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Morpho-physiological characterization of chilli genotypes under NaCl salinity

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Abstract

Salinity is a stringent abiotic stress that limits plant growth and development. Present study was undertaken to explore morpho-physiological responses of different chilli (Capsicum annum L.) genotypes to NaCl-induced salinity. Seedlings of six chilli genotypes (Loralai, Sanam, Desi, Kundri, Asia Bok and Magnum) were subjected to five NaCl salinity levels (0, 25, 50, 75 and 100 mM). A separate control for each genotype was maintained for comparison. Increasing salinity levels significantly reduced shoot and root elongation as well biomass accumulation. Transpiration rate, stomatal conductance, photosynthetic activity and chlorophyll contents were also reduced although genotype-specific responses were evident for these attributes with the exception of photosynthetic rate. At upper limits of salinity (100 mM), Desi and Loralai genotypes produced more shoot and root length and higher fresh and dry biomass than rest of the chilli genotypes. Moreover, comparatively higher stomatal conductance and transpiration rate were also observed for these genotypes at all salinity levels. Desi and Loralai genotypes accumulated less Na^+ and higher Ca^{2+} and K^+ ions than other chilli genotypes. Based on various parameters studied, Desi and Loralai genotypes appeared to be promising salt-tolerant chilli genotypes.

Keywords: *Na^+ ion, photosynthetic activity, salt stress, seedling growth, stomatal conductance*

Introduction

Salinity is one of the most important abiotic constraints limiting crop productivity; 10 percent of world's arable land area is estimated to be salt-stressed (Zhani *et al.*, 2012). Salinity has deleterious effects on growth and development of plants by inducing various biochemical and physiological changes (Munns and Tester, 2008). In Pakistan, nearly 10 million ha area is badly affected by salinity, i.e., 12.9 percent of country's land (FAO, 2008). The arid and semi-arid conditions in Pakistan have aggravated the saline conditions, typically in irrigated areas where availability of fresh water is inadequate.

Hot pepper (*Capsicum annuum* L.) commonly known as chilli is one of the most important vegetable and spice crops of Solanaceae family. It is a crop of much economic significance, as it occupies largest area among vegetable crops in the country, followed by potato and onion. It is mainly grown in Sindh and southern Punjab provinces as a summer crop. During 2011-12, it was grown on an area of 21.8 thousand ha, with an annual production of 37.2 thousand tons (Govt. of Pakistan, 2012). Like other crops, growth and yield of chilli are also adversely affected by salinity (Zhani *et al.*, 2012). Rhoades *et al.* (1992) reported a 14% reduction in chilli yield with each increasing unit of salinity. The harmful implications of salinity arise presumably because of induction of osmotic stress, ionic imbalance and subsequent oxidative stress (Tester and Davenport, 2003). Furthermore, higher concentration of salts under saline conditions causes severe ion toxicity by depositing high concentration of Na^+, which inhibits cell division and expansion, lowers calcium and potassium contents, and causes membrane instability and increased respiration rate (Shahid *et al.*, 2011). Salt stress is also responsible for decreased biosynthesis of chlorophyll and photosynthetic efficiency (Munns, 2002). Under stressful environments, photosynthetic rate declines in response to lower than normal stomatal conductance, depression in carbon uptake and metabolism, inhibition of photochemical capacity (Mundree *et al.*, 2002). Soil salinity affects the growth of plants by altering water relations as a result of salt accumulation in the intercellular spaces (Zhang *et al.*, 2006), injurious effects of toxic ions (Saboora and Kiarostami, 2006), osmotic stress (Almodares *et al.*, 2007) and reduced water-use efficiency (Grewal, 2010).

With dwindling land resources and little scope for horizontal expansion, crop production on saline soils is inevitable and a challenging task. Hence, effective

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utilization of salt-affected soils demands crop-production strategies that can help cope with salinity. Breeding crops for salt tolerance is a long-term and complex process. Therefore, identification of salt-tolerant crop genotypes and determination of inherent genetic variation for salt tolerance would appear to be practical options; and studies have been conducted in this regard (Hussain *et al.,* 2013). Chilli genotypes exhibited immense variation for salt tolerance (Niu *et al*., 2010). Comparing cultivar response of one species to salinity provides a convenient and useful tool for elucidating the fundamental mechanisms involved in salt tolerance. Such information is also crucial for suggesting a suitable crop cultivar for salt-affected soils. Hence, the present study was devised to evaluate the effect of NaCl salinity on some morpho-physiological attributes of different chilli genotypes. Another objective of this research was to determine fluctuations in cationic homeostasis of chilli genotypes in response to NaCl salinity.

Materials and Methods

Plant material and growth conditions

Seeds of six chilli genotypes viz., Loralai, Sanam, Desi, Kundri, Asia Bok and Magnum were collected from a local market (Yousuf Seed Corporation, (Regd.), Faisalabad, Pakistan). Healthy seeds were surface-sterilized with 30% ethanol for 3 min, rinsed three times with distilled water and subsequently dried on filter papers (Srivastava *et al.,* 2010). The study was carried out in a greenhouse at Vegetable Research Area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad (latitude 31.30° N, longitude 73.10° E and 184 m a.s.l.). Seeds were sown in perforated-bottom plastic pots (18 × 29 cm) containing distilled water-rinsed sand and peat in 2:1 ratio (pH 6.2-6.4; field capacity 7.2% and incipient wilting point 1.2% on a volume basis). The number of seedlings per pot was adjusted to five after emergence of first true leaf (15 days after emergence; DAE). Hoagland's nutrient solution of half strength (EC 2 dS m^{-1}, pH 5.8±0.2, temperature 23±2°C) was applied 18 DAE. Saline conditions were simulated by employing aqueous NaCl solutions 30 days after sowing. For this purpose, 25, 50, 75 and 100 mM NaCl solutions were prepared by dissolving analytical grade NaCl (Merck, Darmstadt, Germany) in distilled water. A separate distilled water control was maintained for each chilli genotype. To avoid osmotic shock, NaCl concentrations were adjusted gradually (increasing 25 mM every two days) until desired concentration was reached. An equal volume of distilled water was applied to all pots as and when moisture content decreased.

Experimental design and measurements

The factorial experiment was laid out as a completely randomized design with three replicates and repeated once in time in the same growing season. Chilli samples were collected four weeks after imposing salinity. Plants were separated into roots and shoot and their length and fresh biomass were recorded. For measurement of dry biomass, all samples were oven dried at 70°C till constant weight was achieved.

Photosynthetic pigments (expressed as mg g^{-1} fresh leaf weight) were extracted in 80% ice-cold acetone and quantified at 663 and 645 nm wavelength in a UV-spectrophotometer (UV-4000, ORI, Germany; Lichtenthaler, 1987). Measurements of instantaneous transpiration rate, stomatal conductance and photosynthetic rate were made on the upper-most fully expanded leaf of each plant using an open system LCA-4 ADC portable Infrared Gas Analyzer (IRGA; Analytical Development Company, Hoddesdon, England). Data on physiological attributes were recorded between 10.00-12.00 hours. The quantity of Na^+, K^+, and Ca^{2+} ions was measured in dried and ground leaf tissues after digestion (Wolf, 1990) by comparing the emission on a Flame Photometer (Sherwood Flame Photometer, Model-410) with standard curve (Gomez-Cadenas *et al.,* 1998).

Statistical analysis

Analyses of variance were performed according to a completely randomized design with all data to confirm variability of data and validity of results. Because the results of two runs of experiment were similar, data were pooled for a combined analysis. The differences among treatments were detected using Tukey's HSD (honestly significant difference) test at 0.05 probability level (Steel *et al.,* 1997). Graphical representation of the data was done using MS-Excel and standard errors were computed.

Results

Salt stress adversely affected the performance of the six chilli genotypes as compared with the control. Significant ($p \leq 0.05$) differences regarding different morphological attributes of chilli genotypes were caused by various salinity levels (Table 1; Figure 1). Moreover, chilli genotypes exhibited differential response to salinity levels because of significant interaction ($p \leq 0.05$) between these two factors (Table 1). Maximum shoot length (10.21 cm) and root length (8.56 cm) were recorded for Asia Bok in control pots (Figure 1). Inhibition in shoot and root length was NaCl concentration-dependent and increasing salinity levels diminished the shoot and root length of all chilli genotypes. However, this inhibition was relatively less for Desi and Loralai genotypes than for the rest of the genotypes at upper limits of salinity (100 mM) (Figure 1). Asia Bok and Magnum genotypes appeared to be salt-sensitive because of greater reduction in shoot (68 and

70%) and root (65 and 66%) growth, respectively at higher salinity levels (100 mM). Similarly, significant reduction in fresh and dry biomass of chilli plants was also observed for all genotypes. Nevertheless, the magnitude of reduction varied among chilli genotypes. Increasing salinity levels gradually reduced fresh and dry biomass of chilli plants (Figure 1). Maximum reduction in dry biomass with increase in salinity level was observed for Magnum (50-93%), followed by Asia Bok (52-92%), Sanam (45-87%) and Kundri (23-73%). Desi and Loralai performed somewhat better under NaCl-induced salinity stress and recorded 24-68% and 20-67% reduction in dry biomass, respectively, which was far less than that of other chilli genotypes (Figure 1).

51% compared with the control. Negative impact of NaCl salinity on stomatal conductance was also evident, and increasing salinity levels significantly inhibited stomatal conductance in all chilli genotypes. However, this inhibtion was quite less for Desi genotype even at higher levels of salinity. NaCl salinity had detrimental effects on photosynthetic rate and total chorophyll contents of chilli plants regardless of chilli genotype. Nevertheless, maximum reduction of these attributes was realized at higher salinty levels (100 mM).

Significant ($p \leq 0.05$) differences regarding Na^+, K^+ and Ca^{2+} concentration were recorded for NaCl salinity levels and for chilli genotypes (Figure 3; Table 1). Chilli plants developing under increasing level (25-100 mM) of NaCl

Table 1: ANOVA for combined results for genotypes, different levels of NaCl salinity and their interaction on seedling growth, physiological attributes and cationic homeostasis in chilli

Source of variation	df	Mean squares			
		Seedling growth			
		Shoot length	**Root length**	**Fresh biomass**	**Dry biomass**
Genotype (G)	5	19.199***	4.563***	1.423***	0.052***
Salinity (S)	4	122.26***	100.85***	15.63***	1.453***
G× S	20	3.303***	1.493***	0.316***	0.021***
Error	150	0.140	0.207	0.013	0.0027
CV		5.80	8.07	7.59	16.81
		Physiological attribute			
		Transpiration rate	Photosynthetic rate	Stomatal conductance	Chlorophyll contents
Genotype (G)	5	0.399***	3.379***	0.0412***	2.575***
Salinity (S)	4	25.21***	22.027***	0.305***	3.193***
G× S	20	0.071***	0.702ns	0.005***	0.0328ns
Error	150	0.023	0.544	0.0003	0.045
CV		5.99	8.40	7.55	4.83
		Cationic homeostasis			
		Ca^{2+}	K^+	Na^+	
Genotype (G)	5	0.0162***	248.1***	24.782***	
Salinity (S)	4	0.3210***	12957.2***	453.877***	
G× S	20	0.0014**	149.1***	1.439***	
Error	150	0.0006	16.1	0.096	
CV		11.35	7.82	4.47	

, * denote significance at the 0.01, and 0.001 probability level, respectively, ns= Non-significant

Transpiration rate and stomatal conductance of salt-stressed chilli plants was significantly lower than those of control plants in all chilli genotypes (Figure 2). Interaction between salinity levels and chilli genotypes was significant ($p \leq 0.05$) for these two attributes (Table 1). At the highest salinity level (100 mM), transpiration rate dropped to 1.37 (Magnum) and 1.74 mmol H_2O m^{-2} S^{-1} (Desi) as against 3.61 and 3.65 mmol H_2O m^{-2} S^{-1} observed in the control pots, respectivily; it was a respective decrease of 62 and

salt stress recorded more Na^+ contents than control (Figure 3). Maximum Na^+ concentration was recorded for Magnum (23.56 mg g^{-1}), followed by Asia Bok (22.92 mg g^{-1}) and Kundri (21.94 mg g^{-1}) at 100 mM, whereas plants of Desi (17.04 mg g^{-1}) and Loralai (16.33 mg g^{-1}) recorded less Na^+ contents at the same salinity level. Data showed that K^+ and Ca^{2+} concentrations declined as salinity increased. At 100 mM, maximum K^+ (37.39 mg g^{-1}) and Ca^{2+} (0.17 mg g^{-1}) concentration was recorded in Desi genotype compared to

other genotypes. Asia Bok, Sanam and Magnum appeared to be relatively more sensitive to salinity than rest of the chilli genotypes, as their K^+ and Ca^+ contents were severely declined.

Discussion

Chilli exhibited a typically glycophytic response (significant reduction upon exposure to NaCl-induced salinity as compared to halophytes) for all the morpho-

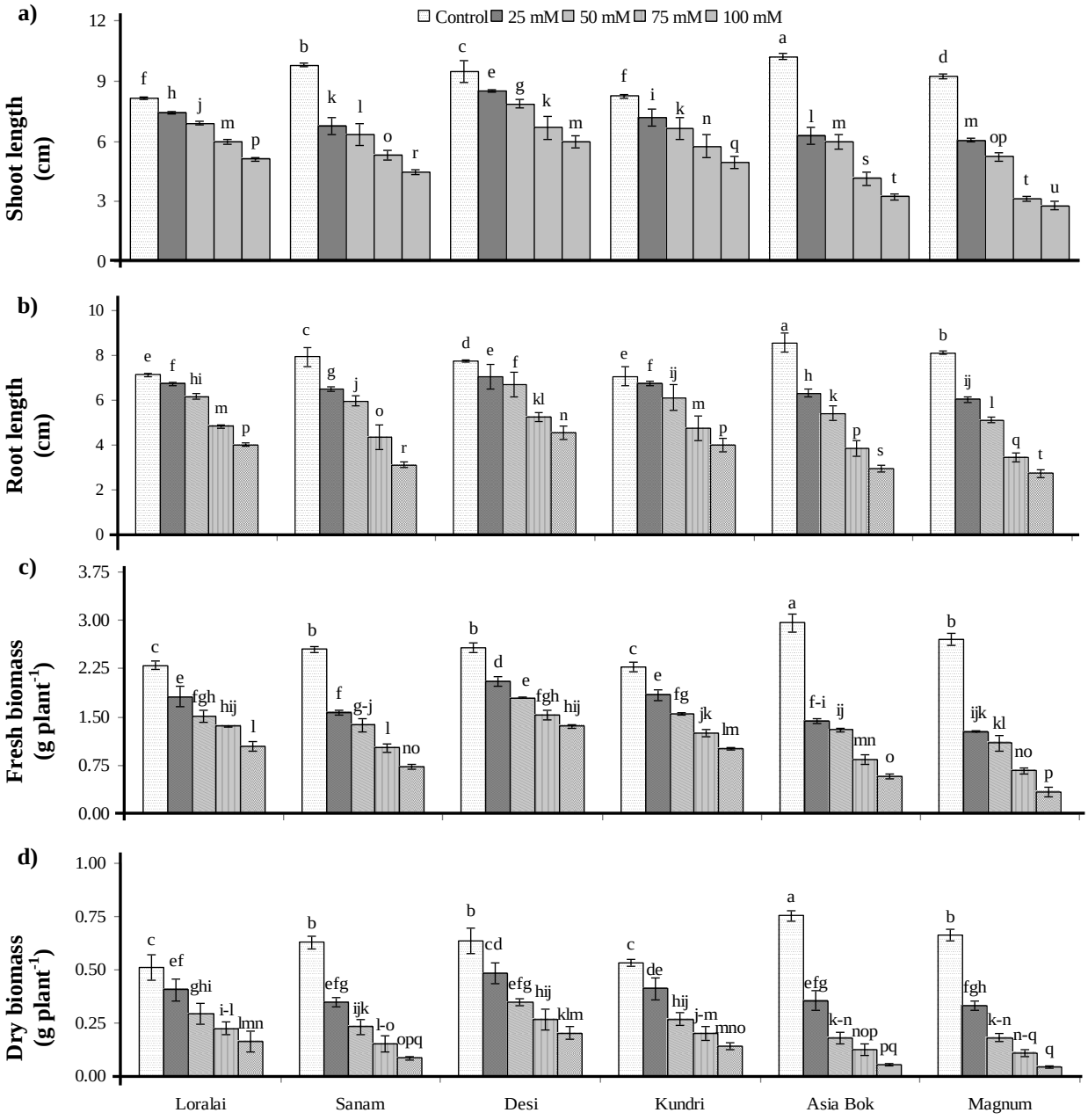

Figure 1: Influence of various salinity levels on (a) shoot length, (b) root length, (c) fresh biomass and (d) dry biomass of six chilli genotypes. Vertical bars above mean denote standard error of three replicates repeated once. Means with different letters differ significantly at 0.05 probability level by HSD test

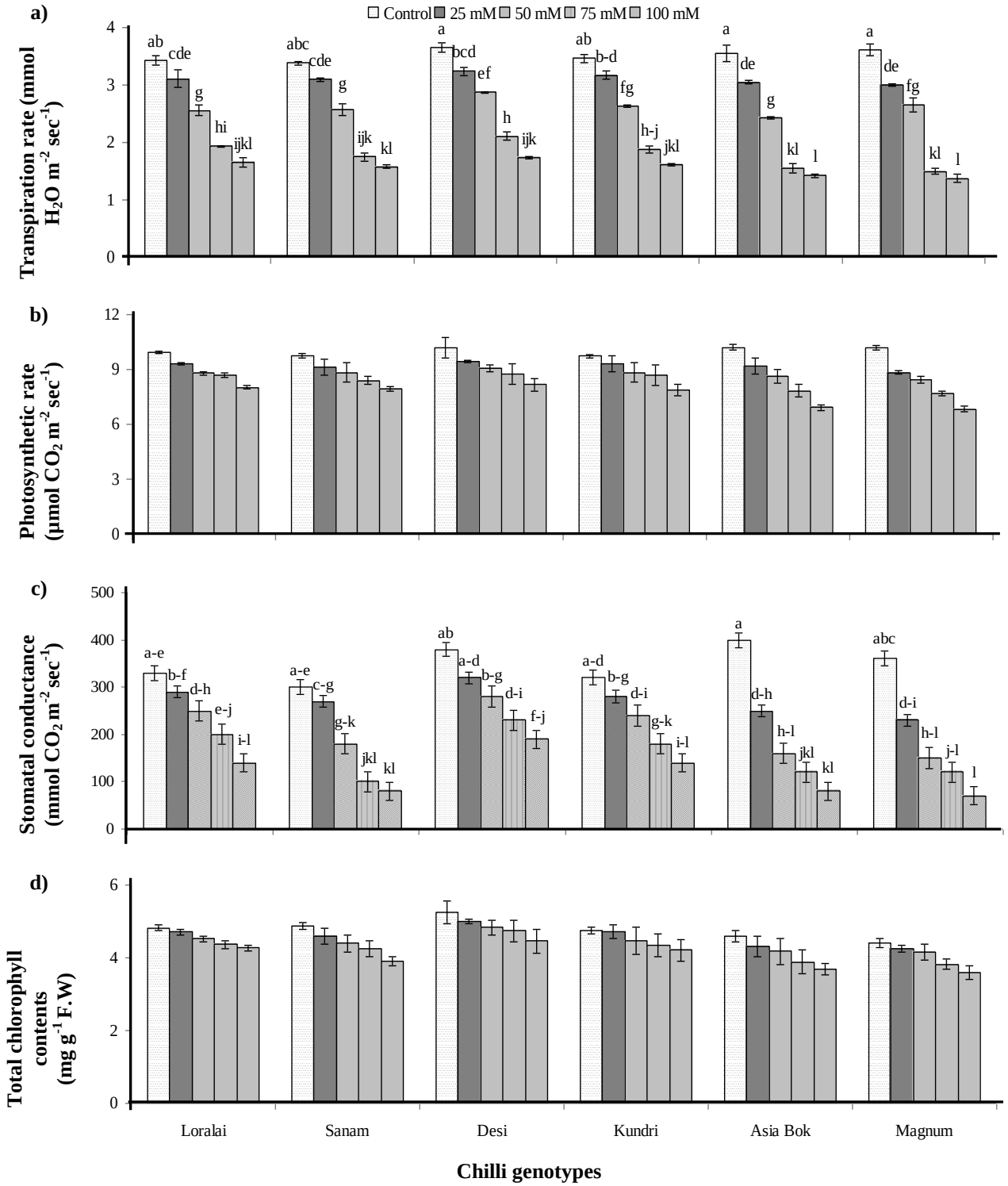

Figure 2: Influence of various salinity levels on (a) transpirational rate, (b) photosynthetic rate, (c) stomatal conductance, and (d) chlorophyll contents of six chilli genotypes. Vertical bars above mean denote standard error of three replicates repeated once. Means with different letters differ significantly at 0.05 probability level by HSD test. F.W: fresh weight

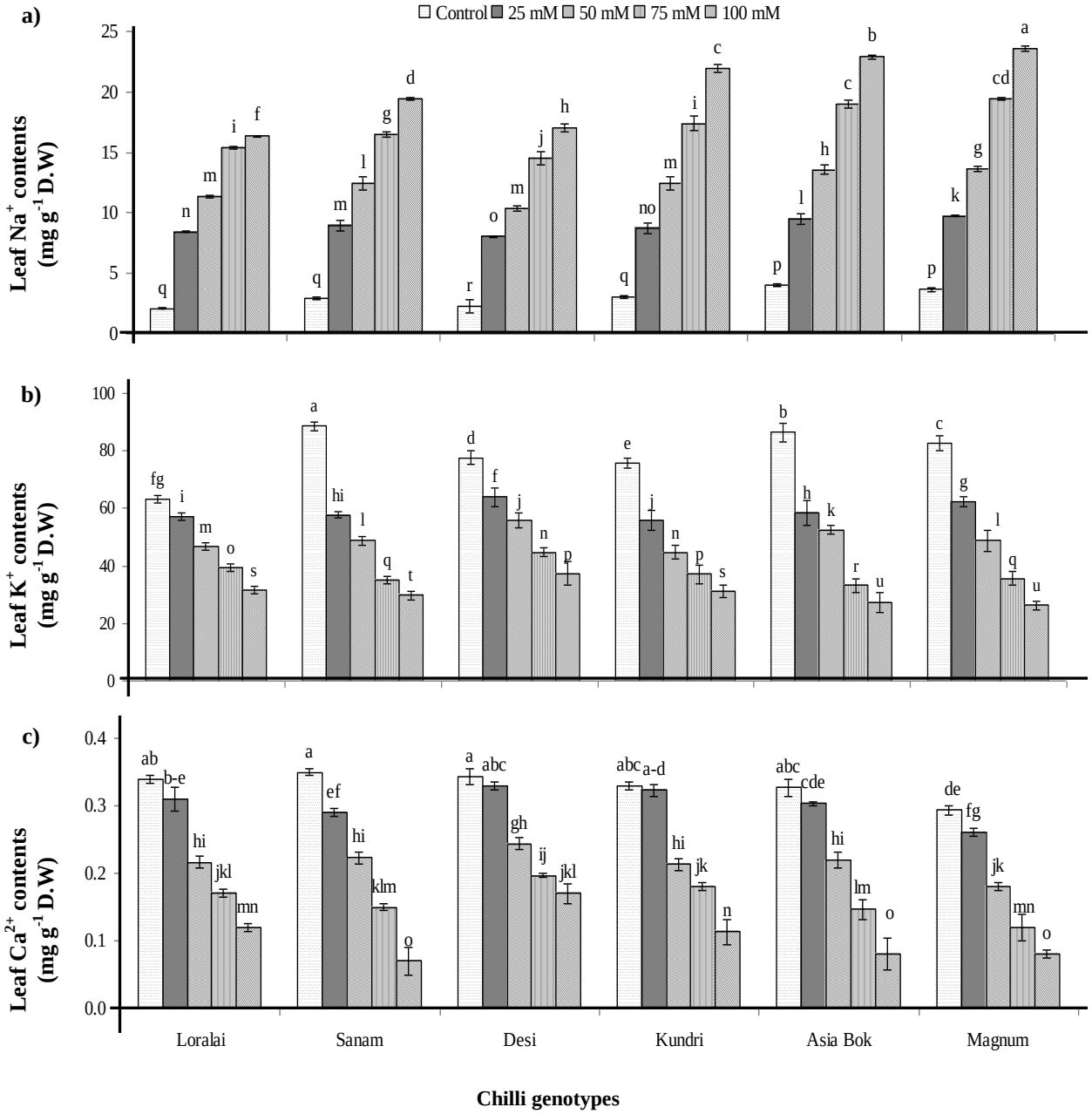

Figure 3: Influence of various salinity levels on (a) Na^+, (b) K^+ and (c) Ca^{2+} concentrations in leaves of six chilli genotypes. Vertical bars above mean denote standard error of three replicates repeated once. Means with different letters differ significantly at 0.05 probability level by HSD test. D.W: dry weight

physiological attributes studied (Figure 1 and 2). Diminished vegetative growth of chilli under salinity stress occurred, e.g., significant ($p \leq 0.05$) reduction in seedling eleongation as well as fresh and dry biomass of plants. Better performance of Desi and Loralai genotypes for these atrributes may be because of less decrease in water potential resulting in less loss of turgor under salinity. The maintenance of turgor even under salinity might have facilitated efficient cell divison and elongation in these genotypes and consequently they had increased biomass. This contrasting response of chilli genotypes for seedling growth could be in part due to differntial regulation of growth related processes at genetic, biochemical and physiological levels.

Table 2: Correlation coefficient (r) denoting association strength between different variables in six chilli genotypes under NaCl salinity (n=5)

X-variable	*Y*-variable	Chilli genotypes					
		Loralai	**Sanam**	**Desi**	**Kundri**	**Asia Bok**	**Magnum**
Na^+	Ca^{2+}	-0.852**	-0.984***	-0.849**	-0.958***	-0.938***	-0.986***
	K^+	-0.862**	-0.984***	-0.898**	-0.975***	-0.980***	-0.994***
	Photosynthetic rate	-0.864**	-0.968***	-0.880**	-0.982***	-0.988***	-0.983***
	Stomatal conductance	-0.847**	-0.969***	-0.897**	-0.995***	-0.962***	-0.968***
K^+	Ca^{2+}	0.997***	0.837**	0.963***	0.819**	0.829**	0.880**
	Photosynthetic rate	0.979***	0.872***	0.928**	0.838**	0.877***	0.890***
	Stomatal conductance	0.991***	0.827**	0.899***	0.755*	0.778*	0.788*
Dry biomass	Transpiration rate	0.983***	0.810**	0.962***	0.867**	0.810**	0.811**
	Photosynthetic rate	0.947***	0.869**	0.896***	0.855**	0.826**	0.861**

*, **, *** denote significance at the 0.05, 0.01 and 0.001 probability level, respectively

Total chlorophyll contents and net photosynthesis rate were reduced with an increase in NaCl salinity (Figure 2). Salinity-induced photosynthetic limitations in present study might be ascribed to less stomatal conductance as well as total chlorophyll contents under stress conditions. Mundree *et al.* (2002) stated that decrease in stomatal conductance, photochemical capacity, and carbon uptake and metabolism under salt stress are the major causes of reduced photosynthesis. Saud *et al.* (2014) also pointed out that net photosynthetic rate in Kentucky bluegrass was strongly linked with stomatal conductance and leaf green color. Parida and Das (2005) suggested that impaired chlorophyll biosynthesis under salinity was a general phenomenon, which decreased chlorophyll content and caused chlorosis in plants. Salinity induced damage is a multi-facet and interwoven effect and reduction in chlorophyll contents might be in part due to reduced nutrient uptake, increased activity of chlorophyll degrading enzymes and formation of reactive oxygen species. Bettaieb *et al.* (2008) also argued that decreased chlorophyll content under salinity is considered as major cause of reduced photosynthetic activity. These two parameters together, clearly explained the reduced amount of biomass production under salinity in present study. Dry biomass of chilli plants had a strong positive (>85%) association with photosynthetic rate (Table 2). Salinity strongly inhibited transpiration rate and stomatal conductance in all chilli genotypes. Higher salt concentration leads to pseudo drought stress, which causes stomatal closure, reduced transpiration rate, lessened photosynthetic activity, and elevated canopy foliage temperature (Halim *et al.,* 1990; Azevedo *et al.,* 2004). Low stomatal conductance resulted in reduction in photosynthetic and transpiration rate in chilli plants (Figure 2).

Changes in plant ionic status are the most obvious effect observed under salinity. Salinity aggravates Na^+ concentration while diminishing K^+ in plants (Akram *et al.,* 2010) and our data also corresponds to the same. Bybordi *et al.* (2010) indicated that a decrease in K^+ contents in salt-sensitive cultivars was caused by an antagonistic relationship between Na^+ and K^+. In present study, the results for ionic content revealed antagonistic relationship of Na^+ with K^+ and Ca^{2+} regarding their uptake, so that increasing NaCl stress was accompanied with a concurrent increase in Na^+ concentration and a simultaneous decrease in K^+ and Ca^{2+} contents (Table 2), although genotypic variation was evident (Figure 3). More Na^+ influx in salt sensitive chilli genotypes might have inhibited K^+ and Ca^{2+} permeability to the cells, thus decreasing concentration of these ions. Previous studies have shown that increased NaCl concentration led to elevated concentrations of Na^+ and Cl^- which were toxic to plant growth (Munns, 2002; Zhani *et al*., 2012). Amador *et al.* (2007) indicated that Ca^{2+} played an important role in regulating ion transfer into plant cells and in amelioration of the adverse effects of NaCl-induced salinity on plants. Regulatory roles of Ca^{2+} in metabolism are well known, therefore, it has been suggested that high Ca^{2+} levels can protect the cell membrane from the adverse effects of salinity (Cramer *et al.,* 1985). Na^+ ions may compete with Ca^{2+} ions for membrane binding sites and this may be one of the possible explanation of the lower Ca^{2+} observed at elevated salinity levels in salt sensitive chilli genotypes especially the Magnum. The divergence in ionic homeostasis of chilli genotypes under salinity may be because of variable genetic architecture and root permeability to these ions. It is noteworthy to mention that salt tolerant chilli genotypes (Loralai and Desi) manifested significantly less Na^+ accumulation even at upper limits of salinity (100 mM) as against highest observed for salinity sensitive chilli

genotype (Magnum) at the same salinity level. Such difference in ability of chilli genotypes towards Na^+ buildup was presumably because of differential Na^+ exclusion capacity. Besides reduced Na^+ buildup, salt tolerant genotypes exhibited improved accumulation of K^+ and Ca^{2+} in most cases. It seems that combination of Na^+ exclusion and maintenance of K^+ and Ca^{2+} might have helped in protecting/improving stomatal conductance and photosynthetic activity that resulted in improved seedling growth and dry matter production under salt stress. The ratio between K^+ and Na^+ under salinity is considered crucial for metabolic activities under salt stress and has been recognized as a vaild physiological criteria explaining salt tolerance. Higher levels of K^+ and Ca^{2+} in salt-tolerant chilli genotypes might also have positively contributed towards increased stomatal conductance, plant water relations and membrane integrity, and higher K^+ levels were positively correlated with stomatal conductance and photosynthetic rate (Table 2). Increase in K^+ levels under salinity might also be a genotypic osmo-regulatory response under stressful environments in quest of favorable plant water balance (Taiz and Zeiger, 2006). Previous study of Zhani *et al*. (2012) established the accumulation of compatible soultes like proline as the basis of salinity tolerance in contrasting chilli genotypes. However, these authors suggessted further studies to evaluate mineral status (Na^+, K^+ and Ca^{2+}) of salt stressed chilli plants. Our study provides an insight, and is a step forward in establishing the role of ionic homeostatis as bases of differntial salinity tolerance amnog chilli genotypes. At the same time, it implies that higher levels of K^+ and Ca^{2+} are actively involved in maintaining biochemical attributes like chlorophyll contents and physiological processes like stoamtal conductance, traspirational and photosynthetic rates of chilli plants exposed to NaCl salinity.

Conclusion

Salinity adversely affected morpho-physiological attributes of chilli genotypes by causing ionic toxicity, osmotic stress, altered stomatal functioning, impaired photosynthesis and limited K^+ availability. Salt-tolerant chilli genotypes (Loralai and Desi) were able to avoid most of the harmful implications of salinity through Na^+ exclusion and maintenance of favorable levels of K^+ and Ca^{2+} that helped stabilize photosynthetic pigments and photosynthetic machinery under salt stress resulting in comparatively better physiological functioning and seedling growth than salt sensitive genotypes. The salt-tolerant chilli genotypes identified in the present work should be further evaluated for their field appraisal. These can also serve as control for future studies dealing with salinity tolerance in chilli, besides their usefulness in breeding programs and general cultivation on soils where salinity seems inevitable.

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