Article

Physiological tolerance and cation accumulation of different genotypes of *Capsicum annum* under varying salinity stress

Muhammad Afzal[1], **Awais Ahmad**[1], **Ali Abdullah Alderfasi**[1], **Adel Ghoneim**[2], **Mohammad Saqib**[3]

[1]Department of Plant Production, College of Food and Agricultural Sciences, King Saud University, 2460, Riyadh, Saudi Arabia

[2]Department of Soil Sciences, College of Food and Agricultural Sciences, King Saud University, 2460, Riyadh, Saudi Arabia

[3]Department of Agronomy, The Islamia University of Bahawalpur, Pakistan

E-mail: Afzal_uaf@hotmail.com

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Abstract

A greenhouse experiment was demonstrated to compare tolerance ability of four genotypes (Desi, Sanam, Kundri, Asia Bok) of *Capsicum annum*. L. under different levels of saline stress (i.e., control, 40, 80 and 120 mM NaCl). Growth parameters (root, shoot; fresh and dry weight) and physiological (Na^+, K^+, Ca^{++}, concentration, stomatal conductance, transpiration rate, photosynthetic rate, chlorophyll a, b contents) indicators were analyzed to determine tolerability of genotypes. The results indicated that, all genotypes tolerated only under low level of salinity stress (40 mM NaCl) while a severe growth suppress in general was observed at higher levels (80 and 120 mM NaCl). Asia Bok was found more sensitive to salinity with 0.626g shoot fresh weight whereas Desi (1.103g) is comparatively salt tolerant under 120mMNaCl. Chlorophyll a and b contents and transpiration rate decreases with increases in salinity level in all genotypes with almost similar trend. Na^+ accumulation increase with increase in salinity level but found maximum (14 mg g^{-1}DW) in Asia Bok while minimum (10.8 mg g^{-1}DW) in Desi. However K^+ contents behave reversely to salt concentration and was recorded maximum in Desi (33 mg g^{-1}DW) at maximum (120 mM NaCl concentration).Stomata conductance and transpiration rate was found maximum in Desi as compare to the all other three genotypes under all salinity levels except control. For all above physiological determinants Sanam and Asia Bok have similar behavior while Desi and Kundri have diversified under all salinity levels. Correlation between varieties and salinity resulted that continuous increase in salinity affected growth, physiological aspects and cation accumulation in chilies.

Keywords salinity; genotypes; growth; physiology; tolerance.

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1 Introduction

Almost 20% of the world's agricultural land (FAO, 2010) is affected by soil salinity (Munns, 2002) which limits crop productivity (Zadeh and Naeini, 2007) and reduces the yield up to 50% (Bray et al., 2000). Soil

salinity is the major stress that adversely affects the overall metabolism of plant (Roychoudhury et al., 2008) and leads to agricultural land deterioration and production reduction (Chinnusamy et al., 2005). Salinity affects soil plant available water (Munns, 2002; Ashraf, 2004), physiological, morphological and metabolic process, including germination, plant growth and nutritional imbalance (Willenborg et al., 2004). These effects are due to low soil osmotic potential and ion accumulation (Marschner, 1995) where NaCl is the major salt (Munns and Tester, 2008) and plants have been induced to switch on mechanism to regulate its uptake and cellular adjustment (Munns and Tester, 2008). Higher NaCl concentration results in osmotic stress and ionic imbalance (Maggio et al., 2000) membrane instability, mineral distribution, increased respiration rate, decreased photosynthesis and Ca^{++} ion replacement (Muhammad et al., 2011). NaCl concentrations 100-200 mM reduce plant growth rate and photosynthetic pigments in chilies (Chookhampaeng, 2011). A significant growth reduction (60 mM NaCl) (Silva et al., 2008) and photosynthetic pigments (100 to 200 mM NaCl) of chilies occurs due to Na^{+} and Cl^{-} accumulation has been reported (Chookhampaeng, 2011). Salt tress results in suppressed germination and seedling growth (Zeng and Shannon, 2000; Ashraf, 2010), reduced leaf expansion which cause low photosynthetic area and dry matter production (Mansour and Salama, 2004). Reduction in plant K^{+} concentration affects the stomatal conductance, xylem stream flow and transpiration rate (Basu et al., 2002). Salinity stress indirectly causes oxidative breakdown of peroxisomes, mitochondria and chloroplast which produces activated oxygen species (AOS) which damage DNA, chlorophyll, cell proteins (Mittova, 2002) and membrane destabilization (Hasegawa et al., 2000).Chilies are a well-known food used as spice, crop defense and irritant weapon all over the world, enriched with pro-vitamin A, B1, B2, B3, E and P, vitamin C and antioxidants (Bosland and Votava, 1999). Chilies have various antioxidant compounds (Chuah et al., 2008) which prevents oxidative damage of human body and prevents it from cardiovascular diseases and cancer (Oboth and Rocha, 2007). It can dilates blood vessels, prevent heart diseases, cause hotness, relieve pain, faster digestion, stop bleeding against frostbite, reduces inflammation and arthritis (Zia, 2006). Pakistan is not only fulfilling its country demands but also exporting a huge amount of chilies to Gulf States, Canada, Sri Lanka, United Kingdom and USA to earn foreign exchange (Zia, 2006) having 21.8 thousand hectors with 37.2 thousand tons annual production (Anonymous, 2011-12). Due to water shortage and salinity its production has been decreased (78.3%) (Qureshi, 2012) in Sindh and Punjab, the leading production states (Anonymous, 2005-06).

Chilies have been graded moderately salt sensitive (Kanber et al., 1992) to salt sensitive crop (Haman, 2000) respond differently at different growth stages (Chartzoulakis and Klapaki, 2000). Salinity, if not controlled or managed properly, affected the chilies seed germination and emergence (Demir and Okcu, 2004) which ultimately resulted in yield reduction up to 14% and economic losses in addition (Rhoades et al., 1992) specific ionic imbalance, toxicity water deficit, salt accumulation damage (Zhu, 2002).Pakistan has 6.3 million hectors of salinity affected land (14% of the total irrigated area) (Anonymous, 2005) which causes 64% yield reduction on average annually (Afzal et al., 2005). Chilies are adding millions of dollar in GDP of Pakistan annually (Anonymous, 2006) but yield (1-2 tons ha^{-1}) is much lower as compare to other producers (20 tons ha^{-1}). Saline irrigation water and soil salinity are the fundamental factors remarkably reducing chilies production. Introduction of the salt tolerant crops is one of the most effective and faster method to overcome the salinity problems. Keeping in view the extent of soil salinity and its effects on growth and physiological parameters of chilies following objectives are formulated for the proposed study, Identification of available Chillies (*Capsicum annum* L.) germplasm for salinity tolerance.Determining morphological and physiological indicators for salinity tolerance in Chillies (*Capsicum annum* L.).

2 Material and Methods

The proposed study was carried out at vegetable area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. The experiment was laid out in Completely Randomized Design (CRD) with three replications. Four chilies varieties are Desi, Sanam, Kundri, Asia Bok cultivars and 4 salt (NaCl) treatments i.e. (Control, 40, 80 and 120 mM NaCl) for inducing salinity in chilies cultivars were used as experimental material. Seeds were sown in 9L, bottom perforated, plastic pots containing sand rinsed with distilled water. After emergence of first true leaves (15 days after germination), the number of seedlings per pot was thinned to three and they were irrigated according to seedlings need. After twenty days of sowing, half strength (0.5) Hoagland's nutrient solution was given to plants. Salt treatments were started one month after sowing. To avoid the osmotic shock NaCl concentrations was adjusted, by gradually increasing 20 mM every two days until desired concentration reached. Each pot (three plants) was considered as one replicate and there were three pots per treatment. Agronomic observations like root length, shoot length, root fresh and dry weight, shoot fresh and dry weight was observed. Chlorophyll contents, photosynthetic rate, transpiration rate, stomatal conductance, K^+, Ca^{++} and Na^+ concentration from leaf sap, was determined. Chlorophyll contents were determined by using the method of Arnon (1949) and Davies (1976). Fresh leaves (0.5 g) were chopped into segments of 0.5 cm and extracted with 5 mL acetone (80%) placed over night at 10^0C. The material was centrifuged at 14000rpmfor 5 minutes and the absorbance of the supernatant was measured at 480, 645 and 663 nm by spectrophotometer. Then a, b contents were measured by using the following formulae:

Chl a = [12.7 (OD 663) -2.69 (OD 645)] × V/1000 × W,

Chl b = [22.9 (OD 645) -4.68 (OD 663)] × V/1000 × W.

where V isvolume of the solution cm^3, W is Fresh weight of the leaf sample g.

Gas exchange characteristics measured by instantaneous net CO_2 assimilation rate (A), Transpiration (E) stomatal conductance (gs) and photosynthetic rate were made on a fully youngest leaf of each plant using an open system LCA-4 ADC portable Infrared Gas Analyzer (IRGA) (Analytical Development company, Huddleston, England). Measurement were performed from 9.00 to 11.00 a.m. with the following specifications/adjustment, molar flow of air per unit leaf area 403.3 mM m^{-2} s^{-1}, atmospheric pressure 99.9 kPa, water vapor pressure into chamber ranged from 6.0-8.9 mbar, PAR at leaf surface was maximum up to 1711 (mol m^{-2}, s^{-1}) temperature of leaf ranged from 28.4 to 32.4 ^{0}C, ambient temperature ranged from 22.4 to 27.9 ^{0}C, concentration was 352 mol mol^{-1}. Plants collected and washed with detergent for surface disinfection and rinsed under tab water. Oven dried plants were ground into fine powder by electric grinder. After grinding digestion took place for Na^+, K^+, and Ca^{++} was done according to method described by Yoshida et al. (1984). One gram oven dried plant samples were transferred to a 100 ml beaker and 10 ml of tri-acid mixture (HNO_3, $HCLO_4$, in a ratio of 2:1) was added and covered it with watch glass. The samples were allowed to settle for about half an hour till the initial reaction subsided. The sample was heated gently till the solid material disappeared. Later on vigorous heating was given by peacing over a hot plate till a colorless solution resulted. When the volume remained 1.5 ml samples were removed and cooled. Then added distilled water and volume was made 100 ml in volumetric flasks. The analysis of Na^+ and K^+ used Flame Photometer (Sherwood Flame Photometer Model-410).

The experiment was laid out according to Factorial Completely Randomized design (CRD- Factorial). Data were analyzed statistically using ANOVA techniques (Steel et al., 1997) and means were compared by using LSD.

3 Results

Recordeddata presented in Table 1 showed that salinity has significant effects on variety. As the salinity level increased shoot fresh weight decrease. Shoot Fresh weight (SFW), Shoot dry weight (SDW), root fresh weight (RFS), root dry weight (RWD) have significant effect while in case of variety SFW, RDW has significant effect while RFW and SDW have non-significant effect on yield. Maximum yield was recorded at T1 (control) where SFW is 1.9483 g while minimum in T4 at which 120 $mmol^{-1}$ salinity were applied have SFW is 0.8475 g. Comparative effect was also significant except SDW. Interactive study Variety cum salinity presented in Table 1. Statistical data represented that maximum SFW was observed in S1 × V4 (control x variety) which was followed by the treatment (S1 × V1) and (S1 × V2) having SFW1.93 and 1.89 respectively. At higher salinity level (S4) where 100 m mol^{-1} NaCl applied variety desi performed better which is high resistant against salinity having SFW.1.103 Which was followed by variety Kundri (V3) having 0.930 at par with sanam (V2) and Asia Bok (V1) having 0.730 and 0.626 g SFW respectively. Data regarding RFW at higher salinity S4 (120 m mol^{-1}) variety desi is more resistant then rest of all. This was statistically similar with Kundri having SFW 0.276 g. Least significant variety is Asia Bok having SFW 0.153 g at higher salinity. Data regarding RDW and SDW presented in table. RDW against salinity level is highly significant as salinity level increased root dry weight decreased while SDW, control treatment has maximum dry weight while other treatments are statistically alike. Effect of varieties in case of RDW is significant while SDW is non-significant. Interactive effect of salinity and variety against RDW is significant while against SDW is non-significant presented in Table 1. Biomass production with reference to salinity is more important aspects in crop plants. Maximum RDW was 0.243 g observed in variety Desi at control (0 m mol^{-1}) is similar with variety Sanam are 0.233 g which was at par with variety Asia bok having 0.213 g biomass production in root. At higher salinity level more resistance was desi variety having 0.123 g more bio mass production in roots which was at par with S3 × V2 (80 m mol^{-1} × Kundri) having 0.073 g while minimum RDW was observed in Asia bok is 0.023 g. Osmotic potential decreased (more negative) significantly with increasing concentration of NaCl. The decrease was also significantly different within the levels of salinity. NaCl induced osmotic stress. Na+ content of the chilies varieties increased with increase in the intensity of stressful environment. Varieties and salinity means showed highly significant differences. Interaction study among salinity and variety showed significant effect among each other presented in (Fig. 1). Chilies variety Asia Bok at maximum salinity level has maximum Na^+ accumulation followed by Sanam at same salinity level. While minimum sodium accumulation was observed named desi variety. K^+ contents in chilies varieties decreased with increasing intensity of NaCl osmotic stress. Data regarding varieties, salinity level and interaction among each other was significant in (Fig. 2). Maximum K^+ concentration was observed in variety Sanam at control (0 m mol^{-1}) while minimum was observed at high salinity level where 120 m mol^{-1} NaCl in Asia Bok was observed. Higher levels of osmotic stress seemed to be more effective in reducing K+ contents. *Data* regarding chlorophyll (a and b) contents among four chilies varieties at four different salinity level were presented in Fig. 3 and 4, respectively. Varieties mean showed highly significant effect against salinity and treatments mean also have significant effect on chlorophyll a contents. Fig. 3 shows that with the increasing salinity level chlorophyll a contents were decreased. Maximum chlorophyll a contents were observed at treatment where 0 NaCl was applied in desi chili variety. While minimum chlorophyll contents was observed in Asia Bok where 120 m mol^{-1} NaCl applied.

Table 1 Effect of NaCl on growth (shoot, root) fresh and dry weight in chilies.

A. Salinity level	Shoot fresh weight (g)	Root fresh weight (g)	Root Dry weight (g)	Shoot Dry weight (g)
1	1.9483 A	0.8083 A	0.2133 A	0.5517 A
2	1.2675 B	0.4275 B	0.1225 B	0.2675 B
3	1.0192 C	0.3342 C	0.1058 C	0.2100 B
4	0.8475 D	0.2358 D	0.0583 D	0.2475 B
LSD	0.0913	0.055	0.0119	0.136
B. Variety				
1	1.4258 A	0.4950	0.1833 A	0.3542
2	1.2575 B	0.4733	0.1308 B	0.3450
3	1.2125 BC	0.4308	0.0933 C	0.2925
4	1.1867 C	0.4067	0.0925 C	0.2850
LSD	0.058	NS	0.88	NS
C. Interaction				
S1V1	1.93 b	0.74 b	0.243 a	0.556
S1V2	1.89 b	0.82 ab	0.233 a	0.540
S1V3	1.74 c	0.74 b	0.163 d	0.470
S1V4	2.23 a	0.92 a	0.213 b	0.640
S2V1	1.43 d	0.50 c	0.193 b	0.340
S2V2	1.21 efg	0.39 cdef	0.130 e	0.250
S2V3	1.27 e	0.46 cd	0.086 f	0.280
S2V4	1.15 fg	0.34defg	0.080 f	0.200
S3V1	1.24 ef	0.42 cde	0.173 a	0.280
S3V2	0.916 h	0.30 efgh	0.123 f	0.160
S3V3	1.08 g	0.40 cdef	0.073 f	0.230
S3V4	0.916 h	0.20 hi	0.053 g	0.170
S4V1	1.103 g	0.31 efgh	0.123 e	0.240
S4V2	0.730 ij	0.20 ghi	0.036 gh	0.340
S4V3	0.930 h	0.27 fghi	0.050 g	0.190
S4V4	0.626 j	0.15 i	0.023 h	0.130
LSD	0.117	0.15	0.0178	NS

SFW = Shoot fresh weight, RFW = Root fresh weight, SDW= Shoot dry weight, RFW= Root fresh weight.
T1; (Control), T2 (40 mM), T3 (80 mM), T4 (120mM) =V1 (Desi), V2 (Sanam), V3 (Kundri), V4 (Asia Bok).
Means not sharing a common letter at LSD 5%.

Table 2 Coefficients of correlation estimated between salinity on physiological aspects of different chilies varieties.

	Ca^+	K^+	Na^+	SC	TRA	Chla	Chlb	Photo
K^+	0.9470**							
Na^+	-0.9577**	-0.9979**						
SC	0.9788**	0.9242**	-0.9383**					
TRA	0.9663**	0.9401**	-0.9505**	0.9863**				
Chl a	0.8224**	0.7804**	-0.8102**	0.8283**	0.7951**			
Chl b	0.8804**	0.8232**	-0.8499**	0.9417**	0.9135**	0.8689**		
Photo	0.9391**	0.9133**	-0.9340**	0.9547**	0.9453**	0.9326**	0.9444**	
Salinity	-0.9651**	-0.9407**	0.9587**	-0.9738**	-0.9683**	-0.8937**	-0.9440**	-0.9895**

Ca^+ = calcium concentration, K^+ = Potassium concentration, Na^+= Sodium concentration, SC= stomatal conductance, TRA, Transpiration rate, Chl a= Chlorophyll a contents, Chl b= Chlorophyll b contents, Photo= Photosynthesis.

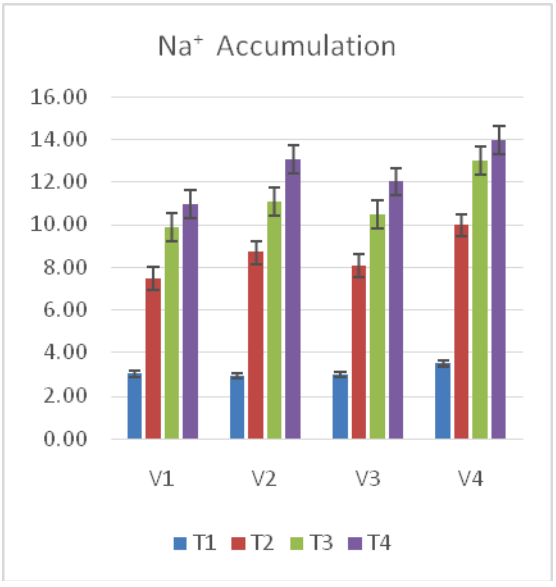

Fig. 1 Effect of salinity on Na^+ accumulation.

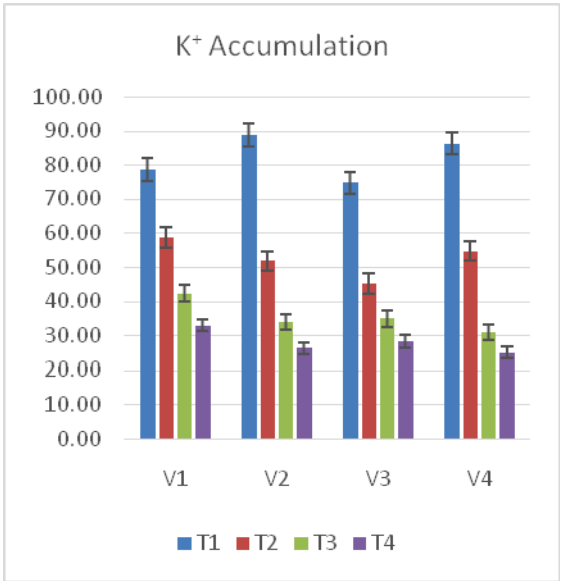

Fig. 2 Effect of Salinity on K^+accumulation.

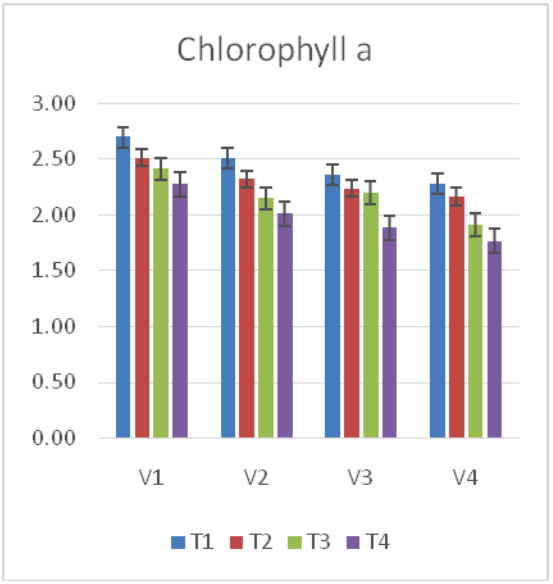

Fig. 3 Effect of salinity on chlorophyll a contents in chilies

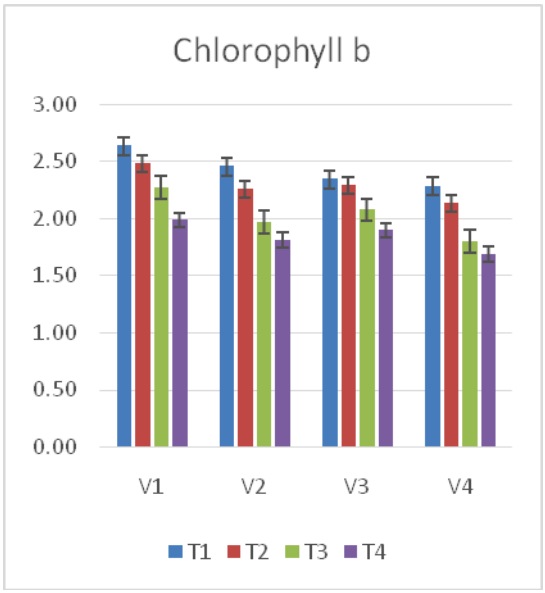

Fig. 4 Effect of salinity on chlorophyll b contents in chilies.

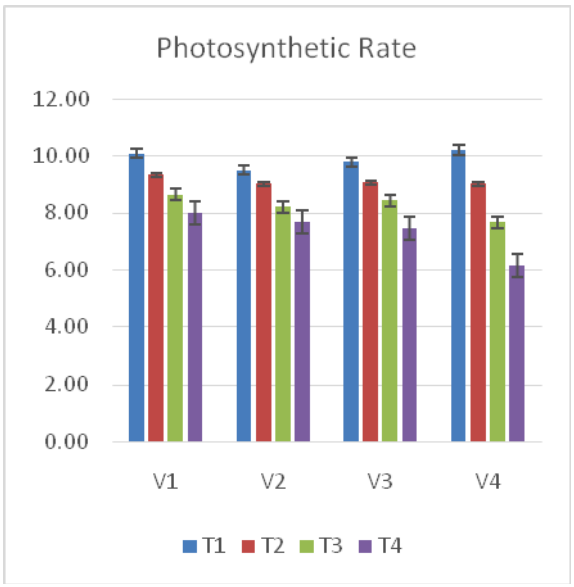

Fig. 5 Effect of salinity on photosynthesis in chilies.

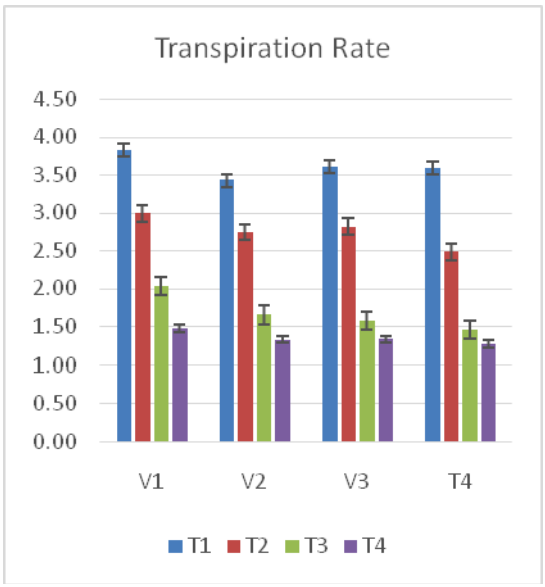

Fig. 6 Effect of salinity on transpiration rate.

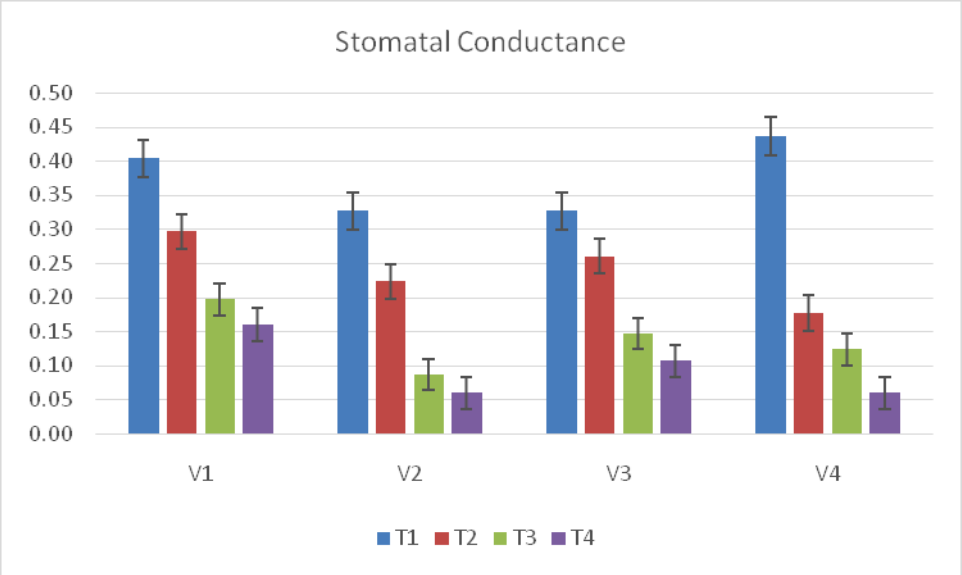

Fig. 7 Effect of salinity on stomatal conductance in chilies.

Effect of salinity on photosynthesis in chilies has a significant effect. With increasing salinity levels photosynthesis activity reduced in chilies. Treatments and varieties mean have highly significant effect on photosynthesis. Interaction between varieties and salinity also has highly significant effect presented in (Fig. 5). Maximum photosynthetic activity was observed in Asia Bok at treatment applied control (0 m mol^{-1}) followed by the Kundri at same level of salinity. Within increase osmotic potential by increasing sodium and chloride contents, photosynthetic activity decreased. Minimum photosynthetic activity was observed in Asia Bok where 120 m mol^{-1} NaCl was applied. While in case of transpiration rate trend also same. As salinity increased transpiration decreased. Treatment and varieties means have significant effect. Desi variety have maximum transpiration rate followed by Kundri. Effect of salinity is significant for transpiration rate in chilies varieties. Mean comparison between salinity and chilies varieties shown in (Fig. 6). Maximum transpiration was observed in Desi variety at control (0 m mol^{-1}) followed by Asia Bok and minimum was observed in Sanam at treatment 100 m mol^{-1} applied. Data regarding stomatal conductance were presented in Fig. 7. Treatments means have highly significant effect on stomatal conductance while variety have significant effect on stomatal conductance. Interaction between variety and salinity also has significance on stomatal conductance. Reported data in Fig. 7 showed that maximum stomatal conductance was observed in Desi variety at control where 0 m mol^{-1} salinity applied. As the concentration of salinity was increased stomatal conductance was reduced among varieties. Minimum stomatal reduction was observed in Sanam at 120 m mol^{-1} NaCl was applied. The following (Table 2) is used to estimate the correlation between salinity and physiological effects on chilies different varieties. In Table 2, data represents about chilies varieties showed that salinity have negative highly significant effect on Ca^{+} concentrations that as salinity increased Ca^{+} concentration decreased gradually. Almost all physiological parameters have same trends i.e. K^{+} concentration, stomatal conductance, transpiration rate, chlorophyll a and b contents and photosynthetic activity have negative highly significant effects except Na^{+}. Results showed that these parameters in four varieties have inverse relation as

concentration of salinity increased they have negatively correlate with each other except Na^+ its concentration increased as salinity level increased.

4 Discussion

NaCl reduced growth by reducing cell division and cell enlargement. Shoot and root growth was observed of stressed chilies plants with 120 m mol^{-1} treatment have negative effect on it by reducing cell elongation and division. Salinity reduced shoot and root elongation as shown in (Table.1). Chilies plants are sensitive to salinity (Chartzoulakis and Klapaki, 2000; Lycoskoufis et al., 2005). Reduction in shoot and root growth due to reduced water absorption, osmotic effect, mineral deficiency account ionic imbalance and reduced metabolic activities (Kumar et al., 2005).The physiological effects were also observed in rice under salt stress (Ozdemir et al., 2004). High salt stress reduced leaf area (Chartzoulakis and Klapaki, 2000); reduction in leaf growth rate is the preliminary sign response of glycophytes exposed to salt stress (Houimli et al., 2008). Reduced leaf area available for transpiration, photosynthetic activity and assimilate production might be a result of osmotic effect (Fig. 1). Water potential and cell volume in cell abruptly decreased due to osmotic dehydration caused by salt stress (Clouse, 1998; Yu et al., 2004). Effect of salinity to the rooting medium had an inhibitory effect on root and shoot growth of the pepper plants (Shahbaz and Ashraf, 2007). Reduction in chlorophyll contents with increase in salinity level in (Fig. 1) this is might be due to excess salinity is often related to reduction in photosynthetic activity. Reduction in photosynthetic activity, due to stomatal or non-stomatal factors or both. Chlorophyll contents give quantitative information about photosynthetic activity (Maxwell et al., 2000).

5 Conclusion

In capsicum annum torabaility against salinity stress varies in different genotypes. Desi variety performed as more tolerant while Asia Bok is relative is more sensitive to saline stress. All genotypes in general have suppressed growth, high sodium accumulation and reduced chlorophyll contents, photosynthesis and transpiration rate. Further molecular study is suggested to identify the tolerance mechanism and optimum level of different genotype under varying saline concentration.

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