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EVALUATION OF DIFFERENT GROWTH AND PHYSIOLOGICAL TRAITS AS INDICES OF SALT TOLERANCE IN HOT PEPPER (*CAPSICUM ANNUUM* L.)

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Abstract

Plant growth and development is hampered by various environmental stresses including salinity. Effect of salt stress on relative water contents, membrane permeability, chlorophyll contents and carotenoids level was evaluated to assess their suitability as reliable indicator of salt tolerance in hot pepper. Cultivars Maha, Tata Puri and Hot Queen were subjected to different NaCl concentrations (2 [control], 4, 6 and 8 dS m^{-1}). Root and shoot length, dry matter contents, relative growth rate, leaf area, specific leaf area and leaf area ratio were significantly reduced by higher salinity levels (6 and 8 dS m^{-1}). Nonetheless, all the aforementioned attributes improved at 4 dS m^{-1} compared with control (2 dS m^{-1}). In contrast, relative leaf water content (RLWC) was markedly affected with an increase in salinity stress. However, leaf chlorophyll contents and carotenoids (CAR) were significantly higher at 6 dS m^{-1} than the control. Salt tolerance index was high for Tata Puri followed by Hot Queen. Changes in RLWC and antioxidant activity were strongly correlated with dry matter, specific leaf area and relative growth rates. While, change in leaf area ratio, chlorophyll contents and membrane permeability was not correlated with the growth traits. In crux, RLWC and CAR contents can be used as reliable index of salt tolerance in hot pepper.

Introduction

Plants are subjected to various kinds of biotic and abiotic stresses including drought, heat, salinity and chilling, which hamper the seedling establishment, allometry and economic yield (Munns, 2002; Sun *et al*., 2002). Salinity, a huge and worldwide problem has affected about 930 million ha of land (Munns, 2002), which accounts about 7% of world's land area. Salt affected area in Pakistan is about 6.67 Mha (Khan, 1998). Critically, this problem is due to the reduction in availability of fresh water as well as precipitation, which forces the growers to use underground water containing salts, particularly NaCl. This has resulted in gradual build up of Na^+ and Cl^- in the root zone.

Genetic engineering of key regulatory genes appears to be one of the most promising strategies to minimize the deleterious effects associated with various stresses including the salt stress. But the conventional approaches in both plant physiology and breeding are also of great significance because most of transgenic plants show growth retardation and alterations in metabolism thus affecting yield of agricultural crops (Vinocur & Altman, 2005). Therefore, the impact of salinity on plant growth and development have been studied intensively and correlated with different morphological and physiological attributes as biomass (Waheed *et al*., 2006), number of leaves (Mohammed *et al*., 1998), leaf area (Marcelis & VanHooijdonk, 1999), plant water relations (Soria & Cuartero, 1997), chlorophyll and carotenoids contents (Lycoskoufis *et al*., 2005).

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Reduced water uptake is the common response of plants subjected to water or salt stress (Munns, 2002). Relative leaf water content (RLWC) have been reported to decline with increase in drought stress in several crops (Siddique *et al*., 2000; Kirnak *et al*., 2001a) but have not been investigated previously in pepper under salt stress. Furthermore, RLWC is considered to be a better indicator of water status than water potential (Sinclair & Ludlow, 1985), although the later is also a reliable trait for quantifying plant response to water stress (Siddique *et al*., 2000).

Absorbed radiation energy from sunlight surpasses the capacity of chloroplasts to use it in CO_2 fixation, and the glut energy is alternatively used to convert O_2 to reactive oxygen species (ROS) under abiotic stresses (Apel & Hirt 2004). ROS levels in plants also increases due to perturbations of chloroplastic and mitochondrial metabolism (Foyer & Fletcher 2001; Neill *et al*., 2002). The ROS are highly reactive and can gravely damage plants by lipid peroxidation, protein degradation, DNA fragmentation and ultimately cell death (Kratsch & Wise, 2000; Ashraf, 2009). The ability to adjust antioxidant systems to changing ROS concentrations may be vital to all species under stress conditions (Foyer *et al*., 2002). For this plants have developed active oxygen-scavenging systems. Recently carotenoids (CAR) have been found as potential quencher of ROS (Verma & Mishra, 2005). Moreover, salt sensitive cultivars show more leakage of electrolyte compared to salt tolerant cultivars in saline environment (Siddiqui *et al*., 2008).

Although, different aforementioned traits are used in stress studies in different crops, it is not yet possible to find any sensitive criterion that could reliably be used by breeders to improve salt tolerance of plants (Ashraf & Harris, 2004). So, it is the dire need to specify distinctive reliable indicators for specific crop plants, in order to develop predictable strategies for selecting salt tolerant genotypes (lines/varieties) for commercially important crops (Ashraf & Harris, 2004). Recently, shoot K concentration has been considered as a reliable parameter for salt tolerance studies in wheat (Bagci *et al*., 2007), photosynthetic capacity (Ashraf *et al*., 2007) and cell membrane stability (Aslam *et al*., 2006) in maize while, such studies are rare in hot pepper.

Hot pepper (*Capsicum annuum*) is one of the most important solanaceous crops, mainly grown in Sindh and Southern Punjab as a summer crop in Pakistan. The arid and semi-arid conditions as well as less availability of fresh water have inflicted the saline conditions in these provinces and are threatening the productivity of this crop, which is considered as salt sensitive (Lycoskoufis *et al*., 2005). Salinity hampers pepper growth more during vegetative phase (Villa-Castorena *et al*., 2003); seedling stage is considered as the most sensitive to salt stress (Rhoades *et al*., 1992). Therefore, the present study was undertaken in hot pepper (1) to ascertain the changes in RLWC, EL, chlorophyll contents and carotenoids and (2) to correlate the changes in aforementioned physiological traits with growth traits in three hot pepper cultivars viz., Maha, Tata Puri and Hot Queen, under NaCl-saline conditions during the vegetative growth stage.

Materials and Methods

Plant material and growth conditions: Three hot pepper cultivars/hybrids viz., Hot Queen (hybrid by Shenyang Tech-Pak Seed Co. Ltd., China), Maha (hybrid by Seminis, 2700 Camino del Sol., Oxnard, CA, USA) and Tata Puri (Local cultivar, fruit for seed were obtained personally from the selected field) were used in this study. Seeds were sown in pots (containing equal parts of top-soil, sand and leaf manure as growing media) in a greenhouse, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan (latitude 31°30 N, longitude 73°10 E and altitude 213 m). Hybrids Maha and Hot Queen were selected because of their high yield potential, while Tata Puri was selected as local open pollinated cultivar, well adapted and commercially grown since many years in Punjab (Pakistan). Uniform seedlings at two true-leaf stage from the grown seedlings were shifted to plastic pots containing half strength Hoagland nutrient solution (EC 2 dS m^{-1}, pH 5.8±0.2, Temperature 23±2°C), in a growth room (27±2°C, 16/8 h day/night conditions). Seedlings at two true-leaf stage were used because the leaves developed prior to the imposition of the salt treatment have different developmental characteristics than leaves developed after salt treatment and such leaves contribute to the lack of correspondence between the salt-induced physiological reduction recorded on young fully expanded leaves developed after the beginning of salt treatments (Romero-Aranda *et al*., 2001). Plants were gradually exposed to different salinity treatments, after their establishment in the hydroponics culture, with an increment of 2 dS m^{-1} per day. The desired electrical conductivities for different salinity treatments (4, 6 and 8 dS m^{-1}) were achieved using NaCl, while the control received a half strength Hoagland nutrient solution (EC 2 dS m^{-1}, pH 5.8±0.2). Electrical conductivity and pH of the solution were daily monitored using a portable EC meter (YOA EC meter, CM 14P, TOA Electronics Ltd., Japan) to avoid build up of EC and pH.

Experimental design and measurements: The experiment was laid out according to completely randomized design in factorial arrangements with four replicates. All the measurements were made three week after the imposition of salt stress, except plant weight and length, which were also measured just before the exposure of plants to salinity. All the measurements were made from four randomly selected plants per replicate. For RLWC and EL measurements, eight plants per replicate were taken.

Plants were dissected into root and shoot to record length and fresh weight of these parts. For measurement of total dry matter accumulation, samples of roots, stem and leaves were weighed (fresh weight), oven dried at 105°C to constant weight and dry weight was recorded. Leaf area (LA) was taken using Image Analysis System (Delta T Devices Ltd., Cambridge, UK). Specific leaf area (SLA) and leaf area ratio (LAR) was then calculated according to Hunt (1990) as: SLA (cm^2 g^{-1}) = LA/leaf dry weight and LAR (cm^2 g^{-1}) = LA/whole plant dry weight. Relative growth rate (RGR) was determined on a dry weight basis (Evans, 1972) as: RGR (g g^{-1} dwt day^{-1}) = $[1n(W_2)-1n(W_1)/(t_2-t_1)]$, where 'W' is the dry weight in g per plant at time 't'.

Leaves were collected at the time of harvest from the mid section of the plant and weighed (FM) for measurement of relative water content. These were then washed with distilled water and floated in distilled water in a closed Petri dish for 24 h, when these were fully imbibed. After being fully imbibed, samples were weighed to get turgid mass (TM) and placed in vacuum oven (EYELA VOC-300SD) at 80°C for 48 h to obtain dry mass (DM). Relative leaf water content (RLWC) was calculated from the following formulae (Yamasaki & Dillenburg, 1999): RLWC (%) = [(FM-DM)/(TM-DM)] × 100.

For chlorophyll estimation, the youngest fully expanded leaf was selected from each plant and chlorophyll from fresh tissue (0.5 g) was extracted using 80% acetone (Arnon, 1949). Concentration of chlorophyll 'a' and 'b' was determined by means of UV/Visible Spectrophotometer (IRMECO QmbH, Germany, Model U2020) at 645 and 663 nm, respectively. For carotenoids estimation in leaf tissues, absorbance of acetone extract of leaf was measured at 440 nm (Ikan, 1969) by means of UV/visible spectrophotometer (IRMECO QmbH, Germany, Model U2020).

Electrolyte leakage (%) was measured to assess membrane permeability, according to the procedure suggested by Lutts *et al*., (1995). Two leaf samples were selected from each of the four plants per replicate per treatment, one from the second leaf below the shoot apex and the second leaf appeared after exposure to salinity treatments was taken as the second sample. Leaf samples were washed with deionized water, cut into 1 cm^2 segments and placed in test tubes containing 10 ml deionized water, covered with polythene. The samples were placed on a rotary shaker (100 g) at room temperature 25±2°C for 24 h. Then electrical conductivity was taken using a portable EC meter (YOA EC meter, CM 14P, TOA Electronics Ltd., Japan) as EC_1 and the samples were incubated at 121 °C for 20 min and EC was determined (EC_2) after cooling the solution in the test tubes. Electrolyte leakage (%) was calculated as: EL = (EC_1/ EC_2) × 100.

The tolerance index (TI: Cano *et al*., 1998) was calculated from the following relation: TI = Fresh weight in NaCl-saline solution/ Fresh weight in NaCl-free solution (control) × 100.

Statistical analysis: The data were subjected to two-way analysis of variance (cultivar × salinity) using general linear model (GLM) of STATISTICA (version = 5.5; Stats Soft) and treatments means were separated using DMR test (α = 0.05). Correlation analysis was conducted to determine the relationships among dry matter accumulation, leaf area, RGR, SLA, LAR Chlorophyll 'a' and 'b', RLWC, CAR, EL and EI.

Results

Plant growth in response to salinity: Root and shoot length was negatively affected by the NaCl concentration in the nutrient solution. Maximum root length was recorded in Tata Puri while minimum in Maha that was similar to Hot Queen. Shoot length was maximum in Maha at all salinity level followed by Tata Puri and Hot Queen (Table 1). Test of significance showed that Maha excelled over the other two cultivars and the difference in shoot length was almost double. Root/shoot ratio decreased gradually with increase in the level of NaCl in nutrient solution except in Maha (Fig. 1A). Maha showed the least value for root/shoot ratio at all salinity levels; root/shoot ratio for Maha at 2 and 4 dS m^{-1} was at par with Hot Queen and Tata Puri at 8 dS m^{-1}. Nonetheless, maximum root/shoot ratio was obtained for Tata Puri at 2 dS m^{-1}. Inversely, shoot/root ratio was maximum in Maha followed by Hot Queen and Tata Puri (Table 1).

Dry weight of root (RDW), stem (SDW) and leaf (LDW) was significantly affected by NaCl-salinity; maximum reduction was recorded in Maha (Table 1). Dry matter accumulation was recorded more in Tata Puri as compared to the other two cultivars at all NaCl-salinity levels, indicating superiority of Tata Puri over the other cultivars. The RDW, SDW and LDW values for Tata Puri were statistically similar to Maha at moderately higher (6 dS m^{-1}) and Hot Queen at moderately higher and higher NaCl-salinity levels (6 and 8 dS m^{-1}).

The NaCl-saline nutrient solution markedly reduced the relative growth rate (RGR) in all the three cultivars, maximum reduction being found in Maha and Hot Queen at 8 dS m^{-1} (Fig. 1B). All the three cultivars showed statistical similarity at 2 dS m^{-1} (control) and the RGR value at this salinity level was at par with the values at 6 dS m^{-1}, indicating that RGR first increased with increase in salinity up to 4 dS m^{-1} and then gradually decreased, minimum at 8 dS m^{-1} in all the cultivars. Tata Puri performed well under increased salinity levels followed by Maha and Hot Queen.

Table 1. Growth and dry matter accumulation of hot pepper cultivars in response to salinity.

Cultivar	Salinity level ($dS\ m^{-1}$)	Dry weight ($mg\ g^{-1}$ FWT)			Root length (cm)	Shoot length (cm)	Shoot: root ratio
		Roots	Stem	Leaf			
Maha	2 $dS\ m^{-1}$ (control)	106.55 cdef	831.30 b	584.47 b	24.25 cde	33.80 b	1.393 b
Tata Puri		162.52 b	699.67 bc	560.30 bc	28.10 b	16.50 f	0.587 ef
Hot Queen		112.75 cdef	636.87 c	443.95 cd	24.65 cd	21.47 de	0.870 cd
Maha	4 $dS\ m^{-1}$	152.95 bc	1130.02 a	602.87 b	25.00 c	37.42 a	1.496 a
Tata Puri		215.45 a	1253.65 a	777.05 a	32.57 a	22.17 de	0.680 de
Hot Queen		106.60 cdef	636.80 c	430.10 d	24.87 c	21.40 de	0.860 cd
Maha	6 $dS\ m^{-1}$	68.97 fg	719.90 bc	457.97 cd	22.00 ef	27.75 c	1.261 b
Tata Puri		143.42 bcd	818.10 b	462.40 cd	30.45 ab	20.37 ef	0.668 de
Hot Queen		99.00 def	609.90 c	405.35 d	25.40 c	20.00 ef	0.787 cd
Maha	8 $dS\ m^{-1}$	47.22 g	444.97 d	284.92 e	21.62 f	24.30 d	1.123 c
Tata Puri		136.87 bcde	617.90 c	429.70 d	31.70 a	17.70 fg	0.558 f
Hot Queen		92.77 ef	592.25 c	390.87 de	22.25 def	16.25 f	0.730 ef

Values in each column sharing same letter are statistically non-significant (α = 0.05)

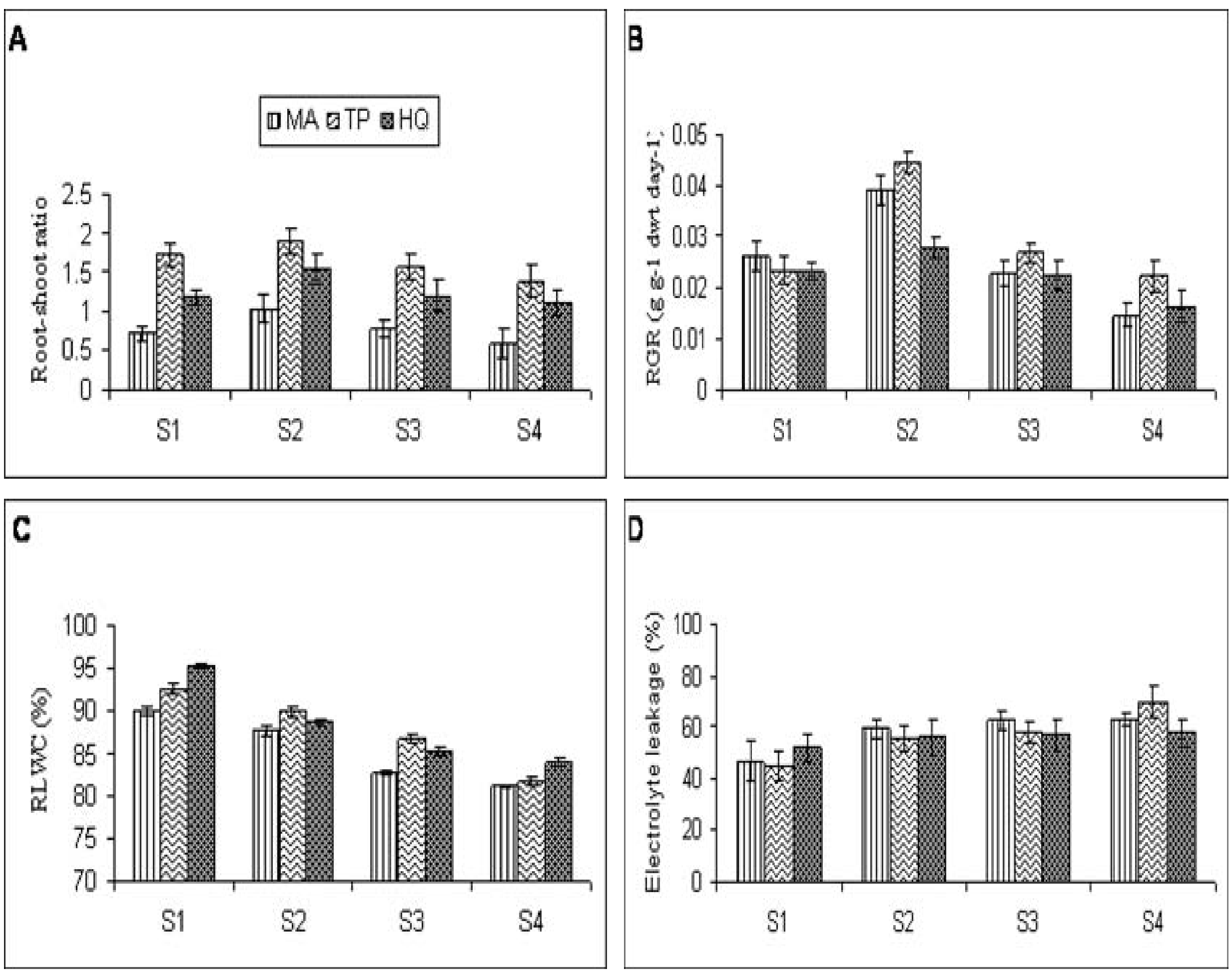

Fig. 1. Variation in (A) root-shoot ratio, (B) relative growth rate (RGR), (C) relative leaf water content (RLWC) and (D) electrolyte leakage (EL) among three hot pepper cultivars in response to salt stress (S1 = 2 $dS\ m^{-1}$, S2 = 4 $dS\ m^{-1}$, S3 = 6 $dS\ m^{-1}$ and S4 = 8 $dS\ m^{-1}$). Bars represent standard error (±S.E) of means.

Number of leaves, leaf area per plant (LA), specific leaf area (SLA) and leaf area ratio (LAR) were significantly restricted by NaCl-concentration in the nutrient solution (Table 2). Number of leaves per plant was the maximum in Tata Puri at 4 dS m^{-1}. But at all other salinity levels, Maha excelled over the other two cultivars. But LA was the maximum and statistically alike in both Hot Queen and Tata Puri. All the cultivars showed a gradual decrease in SLA and LAR with increasing salinity levels. Reduction in SLA was maximum in Maha at 8 dS m^{-1}. While, minimum value of LAR was recorded for Tata Puri. Hot Queen exhibited the highest value for both SLA and LAR.

All the aforementioned parameters initially showed a slight increase in value with increase in NaCl-salinity level upto 4 dS m^{-1} but the further increase in salinity was detrimental.

Physiological changes in response to salinity: Relative leaf water contents were significantly affected by salt stress in all the three cultivars (Fig. 1C). Reduction in RLWC was gradual with increase in salinity above 2 dS m^{-1} (control), maximum reduction being observed at the highest concentration of NaCl in the nutrient solution (8 dS m^{-1}) in all the cultivars. Hot Queen at 2 dS m^{-1} retained maximum value of RLWC. At lower and moderately higher NaCl concentration in the nutrient medium (6 dS m^{-1}) Tata Puri performed better than the other two cultivars, while at highest salinity level (8 dS m^{-1}) Hot Queen surpassed all the cultivars.

Significant differences were obtained for membrane permeability in mature leaves in response to NaCl-salt stress (Fig. 1D), while no significant difference was recorded in developing leaves in the apical portion of the plants (data not shown). Leakage of electrolytes (EL) increased gradually with increase in salinity above 2 dS m^{-1} (control). Leakage of electrolytes was maximum in Tata Puri at 8 dS m^{-1}, although statistically all the cultivars were at par at this salinity level.

The exposure of pepper plants to salinity significantly affected chlorophyll ‘a’ and ‘b’, total chlorophyll content and carotenoids. Moderately higher NaCl- salinity level (6 dS m^{-1}) significantly enhanced the chlorophyll ‘a’ and ‘b’ and carotenoids content over the control except in Maha, but profound reduction was recorded at higher salinity levels (Table 3). The injurious effect of salt concentration on chlorophyll content was more prominent in Maha as compared to Tata Puri and Hot Queen; Hot Queen was being the least affected of all. Carotenoids contents of Tata Puri leaves at 4 and 6 dS m^{-1} NaCl-salinity level were also higher than at 2 (control) and 8 dS m^{-1}.

Salt tolerance index: Salt tolerance of pepper cultivars was significantly different at different levels of NaCl-salinity. All cultivars showed maximum salt tolerance at 4 dS m^{-1} and ability of plants to tolerate salinity gradually decreased with increasing salinity above 4 dS m^{-1}. Salt tolerance index (STI) was highest for Tata Puri at all NaCl-salinity levels followed by Hot Queen, while Maha showed the least tolerance to higher salinity levels.

Correlation analysis: Correlation analysis indicated that all the growth traits viz., dry weight of whole plant as well as individual plant organs (root, stem and leaf), leaf area per plant and SLA were positively correlated with RGR (Table 4). All the aforementioned traits were also significantly correlated with SLA except for root dry weight. Correlation analysis between growth and physiological traits indicated that RGR and SLA were significantly correlated with RLWC and carotenoids, but no significant correlation of these growth determinants with other physiological traits was found (Table 4).

Table 2. Leaf area characteristics of hot pepper cultivars in response to salinity.

Cultivar	Salinity level (dS m^{-1})	No. of leaves per plant	Leaf area $plant^{-1}$ (cm^2)	SLA (cm^2 g^{-1})	LAR (cm^2 g^{-1})
Maha	2 dS m^{-1} (control)	17.00 b	186.20 cd	356.385 cd	122.41 cdef
Tata Puri		14.75 def	182.00 cd	576.54 a	131.07 bcdef
Hot Queen		14.25 def	254.25 a	549.78 a	177.45 ab
Maha	4 dS m^{-1}	17.00 b	177.20 cde	300.42 d	96.90 ef
Tata Puri		19.00 a	252.00 a	299.66 d	88.27 f
Hot Queen		14.00 def	240.50 a	552.01 a	198.21 a
Maha	6 dS m^{-1}	14.00 def	121.00 f	374.66 c	99.58 def
Tata Puri		15.00 cde	207.50 b	294.28 d	108.20 cdef
Hot Queen		13.50 ef	191.20 c	461.68 b	205.38 a
Maha	8 dS m^{-1}	16.75 bc	103.50 g	344.72cd	146.45 bcd
Tata Puri		12.75 f	173.20 de	392.78 c	139.10 bcde
Hot Queen		16.00 bcd	165.00 e	410.88 bc	152.06 bc

Values in each column sharing same letter are statistically non-significant (α = 0.05)

Table 3. Effect of salinity on chlorophyll (Chl) content, carotenoids (CAR), etiolation index and tolerance index of hot pepper.

Cultivar	Salinity level (dS m^{-1})	Chl a (mg g^{-1})	Chl b (mg g^{-1})	Total Chl (mg g^{-1})	CAR (mg ml^{-1})	Tolerance index
Maha	2 dS m^{-1} (control)	34.17 ef	65.57 c	99.74 d	0.8518 i	---
Tata Puri		34.55 e	63.02 e	97.57 e	0.8721 g	---
Hot Queen		36.19 c	65.59 c	101.78 c	0.9491 c	---
Maha	4 dS m^{-1}	36.02 cd	63.18 e	99.20 d	0.936 d	123.87 b
Tata Puri		35.33 d	64.27 d	99.60 d	0.9148 e	185.61 a
Hot Queen		37.38 b	65.82 c	103.20 c	0.9718 b	126.45 b
Maha	6 dS m^{-1}	33.98 ef	61.34 f	95.32 f	0.8704 g	81.90 c
Tata Puri		36.95 b	66.83 b	103.78 b	0.9158 e	125.75 b
Hot Queen		38.39 a	69.23 a	107.62 a	107.62 a	92.83 c
Maha	8 dS m^{-1}	33.52 f	62.04 f	95.56 f	0.8524 i	51.04 d
Tata Puri		34.52 e	61.22 f	95.74 f	0.8621 h	107.34 bc
Hot Queen		35.65 cd	65.08 cd	100.73 d	0.8912 f	98.31 c

Values in each column sharing same letter are statistically non-significant (α = 0.05)

Discussion

The growth and dry matter accumulation of hot pepper was severely hampered at highest salinity level (8 dS m^{-1}), indicating hot pepper to be a salt sensitive crop. But, there was a considerable increase in root and shoot length and dry matter accumulation in different plant organs at lower salinity levels (4 dS m^{-1}). Root length, although statistically significant, yet showed a very slight reduction at all salinity levels in all the cultivars (Table 1). The findings of Sharp (1996) and Kirnak *et al*., (2001b) support our results who observed that roots continue to grow at low soil water potential that completely inhibited shoot growth. This may be due to the reason that many traits like root size and depth that explain adaptation to water stress (which induce osmotic stress similar to salt stress) are associated with plant development and structure and are constitutive rather than stress induced (Chaves *et al*., 2003). Significant reduction in plant height in bell pepper hybrids (Chartzoulakis & Klapaki, 2000) at salinity level higher than 25 mM NaCl (equivalent to 4.1 dS m^{-1}) was in confirmation to decreased shoot length at 6 and 8 dS m^{-1} in this study.

Table 4. Correlation analysis between different growth and physiological traits of hot pepper cultivars. RDW, root dry weight; SDW, stem dry weight; LDW, leaf dry weight; Pl DW, plant dry weight; LA, leaf area plant^{-1}; SLA, specific leaf area; LAR, leaf area ratio; RGR, relative growth rate; RLWC, relative leaf water content; Chl, chlorophyll; CAR, carotenoids; EL, electrolyte leakage; EI, etiolation index.

	RDW	SDW	LDW	PlDW	LA	SLA	LAR	RGR	RLWC	Chl a	Chl b	CAR	EL
RDW	1												
SDW	0.498 *	1											
LDW	0.416 *	0.864 *	1										
Pl DW	0.571 *	0.977 *	0.929 *	1									
LA	0.250 *	0.109 *	0.151 *	0.150 *	1								
SLA	0.016	0.276 *	0.220 *	0.233 *	0.086 *	1							
LAR	0.149 *	0.119 *	0.068	0.115 *	0.033	0.144 *	1						
RGR	0.454 *	0.784 *	0.608 *	0.751 *	0.254 *	0.152 *	0.114 *	1					
RLWC	0.171 *	0.057	0.094 *	0.087 *	0.537 *	0.186 *	0.041	0.138 *	1				
Chl a	0.008	0.004	0.006	0.003	0.240 *	0.121 *	0.097 *	0.005	0.059	1			
Chl b	0.000	0.001	0.002	0.000	0.257 *	0.021	0.194 *	0.002	0.084 *	0.491 *	1		
CAR	0.010	0.007	0.001	0.006	0.509 *	0.145 *	0.088 *	0.101 *	0.190 *	0.589 *	0.277 *	1	
EL	0.016	0.008	0.028	0.015	0.075 *	0.013	0.080	0.071	0.338 *	0.019	0.054	0.007	1
EI	0.000	0.002	0.001	0.002	0.010	0.002	0.000	0.001	0.008	0.002	0.000	0.002	0.001

Values with * are statistically significant at $\alpha = 0.05$

There was an increase in dry matter accumulation in root, stem and leaves (Table 1) and relative growth rate (RGR; Fig. 1B) at lower NaCl-salinity level in the nutrient solution (4 dS m^{-1}), which is contrary to the findings observed by Villa-Castorena *et al*., (2003). But the difference in result may be due to the growing medium (as it was soil in former case). Rodriguez *et al*., (2005) also found lower biomass production in salt-stressed plants, which is in line with our findings of lower values of RDW, SDW and LDW at higher salinity levels. Dry matter accumulation, regarded as the best integral of all growth parameters correlated with yield (Romero-Aranda *et al*., 2001), was highest in Tata Puri. The value of relative growth rate, which provides a more appropriate comparison of plant growth among salinity treatments than the absolute growth rate (Cramer *et al*., 1994), was also high for Tata Puri, depicting its superiority over the other cultivars under salt-stress conditions.

Specific leaf area (SLA) is an important variable in crop growth models, as it relates dry matter production to leaf area expansion and consequently to light interception and photosynthesis (Gary *et al*., 1993). Similarly, leaf area ratio (LAR) is an index of leafiness of the plant (Hunt, 1990). Higher salinity levels significantly reduced number of leaves per plant, leaf area per plant, SLA and LAR in all the cultivars; more reduction was recorded in Maha (Table 2). Reduction in leaf area has been reported in plants under saline conditions (Lycoskoufis *et al*., 2005; Rodriguez *et al*., 2005). Higher NaCl-concentration in the nutrient solution increased the osmotic stress (Munns & Termaat, 1986), which significantly reduced uptake of water ultimately affecting the relative leaf water content (RLWC) at higher salinity levels (Fig. 1C). The decreased RLWC not only decreased leaf area due to a reduction in turgidity of leaves (causing less light interception) but may also suppress stomatal conductance as stated by Lycoskoufis *et al*., (2005). Therefore, decrease in RLWC indirectly restricted the photosynthetic rates (data not recorded) and ultimately resulted in reduced plant growth rate (RGR) and dry matter accumulation. This reduction in accumulation of dry matter in leaves lowered the SLA as well as LAR values in hot pepper plants at higher salinity levels. It is in confirmation to Curtis & Lauchli (1986), who related osmotic stress due to salts with reduced dry matter accumulation and reported decrease in SLA. Marcelis & VanHooijdonk (1999) concluded that reduced dry matter accumulation in radish at higher salinity levels was more (about 80%) due to the reduced leaf area, that decreased light interception and to a lesser extent (about 20%) by a decrease in stomatal conductance, which confirms our results of positive correlation between SLA and dry matter and SLA and RGR (Table 4).

Our results were in agreement with the findings from Chaudhuri & Choudhuri (1997), who observed that salt stress decreased water uptake in Jute with a concomitant reduction in relative leaf water content (RLWC). Decrease in RLWC under water and saline stress in *Asteriscus maritimus* (Rodriguez *et al*., 2005) and recently in tomato (Yokas *et al*., 2008) further strengthen our hypothesis that salt stress reduces RLWC in hot pepper in a similar manner as drought stress due to its osmotic component. Moreover, reduction of photosynthesis in hot pepper was not due to the toxic effect of salts caused by their accumulation in leaves as supposed by Munns & Termaat (1986), as pepper is regarded as Na-excluder (Lycoskoufis *et al*., 2005). Furthermore, significant correlation between RLWC and RGR as well as between RLWC and SLA (Table 5) provides evidence that RLWC can be used as an index for ranking genotypes according to their salt stress tolerance in hot pepper.

Salt stress reduces the life-span of leaves by chlorophyll degradation, leading to accelerated senescence of leaves (Yeo & Flowers, 1984). But the increased leaf chlorophyll contents at 6 dS m^{-1} (Table 3) may be due to the reason that the threshold of Na^+ concentration should exceed in the leaves before chlorophyll degradation while, pepper being Na-excluder (Lycoskoufis *et al*., 2005) restricts the build of Na^+ in the leaves. Therefore, Na^+ concentration at 6 dS m^{-1} might not be high enough to cause chlorophyll degradation (Asch *et al*., 2000). Moreover, high chlorophyll contents at 6 dS m^{-1} than control and 4 dS m^{-1} might be due to an increase in the number of chloroplasts in the leaves of stressed plants (Aldesuquy & Gaber, 1993).

Carotinoids (CAR), being antioxidant, have the potential to detoxify the plants from the ill effects of ROS (Verma & Mishra, 2005). The use of moderately high saline water (6 dS m^{-1}) increased leaf CAR contents over the control but higher level (8 dS m^{-1}) induced severe reduction (Table 3). Increased antioxidant activity in pepper (Navarro *et al*., 2006) and tomato fruit (De Pascale *et al*., 2001) by the use of moderately saline water, strengthened our findings. Observation of Verma and Mishra (2005) that low level of salt (50 mM/L NaCl) increased CAR level in *Brassica juncea* leaves over the control, but higher levels reduced, was in confirmation to our results. Carotenoids is suggested to be one of the required factors for salt tolerance in crop plants (Hernandez *et al*., 1995) and therefore, CAR contents may be helpful to differentiate between salt sensitive and tolerant cultivars. Higher CAR contents in Hot Queen and Tata Puri depict that these genotypes have potential to quench ROS and thus can be regarded as relatively salt tolerant. Furthermore, significant correlation between CAR and RGR, CAR and SLA (Table 4) signify CAR as a reliable parameter for salt stress studies in hot pepper.

Membrane damage by abiotic stresses owing to ROS production is well documented and therefore, has been widely used to differentiate stress tolerant and susceptible cultivars in different crops (Mansour & Salama, 2004). Higher EL value at high salinity levels indicates more permeability of membranes leading to excessive leakage of solutes (Siddiqui *et al*., 2008). Although, an increase in EL was recorded but it was inconsistent in Maha, gradually increased in Tata Puri and remained at par with control in Hot Queen (Fig. 1D). Correlation of EL with RGR, SLA (Table 5) was non-significant, which also revealed that EL to be a weak parameter for salinity tolerance estimation in hot pepper. It can be assumed from the inconsistency in data that salts present in the leaves, used for EL estimation, may contribute to high electrical conductivity so making it an unreliable trait for estimation of salt tolerance.

Finally, salt tolerance index indicated Tata Puri to be relatively salt tolerant as compared to the other genotypes.

Conclusion

The hampered growth of hot pepper under salt stress can be best evaluated by the relative growth rate, relative leaf water contents and carotenoids levels. Moreover, under saline conditions, reduced pepper plant growth is primarily due to osmotic stress rather than ionic toxicity, because pepper is regarded as Na^+ excluder. That's why RLWC can be used as indicator of salt stress in hot pepper. Furthermore, these simple traits (RLWC and CAR contents) can be used to screen hot pepper germplasm for salt tolerance. It is suggested that use of indigenous cultivars could be more appropriate than the exotic cultivars because indigenous germplasm has potential to perform better than the exotic cultivars due to their adaptive-ness.

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