosynthetic rate and ultimately results in reduced growth rate (Ziaf et al. 2009).

Pepper is an important agricultural crop, because of its economic importance and the nutritional value of fruits (Navarro et al. 2006). In most of reports, it has been found that salinity reduces the photosynthetic rate and related parameters such as, stomatal conductance and intercellular CO_2 concentration in pepper (Azuma et al. 2010). It has been demonstrated that salinity stress causes a great reduction in stomatal conductance, leaf chlorophyll content, transpiration rate and net assimilation of CO_2 in pepper

(Martinez-Ballista et al. 2004, Lycoskoufis et al. 2005). Navarro (2003) and Martinez-Ballista et al. (2004) found that pepper is susceptible to high salinity, because of reduced stomatal conductance, but they also suggest that growth suppression after longer exposure to salinity is also because of inhibition of photosynthesis at chloroplast level. Some eco-physiological characteristics have been reported which can be useful to be used as selection indices for salt tolerance in different crops (Ashraf and Harris 2013). So far, it has not been investigated whether photosynthetic parameters could be used as effective selection indicators for screening pepper cultivars for salt tolerance. Salt stress disturbs pepper growth more during vegetative phase and seedling stage is more sensitive to salt stress (Ziaf et al. 2009). Therefore, the objective of this study was to investigate the tolerance of 26 pepper cultivars to salt stress by studying the photosynthesis parameters, chlorophyll and RLWC during the seedling stage under salinity stress conditions.

MATERIALS AND METHODS

Plant material

This experiment was conducted on 26 commercial pepper (*Capsicum annuum* L.) cultivars (different types) (Table 1). Seeds were surface-sterilized in sodium hypochlorite solution (5% v/v) for 10 min, then washed with de-ionized water and were incubated at a constant temperature of 28°C. After 12 days, the seedlings were transferred to greenhouse, with the temperature ranged between 16-32ºC and the relative humidity of 60-90% (day/night). They were selected according to their uniformity and transplanted into 15 L black plastic containers, containing aerated full nutrient solution consisted of macronutrients (4 mM N, 2 mM K, 0.25 mM P, 2 mM Ca, 1 mM Mg, 1.88 mM S) and micronutrients (10 µmol B, 0.5 µmol Mn, 1µmol Zn, 100 µmol Fe, 0.2 µmol Cu and 0.02 µmol Mo). The solution was completely renewed every 3-day (Aktas et al. 2006).

Treatment and experimental design

Pepper plants, at 6 –7 true leaf stage, were treated with a salty solution containing 0 and 100 mM NaCl for two weeks. The evaluation was carried out after 14 day salt stress treatment. The experiment was conducted in a completely randomized

Table 1. The name of seed company and pepper cultivars which were used in this experiment

Number	Cultivar	Company	Number	Cultivar	Company
1	Ethem	Petoseed	14	Exp. 10	Vilmorin
2	Dulce	Petoseed	15	Tyson	Vilmorin
3	Shanghai(SQ-Y)	Petoseed	16	Daytona	Nunhems
4	Luzon	Bruinsma	17	Magic	Axia
5	PaxRGH	Bruinsma	18	Defender	Nunhems
6	Paramo	Bruinsma	19	Figaro	Vilmorin
7	Lorca F1	Bruinsma	20	Radin	Axia
8	Mentor	Bruinsma	21	ACX 248	ABBOT&COBB
9	Snooker	syngenta	22	Maral	Axia
10	Efests	Nunhems	23	Wanado	Axia
11	Semerkand	Nunhems	24	Octavio	Vilmorin
12	SPADI	Vilmorin	25	Sereno	Vilmorin
13	ACX 270	ABBOT&COBB	26	Exp. 4	Vilmorin

design, in a factorial, with two salinity treatment (0 and 100mM NaCl) and 26 pepper cultivars, with three replications (30 plants represented one replicate).

Growth parameters

After measuring all physiological parameters, the plants were harvested and separated into shoots and roots parts. Fresh mass of shoots and roots were dried a minimum of 72 h at 70ºC. Shoot (*SDW*), Root (*RDW*) and Total dry weights (*TDW*) amounts were recorded using analytical balance. Three plants per replicate (nine plants totally) were randomly measured for each cultivar and treatment.

Leaf gas exchange

Gas exchange in the youngest fully expanded leaves was measured after two weeks salt stress treatment. Assimilation rate (*A*), stomata conductance (GCO_2), water vapor conductance (GH_2O), intercellular CO_2 concentration (*Ci*), mesophyll conductance (*MC*), and transpiration rate (*E*) were simultaneously recorded from 08:30 to 10:30 am using a portable Gas Exchange Fluorescence System (GFS-3000, Walz, Germany), under natural conditions (28 - 32°C, 900 µmol photons m^{-2} s^{-1}, 400 µmol CO_2 mol^{-1},70% relative humidity). All measurements were taken in

14 days after treatment. Each set of data (replicate) was the mean of two measurements which was taken from two different plants. All measurements were made in triplicate.

Photosynthesis Water Use Efficiency (PWUE)

Photosynthesis water use efficiency (PWUE) is equal the ratio of CO_2 assimilation rate (A) over stomatal conductance (GCO_2).

Chlorophyll content

The Chlorophyll content was measured on the same leaves used for photosynthetic gas exchange measurements using the method described by Saida et al (2014). One gram of fresh leaf from the youngest fully expanded leaf was thoroughly homogenized in 2 mL of 85% acetone with a mortar and pestle. The extracts were centrifuged at 10,000 rpm for 5 min. Absorbance of the supernatant were recorded at 663.2 and 646.8 nm wavelengths using a PG Instruments LTD – T80+ UV/VIS spectrophotometer. Chlorophyll *a, b* and total chlorophyll concentration were calculated using the below formulas:

$Chl.a$ (mg/g FW) = 12.25(A663.2) − 2.72(A646.8),

$Chl.b$ (mg/g FW) = 21.5(A646.8) − 5.10(A663.2),

$TChl.$ (mg/g FW) = 7.15(A663.2) +18.71(A646.8).

Where, A663.2 and A646.8 represent absorbance values read at 663.2 and 646.8 nm wavelengths, respectively.

Relative leaf Water Content (RLWC)

Relative leaf water content (*RLWC*) was measured in fully expanded leaves of four plants per replicate according to Ziaf et al (2009), where

RLWC (%) = [(Leaf fresh mass – Leaf dry mass)/ (Leaf turgid mass– Leaf dry Mass)] ×100 based.

Salt Tolerance Index (STI)

The stress tolerance index was calculated using the Fernandez (1992) formula.

$$STI = (Y_s \times Y_p)/(Y_{\bar{p}})^2$$

Y_s = total dry weight in salt treatment, Y_p= total dry weight in control, $Y_{\bar{p}}$ = Total dry weight of all cultivars in control. Three plants per replicate (nine plants in total) were randomly measured for each cultivar and treatment.

Statistical analysis

Data were subjected to analysis of variance (*ANOVA*) followed by LSD test ($P < 0.05$) to distinguish differences among the treatments. Statistical analyses and Pearson correlation coefficients between traits were analyzed using SPSS software 16.00.

RESULTS AND DISCUSSION

Growth parameters

Shoot dry weight (*SDW*) of all pepper cultivars decreased significantly under salt stress condition (Table 2). There was a significant difference between pepper cultivars for *SDW.* The cultivars, Parano, Efests and Sereno showed the higher *SDW*, while PaxRGH, Mentor and Exp. 10 had lower *SDW* than other cultivars when exposed to salt stress (Table 2). Correlation analysis indicated that *SDW* was positively correlated with gas exchange parameters, chlorophyll content and *RLWC* (Table 4).

Root dry weight (*RDW*) also significantly decreased under salinity stress (Table 1). Under salt stress conditions, maximum *RDW* was found in Paramo, Sereno and Snooker respectively, while minimum one was noted in PaxRGH, Mentor and Ethem (Table 2). There was a significant correlation between *RDW* and gas exchange parameters, chlorophyll content and *RLWC* (Table 4).

The whole plant biomass (*TDW*) also declined in pepper seedling under 100 mM NaCl. The decrease was mostly derived from a reduction in shoot biomass (*SDW*) than to root (Figure 1). This decline was completely dependent to pepper cultivars. Paramo, Efests and Sereno cultivars maintained the highest *TDW* under salt stress conditions, while it was the least in PaxRGH, Mentor and Exp. 10 (Table 2). A positive correlation was found between *TDW* and gas exchange parameters, chlorophyll content and *RLWC* (Table 4).

Results of this study showed that salinity stress significantly decreased the growth parameters of all pepper cultivars, however, cultivars behaved differently in this attribute. These results were in conformity with finding of Niu et al. (2010), Niu & Rodrigueza (2010), Zhani et al. (2012), and

Table 2. Growth parameters and chlorophyll content of pepper cultivars in salt stress.

Cultivar	RDW(g)	SDW(g)	TDW(g)	Chl a (mgg^{-1} FW)	Chl b (mgg^{-1} FW)	TChl (mgg^{-1} FW)
Ethem	0.31 jk	0.94 hi	1.25 g-i	4.04 e-i	1.91 f-h	5.94 gh
Dulce	0.43 d-g	1.20 d-g	1.63 c-f	4.36 b-h	1.99 f-h	6.34 e-g
Shanghai	0.38 g-j	1.12 f-i	1.50 e-i	4.08 e-i	2.34 b-g	6.41 d-g
Luzon	0.36 g-k	1.07 g-i	1.42 e-i	4.09 d-i	1.99 f-h	6.08 f-h
PaxRGH	0.13 l	0.36 j	0.49 j	3.52 i	1.45 h	4.96 h
Paramo	0.61 a	1.72 a	2.33 a	5.03 ab	2.99 a	8.02 a
Lorca F1	0.51 b-d	1.37 b-f	1.88 bc	4.66 a-g	2.08 e-g	6.74 b-g
Mentor	0.29 k	0.88 i	1.17 i	4.01 f-i	1.91 f-h	5.91 gh
Snooker	0.56 a	1.55 ab	2.11 ab	4.75 a-f	2.62 a-e	7.38 a-e
Efests	0.56 a	1.62 ab	2.18 ab	4.98 a-c	2.76 a-d	7.75 a-c
Semerkand	0.35 g-k	1.06 g-i	1.42 e-i	4.35 b-h	2.28 b-g	6.64 b-g
SPADI	0.50 b-e	1.37 b-e	1.87 b-d	4.80 a-e	2.49 a-f	7.29 a-f
ACX 270	0.52 bc	1.54 ab	2.06 ab	4.99 a-c	2.86 ab	7.84 ab
Exp. 10	0.31 i-k	0.89 i	1.21 hi	3.61 hi	2.08 e-g	5.70 gh
Tyson	0.38 g-j	1.12 e-i	1.50 e-h	4.49 a-g	2.39 a-g	6.89 a-g
Daytona	0.52 bc	1.46 bc	1.98 b	4.67 a-g	2.63 a-e	7.31 a-f
Magic	0.35 g-k	1.02 g-i	1.37 e-i	4.22 c-i	2.18 d-g	6.40 d-g
Defender	0.38 g-j	1.17 d-h	1.55 d-g	4.32 b-h	2.19 c-g	6.50 c-g
Figaro	0.42 e-h	1.22 c-g	1.64c-e	4.38 b-h	2.37 b-g	6.75 b-g
Radin	0.35 g-k	1.06 g-i	1.41 e-i	3.97 g-i	1.90 gh	5.86 gh
ACX 248	0.47 c-f	1.42 b-d	1.89 bc	4.87 a-d	2.77 a-c	7.65 a-d
Maral	0.40 f-i	1.06 g-i	1.46 e-i	4.26 b-i	2.16 e-g	6.42 d-g
Wanado	0.34 h-k	0.97 g-i	1.31 f-i	4.25 b-i	2.18 d-g	6.43 d-g
Octavio	0.35 g-k	1.01 g-i	1.36 e-i	4.23 c-i	2.22 c-g	6.46 d-g
Sereno	0.57 a	1.60 ab	2.17 ab	5.27 a	2.59 a-e	7.86 ab
Exp. 4	0.36 g-k	1.12 g-i	1.48 e-i	4.33 b-h	2.32 b-g	6.64 b-g

Numbers with the same letters are not statistically different ($P < 0.05$)

Kaouther et al. (2012). In general, salt stress decrease plant growth due to osmotic stress, altered metabolism, inability of apoplastic acidification and lack of turgor (Munns and Tester, 2008). Dry matter accumulation accounts as the best index of all growth parameters correlated with yield (Ziaf et al. 2009). Kumar et al. (2009) noted that salt resistant cultivars generate larger biomass (dry weight) than sensitive ones under salt stress condition. Therefore, higher seedling dry weight in Paramo, Efests and Sereno cultivars under salinity could be associated with their higher salinity tolerance (Tables 2). We found a reduction in growth parameters that can be partially attributed to the inhibition of carbon assimilation under stress (Lovelock and Ball, 2002).

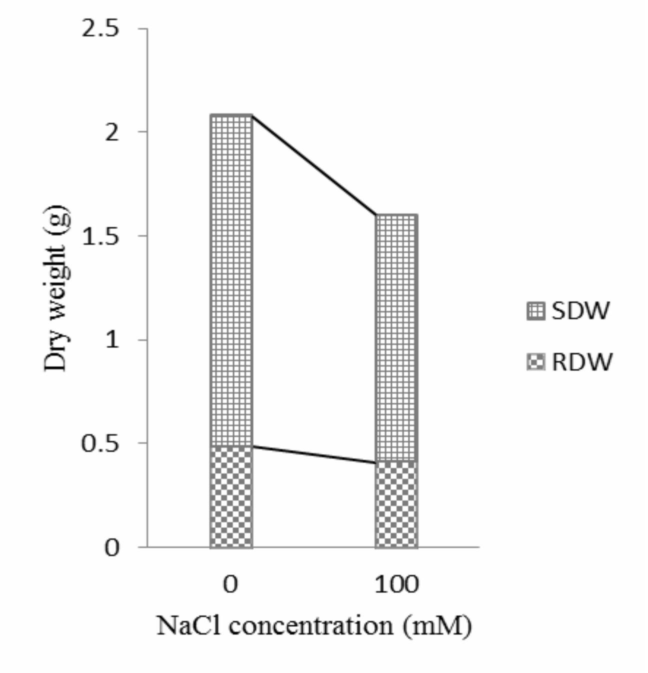

Figure 1. Effects of salinity (NaCl) treatment on mean dry weight of root and shoot in all pepper cultivars ($P < 0.05$).

Chlorophyll content

Salt treatment significantly affected chlorophyll *a* and *b* and *TChl.* of pepper cultivars. Both *Chl. a* and *b* contents decreased significantly at 100 mM NaCl, however there was a significant difference among pepper cultivars for *Chl. a* and *Chl. b*. The reduction in *Chl. b* was proportionally higher than that of *Chl. a* and subsequently the *Chl. a/ Chl. b* ratio increased significantly in salinity (Figure 2), however no significant differences was found among pepper cultivars.

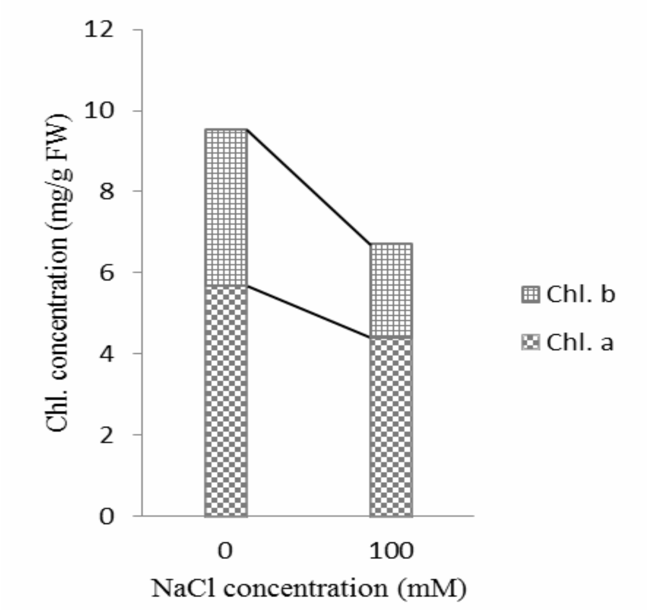

Figure 2. Effects of salinity (NaCl) treatment on Chl a and b in all pepper cultivars ($P < 0.05$).

TChl. decreased at 100 mM NaCl compared with the *TChl.* content in control. All cultivars showed a significant variation in this parameter. More amount of *Chl. a* and *TChl.* were belonged to Sereno, Paramo and ACX 270 cultivars, while the lowest value was observed in PaxRGH, Luzon and Radin cultivars. The highest value in *Chl. b* was observed in Paramo, ACX 270 and ACX 248 under salt stress, while the lowest was observed in PaxRGH, Radin and Ethem cultivars respectively (Table 2). A positive significant correlation was found between chlorophyll content, gas exchange and growth parameters (Table 4).

Table 3. Some photosynthesis parameters and relative leaf water content of pepper cultivars in salt stress conditions

Cultivar	E (mmol m^{-2} s^{-1})	GH2O (mmol m^{-2} s^{-1})	GCO2 (mmol m^{-2} s^{-1})	A (µmol m^{-2} s^{-1})	PWUE (µmol mol^{-1})	RLWC (%)
Ethem	1.59 rs	0.14 op	0.09 qr	5.61 q-t	63.34 c	80.96 c-g
Dulce	1.69 qr	0.15 no	0.09 pq	6.60 d-j	70.27 b	82.46 a-g
Shanghai	2.71 hi	0.24 gh	0.15 gh	5.69 p-t	37.67 ij	81.10 c-g
Luzon	1.96 no	0.17 lm	0.11 mn	5.99 l-r	55.05 d	79.50 fg
PaxRGH	1.27 u	0.11 q	0.07 s	5.38 t	76.45 a	79.12 g
Paramo	3.59 a	0.31 a	0.20 a	7.35 a	36.75 ij	84.65 a-c
Lorca F1	2.79 gh	0.24 fg	0.16 fg	6.68 c-i	42.97 gh	80.67 d-g
Mentor	1.44 t	0.12 pq	0.08 r	5.46 st	68.56 b	79.59 e-g
Snooker	3.07 de	0.27 de	0.17 cd	6.91 a-f	40.39 hi	82.79 a-g
Efests	2.32 k	0.20 j	0.13 j	7.14 a-c	55.19 d	83.35 a-e
Semerkand	3.34 bc	0.29 bc	0.19 b	6.30 h-n	33.84 j	81.83 b-g
SPADI	3.20 cd	0.28 cd	0.18 bc	6.99 a-e	39.19 hi	84.44 a-d
ACX 270	3.22 bc	0.28 b-d	0.18 bbc	7.06 a-d	39.29 hi	85.72 a
Exp. 10	1.47 st	0.13 p	0.08 r	5.54 r-t	67.53 bc	79.72 e-g
Tyson	2.57 ij	0.22 hi	0.14 hi	6.53 e-k	45.64 fg	82.05 a-g
Daytona	2.95 ef	0.26 ef	0.16 de	6.83 b-g	41.54 g-i	82.69 a-g
Magic	1.81 pq	0.16 mn	0.10 no	5.84 n-t	58.00 d	80.08 e-g
Defender	2.25 kl	0.20 j	0.13 jk	6.23 i-o	49.66 ef	81.21 c-g
Figaro	1.73 p-r	0.15 no	0.10 op	6.45 f-l	67.10 bc	81.90 b-g
Radin	2.51 g	0.22 i	0.14 i	5.77 o-t	41.30 g-i	80.80 d-g
ACX 248	1.73 p-r	0.15 no	0.10 op	6.75 b-h	70.25 b	83.15 a-f
Maral	2.89 fg	0.25 fg	0.16 ef	6.07 k-q	37.71 ij	80.67 d-g
Wanado	2.05 mn	0.18 kl	0.11 lm	6.15 j-p	53.91 de	81.24 c-g
Octavio	2.16 lm	0.19 jk	0.12 kl	5.92 m-s	49.21 ef	81.13 c-g
Sereno	1.87 op	0.16 mn	0.10 no	7.22 ab	69.37 b	85.25 ab
Exp. 4	3.36 b	0.29 b	0.19 b	6.38 g-m	34.02 j	82.35 a-g

Numbers with the same letters are not statistically different ($P < 0.05$)

The reduction in Chl. *a* and *b* and *TChl.* by salinity has been described previously in pepper (Lycoskoufis et al. 2005, Mota-Cadenas et al. 2010, Kaouther et al 2012, Zhani et al. 2012, Lopez-Aguilar et al. 2012). Salt stress can reduce leaves chlorophyll by degradation or inhibition of chlorophyll synthesis (Ashraf and Harris 2013). High salt stress increases activity and synthesis of chlorophyllase, chlorophyll degrading enzyme, and increase destruction of number, size and chloroplast structure by oxidative stress, which causes a reduction of chlorophyll content (Santos 2004, Khafagy et al. 2009, Hayat et al. 2013). Therefore, changes in chlorophyll content are suitable indicators for stress (Naumann et al. 2008), because chlorophyll content is decreased in salt-sensitive species under salinity conditions (Ashraf and Harris 2013). Highly positive correlation between chlorophyll content and assimilation rate confirms that high salinity inhibits photosynthesis which is partly associated with reduction in chlorophyll concentration (Ashraf 2004, Lycoskoufis et al. 2005). In this study, salt stress markedly reduced chlorophyll *a, b* and *TChl.* in all cultivars. Reduction in chlorophyll contents reduces photosynthetic processes which ultimately decrease biomass production under saline conditions (Mukhtar etal. 2013). These results are conformity with the reports for different crops e.g., wheat (Raza et al. 2006), pea (Yildirim et al. 2008) and safflower (Siddiqi et al. 2009), canola (Mukhtar etal. 2013). In this study, salt stress increased *Chl. a/Chl. b* ratio in all cultivars, because salinity increase chlorophyll degradation and during this process, *Chl. b* is converted in *Chl. a* (Santos 2004), and this may explain the increase of the *Chl. a/Chl. b* ratio in salt stress.

Leaf gas exchange

Salt stress affected the assimilation rate (*A*), stomata conductance (GCO_2), water vapor conductance (GH_2O), intercellular CO_2 concentration (*Ci*), mesophyll conductance (*MC*), and transpiration rate (*E*) (Table. 3).

The exposure of pepper cultivars to salinity, significantly reduced *A*. The cultivars showed a significant variation in response to salt stress in this attribute. Comparison of the cultivars shows that Paramo, Sereno and Efests had the highest values, while PaxRGH, Mentor and Exp. 10 had the lowest one in *A* under salinity stress. There was a positive correlation between *A* and growth parameters (r=0.92 **).

Table 4. Bivariate correlations analysis between photosynthesis parameters, growth parameters and relative leaf water content on pepper cultivars in salt stress

	E	GH2O	GCO2	A	Ci	MC	PWUE	Chl. a	Chl. b	TChl.	Chl. a/ b	RDW	SDW	TDW	RLWC	STI
E	1															
GH2O	1.00**	1														
GCO2	1.00**	1.00**	1													
A	0.55**	0.55**	0.55**	1												
Ci	0.34	0.34	0.34	0.72**	1											
MC	0.52**	0.52**	0.52**	0.89**	0.35	1										
PWUE	-0.94**	-0.94**	-0.94**	-0.30	-0.14	-0.32	1									
Chl. a	0.49*	0.49*	0.49*	0.95**	0.73**	0.82**	-0.27	1								
Chl. b	0.57**	0.57**	0.57**	0.84**	0.69**	0.68**	-0.39	0.87**	1							
Total Chl.	0.55**	0.55**	0.55**	0.93**	0.74**	0.78**	-0.34	0.97**	0.96**	1						
Chl. a/ b	-0.49*	-0.49*	-0.49*	-0.42*	-0.40*	-0.31	0.44*	-0.44*	-0.82**	-0.63**	1					
RDW	0.54**	0.54**	0.54**	0.92**	0.74**	0.78**	-0.35	0.92**	0.86**	0.93**	-0.52**	1				
SDW	0.54**	0.54**	0.54**	0.92**	0.74**	0.77**	-0.36	0.93**	0.89**	0.95**	-0.57**	0.99**	1			
TDW	0.54**	0.54**	0.54**	0.92**	0.75**	0.77**	-0.36	0.93**	0.89**	0.94**	-0.56**	0.99**	1.00**	1		
RLWC	0.52**	0.52**	0.52**	0.89**	0.81**	0.69**	-0.28	0.89**	0.84**	0.90**	-0.49*	0.81**	0.83**	0.83**	1	
STI	0.53**	0.53**	0.53**	0.97**	0.75**	0.84**	-0.30	0.95**	0.86**	0.94**	-0.48*	0.96**	0.96**	0.96**	0.90**	1

*, ** Correlation is significant at the 0.05 and 0.01 level 2-tailed respectively.

Assimilation rate (A), stomata conductance (GCO2), water vapor conductance (GH2O), intercellular CO2 concentration (Ci), transpiration rate (E), photosynthetic water use efficiency (PWUE) and mesophyll conductance (MC), chloropyll a (chl. a), chlorophyll b (chl. b) and total chlorophyll (TChl.), shoot dry weight (SDW), root dry weight (RDW) and total dry weight (TDW) and relative leaf water content (RLWC).

In our experiment, salt stress significantly decreased *A*, this ultimately reduces biomass production (Total dry weight). These results were in agreement with Martinez-Ballesta et al. (2004), Lycoskoufis et al. (2005) and Mota-Cadenas et al. (2010) in pepper, Siddiqi et al. (2009) in safflower, Ulfat et al. (2007) and Mukhtar et al. (2013) in canola. The *A* was generally reduced by salinity (Chaum & Kirdmanee, 2009, Mota-Cadenas et al. 2010, Colla et al. 2010). Salinity stress might affect the biochemistry of photosynthesis by causing disorientation of the lamellar system of chloroplasts and loss of chloroplast integrity leading to a decrease in the activity of photosystems (Desingh, and Kanagaraj, 2007).The reduction in photosynthetic parameters under salt stress, reduces crop growth and yield (Ashraf, 2004, Ulfat *et al.* 2007, Siddiqi *et al.* 2009). We could assume that the reduced CO_2 assimilation due to salt stress decreased the biomass in all cultivars. The sensitivity of *A*, is an effective salt tolerance indicator under salt stress (Ashraf 2004, Deng et al. 2010, Mukhtar et al. 2013). Some papers confirm that pepper is sensitive to salinity and indicate that photosynthesis rate as well as stomatal conductance is adversely affected by high salinity (De Pascale et al. 2003, Lycoskoufis et al. 2005, Azuma et al. 2010). We observed a strong correlation between assimilation rate and growth parameters. The assimilation rate as a selection index for stress tolerance can be used in species which there is a positive relationship between the photosynthesis rate and the growth under salt stress conditions (Ashraf and Harris, 2013).

Salt stress also reduced transpiration rate *(E)* of all cultivars. However, pepper cultivars showed different responses for *E*. The Lowest value of *E* was observed in PaxRGH, Mentor and Exp. 10 and the highest value was observed in Paramo, Exp. 4 and Semerkand respectively. Calculated correlation coefficients showed significant positive correlation between *E* and growth parameters among cultivars (Table 4).

This results was correlated with other previous studies on pepper (Lycoskoufis et al. 2005, Lopez-Aguilar et al. 2012), wheat (Ashraf and Shahbaz, 2003, Raza et al. 2006), Sunflower (Noreen & Ashraf 2008), safflower (Siddiqi et al. 2009), and canola (Mukhtar etal. 2013). It is reported that the transpiration rate usually tends to decrease, with increasing salt concentration in root zone and this could be associated with lower water potential in roots and the transport of abscisic acid (*ABA*) from root to shoot to influence stomatal closure (Turan et al. 2009) .

When pepper plants exposed to salinity stress, GCO_2 and GH_2O decreased significantly in all cultivars. A considerable variation was recorded in this attribute. Paramo, Exp. 4 and Semerkand cultivars showed the higher, while PaxRGH, Mentor and Exp. 10 showed the lower reduction of GCO_2 as compared with other cultivars. A weak, but positive correlation was observed among GCO2 and GH2O with *A* and growth parameters (Table 4).

GCO_2 significantly decreased in all cultivars under salinity stress and also showed inter-cultivar variations. These observations confirm other results in pepper (Martinez-Ballesta et al. 2004, Lycoskoufis et al. 2005, Mota-Cadenas et al. 2010, Azuma et al. 2010), wheat (Ashraf & Shahbaz 2003, Raza et al. 2006) and canola (Mukhtar etal. 2013). The stomatal closure affected the photosynthesis process because the path of CO_2 and O_2 was blocked (Lopez-Aguilar et al. 2012). In this study, results showed that there was a positive correlation between GCO_2 and *A*. It was found to be a strong negative correlation between GCO_2 and NaCl stress (Turan et al. 2007). Similarly, a positive correlation was observed between GCO_2 and photosynthesis (Eisa et al. 2012).

Salinity caused a high increase in photosynthetic water use efficiency (*PWUE*) in all cultivars. Maral, Exp. 4 and Paramo cultivars had significantly higher amount, while PaxRGH, Dulce and ACX 248 showed the lower *PWUE* in compared with other cultivars. In contrast, no significant correlation was found between *PWUE* with growth parameters (Table 4).

Mesophyll conductance *(MC)* in leaves reduced significantly due to salt stress. There was no significant difference in *MC* values among pepper cultivars. A high correlation was found between growth parameters and *MC* (Table 4).

Salinity decreased *MC* in all cultivars but a significant inter-cultivar variation was not observed. *MC* reduction was associated with changes in leaf anatomy or reduction of the intercellular spaces in the mesophyll (Delfine et al. 1999). Results of this study present a strong correlation between *MC* and *A* that indicated that non stomatal factors are more important than stomatal factors in high salinity. These results are in good agreement with that obtained by Deng et al. (2010), who found that stomatal restriction is more significant at intermediate salinities and non-stomatal limitation is more represented at high salinity.

Ci was significantly reduced in all pepper cultivars due to salinity ($P < 0.05$) but *Ci* didn't show significant differences among pepper cultivars. A high correlation was found between *Ci* and *STI* ($r = 0.75$ **). The present result was in agreement with Lycoskoufis et al. (2005) in pepper, Yildirim et al. (2008) in pea, Siddiqi et al. (2009) in safflower and Mukhtar et al. (2013) in canola. Salt stress decline of GCO_2, which reduce *Ci* in plants (Parida and Das 2005, Qu et al. 2009).

Our results showed that salinity stress had a negative effect on *A*, GCO_2, *Ci* and *MC*. The percentage of GCO_2 reduction under 100 mM NaCl was more than Ci that indicates the decline in GCO_2 did not decrease Ci and the reduction in *A* was not only dependent on GCO_2 and *Ci.* In other research, non-stomatal inhibition of photosynthesis has been observed for several species. This could be caused by the effect of NaCl on photosynthetic mechanisms unrelated to stomatal closure (Flexas et al., 2008). The strong correlation was observed between *A* and *MC*. Presumably, Stomatal and non-stomatal factors contribute to the effect of salt stress on the reduction of *A* in pepper cultivars. Similar results have been reported in pepper (Lycoskoufis et al. 2005, Azuma et al. 2010).

It has been shown that species with relatively higher salt tolerance would have less affected gas exchange parameters (Mota-Cadenas et al. 2010). Our results agree with these reports and conclude that the better photosynthetic machinery may be correlated with the salt tolerance ability of the tolerant pepper cultivars.

Leaf relative water content (RLWC)

Pepper cultivars treated with 100mM NaCl showed a significant decrease in *RLWC* as compared to the control. A considerable variation was found in all pepper cultivars pepper cultivars in this attribute. Results of this study showed cultivars PaxRGH, Luzon and Mentor has the lowest values of

RLWC in the salinity stress, while cultivars ACX 270, Sereno and Paramo has the highest. The correlation coefficient of *RLWC* with growth parameters was positive and highly significant (Table 4).

Our results were in agreement with Ziaf et al. (2009). Salinity stress reduced water uptake with a concomitant reduction in *RLWC* (Ziaf et al. 2009). The direct effect of salt accumulation on water potential and osmotic adjustment cause reduced relative water content (Parida and Das 2005). Salt accumulated in the root zone result in low water potential which this inhibits the water absorbance of roots and results in a lack of water supply to the mesophyll. Accordingly stomatal opening and the photosynthetic biochemical reactions altered (Yang et al. 2010). A highly positive and high significant correlation between *RLWC* and growth parameters as well as *RLWC* and photosynthetic parameters (Table 4) provides evidence that *RLWC* can be used as an indicator for screening cultivars according to their salt stress tolerance in pepper. Ziaf et al. (2009) suggested that *RLWC* can be used as an index for salinity tolerance in pepper.

Salt tolerance index (STI)

STI was significantly different among pepper cultivars under salt stress condition (Table 1). According to this indicator, Paramo, Sereno and Efests were more tolerant cultivars and PaxRGH, Exp. 10 and Mentor were more sensitive cultivars to salt stress.

Correlation analysis indicated that *STI* were positively correlated with gas exchange parameters, chlorophyll content and *RLWC* (Table 4).

STI could be used for screening of cultivars with high yield potential and higher tolerance to stress. This criterion is based on the selection of cultivars with high yield in stress and non-stress condition (Fernandez, 1992).

CONCLUSION

In conclusion, our study showed that salt stress (100 mM NaCl) is harmful to vegetative growth of pepper since the root, shoot and total dry weight decreased significantly. In addition, gas exchange parameters, chlorophyll content and *RLWC* were adversely affected by salinity. The gas exchange parameters, chlorophyll content and *RLWC* in pepper cultivars were the effective parameters, which related to overall growth reduction under salt stress. Since a strongly positive correlation between *A* and *STI* can be observed in most of the cultivars, it can be concluded that the photosynthetic rate is a rapid, non-invasive, non-destructive and accurate technique for assessment of salinity tolerance in pepper cultivars. PaxRGH cultivar was classified as salt susceptible, whereas CV. paramo was selected as salt tolerant, based on various biochemicals, physiological and growth parameters appraised in the present study.

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