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ASSESSMENT OF SALINITY TOLERANCE IN BELL PEPPER (*CAPSICUM ANNUUM* L.) GENOTYPES ON THE BASIS OF GERMINATION, EMERGENCE AND GROWTH ATTRIBUTES

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Abstract

Abiotic stresses are principal threat to crop growth and productivity all over the world. The most devastating one is soil salinity which adversely affects the plants, so a comprehensive study was conducted to categorize different available bell pepper (*Capsicum annuum* L.) genotypes into salt tolerant, moderately tolerant and sensitive ones on the basis of germination and emergence parameters. Genotypes were exposed to different saline treatments (2, 4, 6 and 8 dS m^{-1}) along with control (0 dS m^{-1}). Germination test, conducted in petri dishes in incubator, revealed that salinity stress significantly decreased final germination percentage, germination index and embryo axis length of tested genotypes. On the other hand, mean germination time and time to 50% seeds germination were increased with the increasing salinity level from 2 to 8 dS m^{-1}. Emergence test of bell pepper genotypes conducted in pots under greenhouse conditions, shown that salinity decreased the seedlings fresh and dry biomass, number of leaves, leaf area and root and shoot length. On the basis of overall percent decrease ranking table, genotypes were grouped into comparatively salt tolerant (Zard, Tasty, Super shimla, Aristotle), moderately tolerant (Capistrano, CW-03, Kaka-01, Orable, Yolo wonder, Crusadar) and sensitive ones (PEP-311, Admiral, Lafayette, Colossol). From these results, it can be extracted that germination and emergence tests are reliable screening tools for evaluating pepper genotypes for salt stress at seedling stage. Moreover, results of this study can be useful for local farmers to utilize their marginal soils by growing relatively salt tolerant bell pepper genotypes.

Key words: *Capsicum annuum*, Emergence, Germination, Salinity, Screening.

Introduction

Growth and productivity of the plants are affected due to many abiotic stresses like salinity, heat, cold and drought etc. and biotic stresses such as bacteria, virus, fungi and insects (Winfield *et al*., 2010) which are leading towards hundreds of billions of crop losses each year (Atkinson & Urwin, 2012). Soil salinity is the most devastating among them (Shahbaz & Ashraf, 2013), which not only limits plant growth and metabolism but also poses a foremost intimidation to sustainable agricultural production throughout the world particularly in arid and semi-arid areas (Tayyab *et al.,* 2016). More than 400 million hectares of the total geographical area of the world are affected by high concentration of the soluble salts (Al-Sadi *et al*., 2010) that may arise from some natural processes like weathering of rocks or man induced activities such as mismanagement in the use of irrigation practices, uncontrolled use of fertilizers and resulting in the yield reduction of many valuable crops (Yiu *et al*., 2012). Clearing of vast area of land for construction or other purposes results in rising of water table and ultimately affected by salinity (Varshney *et al.,* 2011). Whenever, plants expose unfavorable conditions during their life cycle they exhibit plasticity in cells to combat these conditions by adopting various mechanism at cellular, tissue and whole plant level. Maintenance of crop yields under adverse environmental stresses is probably the major challenge faced by modern agriculture. Due to increase in salinity, the other methods of plant growth and development at saline soils become limited and one has to choose salt tolerant cultivars, which can be easily grown on saline soils. Screening of various crop genotypes depends on salt tolerant germplasm for breeding purposes to get high yield crop plants having tolerance against salt stress. In field conditions salinity is present in the patches rather than regular pattern so soils have different physico-chemical properties and great heterogeneity. Due to this reason it is not an easy approach to screen out the genotypes in field conditions (Akram *et al*., 2010). Screening of tolerant and sensitive cultivars of any plant can be carried out on the basis of their performance and functioning under drastic and harsh climatic conditions (Ashraf & Leary, 1996; López-Arredondo *et al*., 2015). To cope with significance of bell pepper and exploitation of salt affected soils for production of crop, a study was planned to screen out the maximum available pepper genotypes, on the basis of their performance at various salinity levels at seedling stage.

Materials and Methods

Seeds of 14 bell pepper genotypes from Ayyub Agriculture Research Institute, Faisalabad were collected. Seeds were disinfected with 15% sodium hypochlorite solution.

Germination test: The germination tests were accomplished in Petri dishes in a seed incubator. Each Petri dish had twenty five seeds in double Whattman filter paper, soaked with relevant salt solution (2, 4, 6 and 8 dS m^{-1}) to maintain the desired salinity level. Control (0 dS m^{-1}) plants were treated with distilled water. Final germination percentage (days) and germination index was determined followed by protocol of Association of Official Seed Analysts (Anon., 1983). Mean germination time (days) was calibrated by Ellis & Roberts (1981) equation. Time to 50% seed germination was governed following the equation derivative by Coolbear *et al*. (1984) improved by Farooq *et al*. (2005). Embryo axis length (plumule + radical) was determined with the help of graded scale in centimeters (cm).

Emergence test: For emergence test, seeds were sown in plastic pots placed in greenhouse of Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Twenty seeds per pot were sown having Astatula fine sand (hyperthermic, uncoated typic quartzipsamments) individually as growth medium. The sand had pH of 6.0-6.5, with field capacity (7.2%) and incipient wilting at 1.2% (volume basis). NaCl at different concentrations (2, 4, 6 and 8 dS m^{-1}) was applied before sowing of seeds, while 0.5 strength Hoagland solution was used as nutrient solution. The seedlings were watered according to the need by observing moisture contents of sand. The salinity level was maintained by frequently assessing salt level, using EC meter. Control was without salt stress and irrigated only with half strength Hoagland solution.

Measurement of seedling fresh and dry biomass (g): After twenty days of growth, seedlings were uprooted and washed with distilled water to remove the foreign particles of sand. Fresh weight was measured by digital balance immediately after harvest. For dry weight, seedlings were kept in oven at 70°C for a week.

Measurement of seedling shoot and root length (cm): Shoot length of five randomly selected seedlings from each replicate was measured from the base of hypocotyls to the tip of the shoot with the help of meter rod. While, root length was measured from the base of hypocotyls to the tip of the longest root.

Measurement of number of leaves per plant and leaf area (cm^2): Number of leaves per plant was calculated by randomly selected five plants from each pot. For leaf area, five randomly selected leaves from two plants per replication were separated. These sampled leaves were placed on an electronic leaf area meter (Ll-3100; LI-COR, Inc., Lincoln, Nebr.) to calculate the leaf area (LA).

Ranking of genotypes: For ranking of genotypes, percent decrease of each parameter was calculated by using the formula, % decrease = attribute (control) – attribute (salinity) / attribute (control) * 100. After this calculation, each attribute had been arranged in an ascending order. Cumulative scoring was carried out by adding the numbers of each salinity level (2, 4, 6 and 8 dS m-1). Rankings were based on evaluation of each parameter with number 1 being the most tolerant cultivar.

Experimental design and statistical analysis: Experiment was designed following Complete Randomized Design (CRD) with two factor factorial arrangements. Collected data were analyzed statistically by employing the Fisher's analysis of variance technique and significance of treatments were essayed by using HSD (Tukey Test). Statistical analysis was estimated by using Statistix 8.1.

Results

Effect of salt stress on final germination percentage: Salt stress significantly affected final germination percentage (FGP) of all the tested bell pepper genotypes. Seedlings showed maximum mean value of FGP under non saline condition as compared to those subjected to NaCl stress. There was reduction in FGP in almost all the genotypes under 8.0 dS m^{-1}, followed by 6.0, 4.0, 2.0 and 0 dS m^{-1} (Fig. 1a). The interaction between salt stress and cultivars was significant ($p \leq 0.05$). On the basis of statistical comparison of means of FGP it is clear that maximum mean value was noted in genotype Zard followed by Tasty and Kaka-01 while genotype Colossal showed minimum mean value of FGP (Table 1).

Effect of salt stress on germination index: Salinity influenced adversely to germination index (GI) of all the tested bell pepper genotypes (Fig. 1b). Varietal means differed significantly, indicating that Zard (4.89) and Super Shimla (4.59) had the maximum GI values and statistically at par behavior under saline condition, followed by Tasty (3.91) and Aristotle (2.89). Least mean value for GI was noted in Colossal (1.77), Admiral (1.96) and PEP-311 (2.32). Statistical comparison of means revealed that highest germination index was found in Super Shimla both under saline as well as non saline conditions (Table 1).

Effect of salt stress on mean germination time: Salt stress significantly increased the mean germination time (MGT) of almost all the bell pepper genotypes used in this study. However, higher values of MGT were noted when seeds were subjected to higher salt level (8.0 dS m^{-1}) as compared to other treatments (6.0, 4.0, and 2.0 dS m^{-1} NaCl) (Fig. 1c). Crusadar, Admiral and Capistrano remained statistically at par and took minimum time for seed germination under saline and non saline conditions while Zard and Super Shimla also remained statistically at par with each other and revealed minimum mean value of MGT as compared to other genotypes.

Effect of salt stress on time to 50% germination: Seeds grown under saline conditions took more time for 50% seeds to germinate (T_{50}) as compared to those grown under non saline environment (Fig. 2a). The comparison of means of different cultivars at various saline concentrations revealed that Admiral took maximum time for 50% seed germination which was statistically at par with Crusadar. The cultivars like Zard and Super Shimla demonstrated significantly at par behavior by taking minimum time for 50% seed germination.

Effect of salt stress on embryo axis length: Salinity offered a remarkable decrease in embryo axis length (EAL) in all the tested bell pepper genotypes (Fig. 2b). Statistically compared means of all cultivars demonstrated that highest values of EAL was noted with Super Shimla, Zard, Orable and Lafayette being statistically at par with Colossal, while minimum mean values were found in case of Kaka-01 and Tasty. However, genotype PEP-311, CW-03 and Yolo Wonder demonstrated statistically at par results.

Effect of salt stress on seedling fresh and dry biomass: The seedlings grown under +NaCl environment exhibited a decline in fresh and dry biomass as compared to those under –NaCl conditions (Fig. 2c and Fig. 3a), furthermore, in present study this decrease was proportionate to the increase in salinity levels from (2.0 dS m^{-1}) to higher (8.0 dS m^{-1}). Maximum mean value for dry weight was noted when plants were sown under normal conditions without any salt application. Genotype Zard (4.56 g) showed maximum mean value of dry weight followed by Super Shimla (4.31 g) and Tasty (3.96 g).

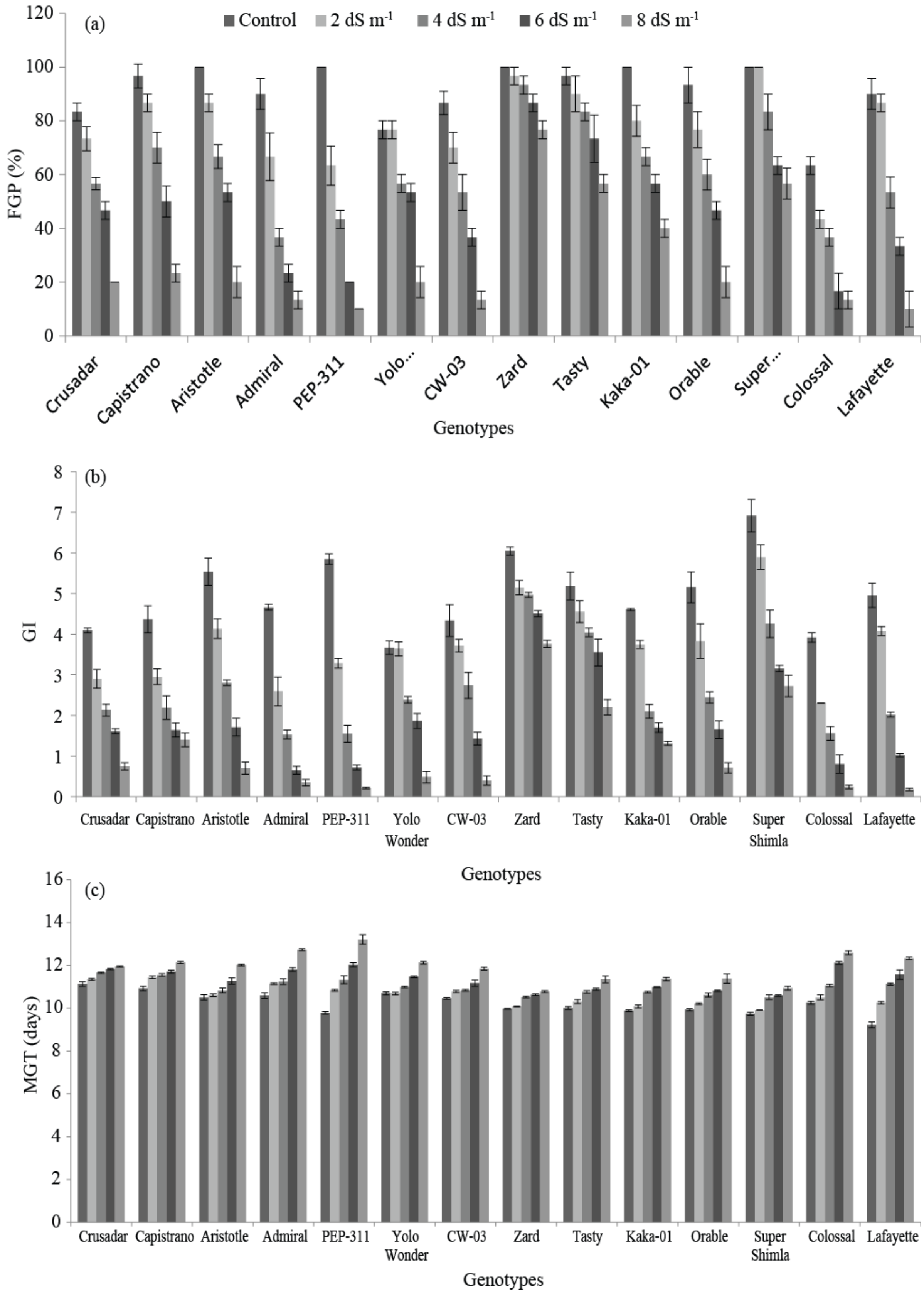

*Fig. 1. Effect of salt stress on (a) final germination percentage (FGP), (b) germination index (GI), (c) mean germination time (MGT) of 14 bell pepper genotypes when exposed to different saline treatments (2, 4, 6 and 8 dS m^{-1}) along with control (0 dS m^{-1}).

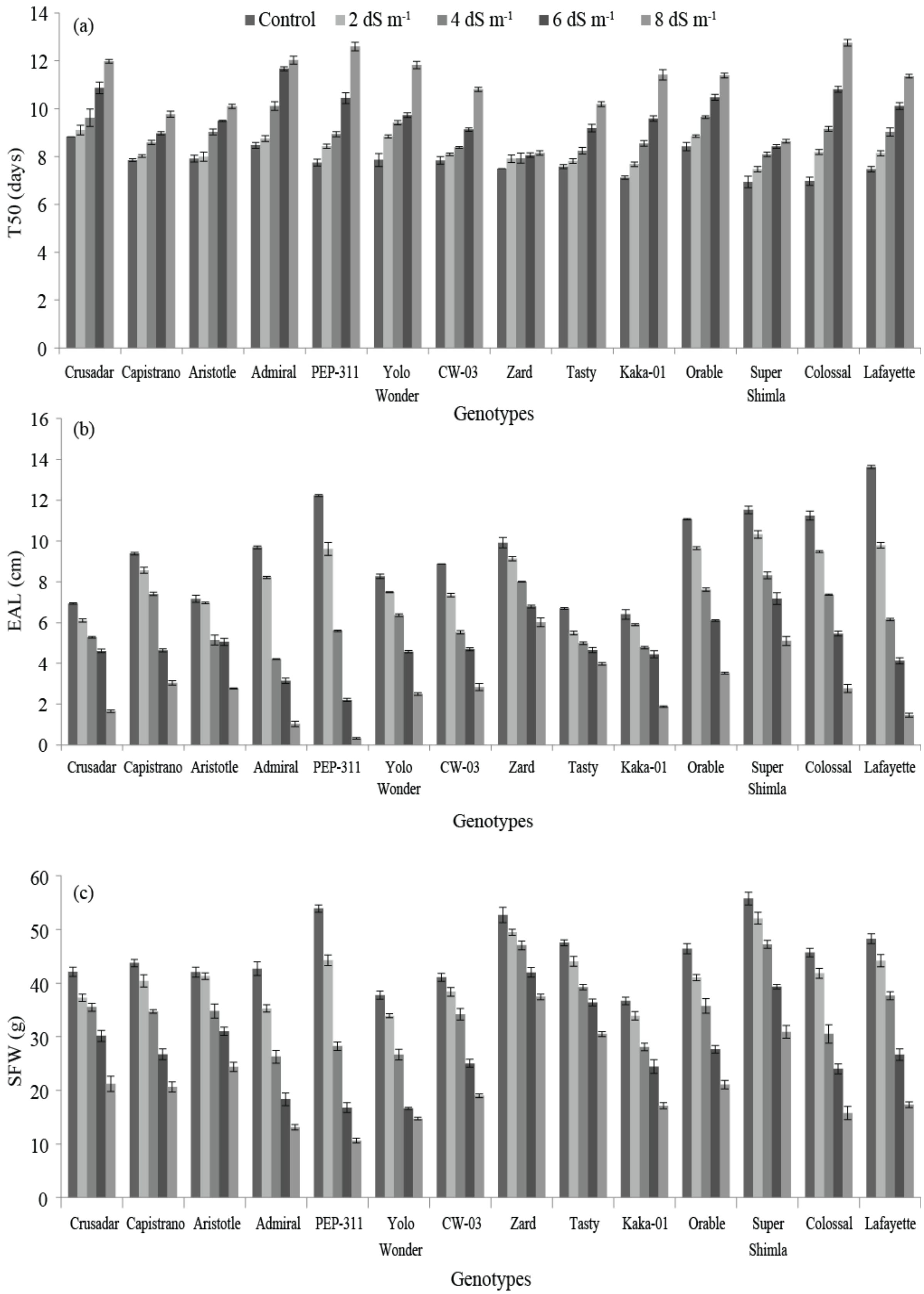

*Fig. 2. Effect of salt stress on (a) time to 50% seed germination (T_{50}), (b) embryo axis length (EAL), (c) seedling fresh weight (SFW) of 14 bell pepper genotypes when exposed to different saline treatments (2, 4, 6 and 8 dS m^{-1}) along with control (0 dS m^{-1}).

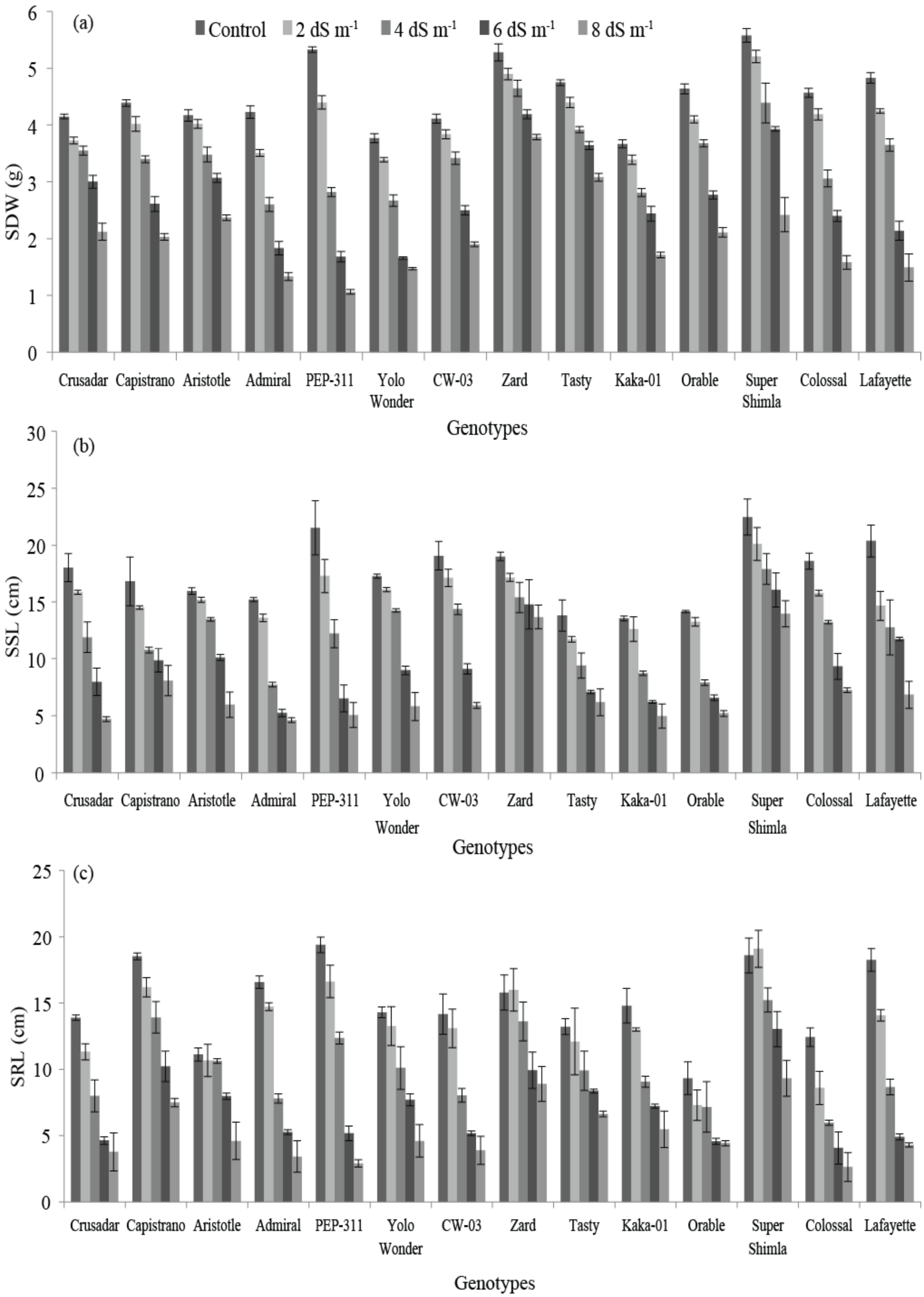

*Fig. 3. Effect of salt stress on (a) seedling dry weight (SDW), (b) seedling shoot length (SSL), (c) seedling root length (SRL) of 14 bell pepper genotypes when exposed to different saline treatments (2, 4, 6 and 8 dS m^{-1}) along with control (0 dS m^{-1}).

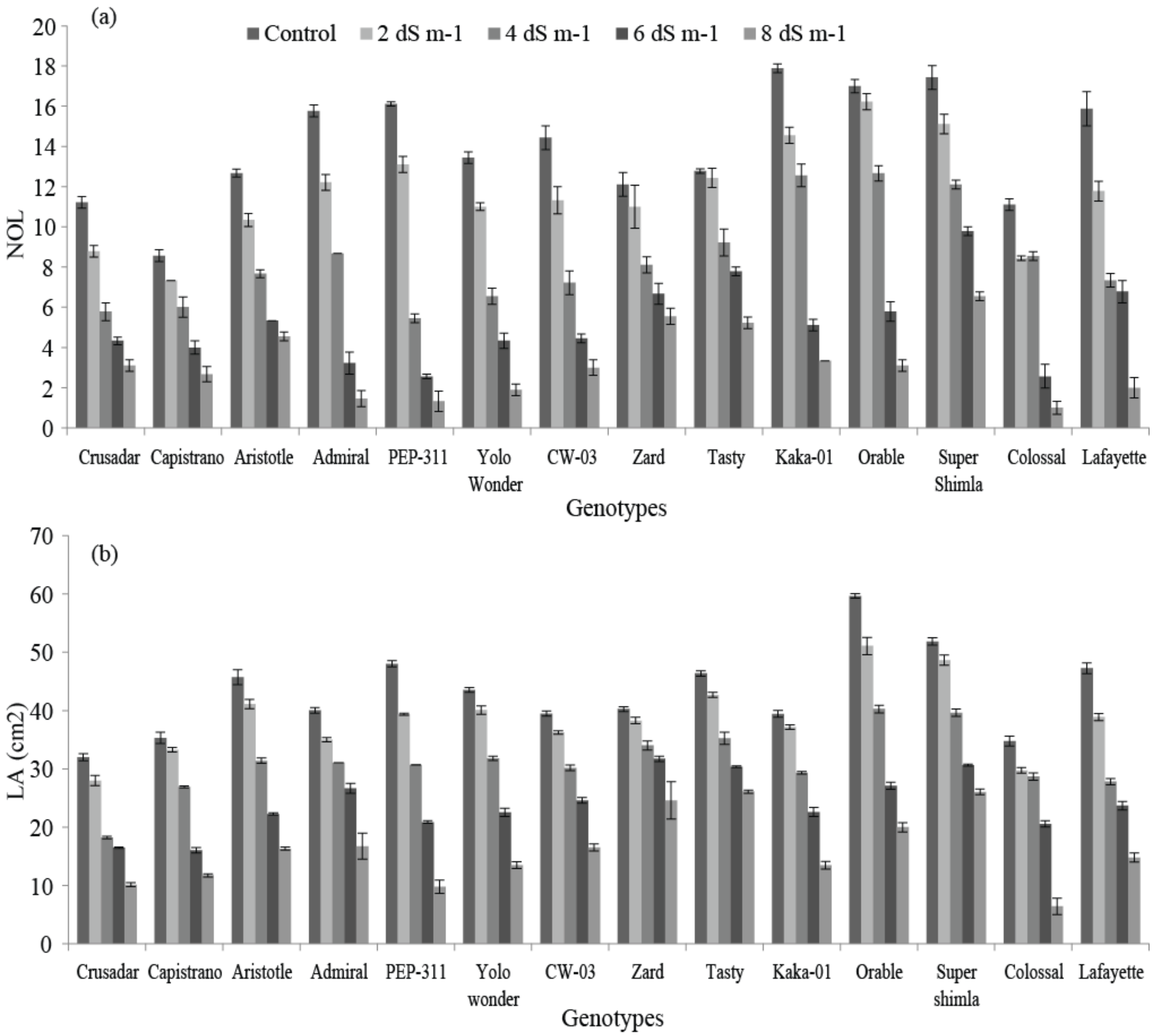

*Fig. 4. Effect of salt stress on (a) number of leaves (NOL), (b) leaf area (LA) 14 bell pepper genotypes when exposed to different saline treatments (2, 4, 6 and 8 dS m^{-1}) along with control (0 dS m^{-1})

*Each value in the above figure is the mean of 4 replicates and the vertical bars give the standard error (SE) of the mean. HSD (Tukey Test) for genotypes and treatments were significant at P= 0.05.

Effect of salt stress on seedling shoot and root length: Salt stress negatively affected plant vegetative growth as it was reported in the described study that seedlings of bell pepper under salt stress showed reduction in shoot and root lengths as compared to those grown under non saline environment (Figs. 3b and 3c). Maximum mean value of shoot and root lengths was noted in all genotypes under control conditions. Statistical comparison of means revealed that maximum mean value was found in Super Shimla, followed by Zard, Lafayette and CW-03 being statistically at par. While lowest mean value was recorded with Orable and Kaka-01 (statistically at par). Data regarding mean root length depicted the higher rank of Super Shimla, followed by Zard being statistically at par with Capistrano. Minimum mean value of root length was found in case of Orable and Colossal (Table 1).

Effect of salt stress on number of leaves and leaf area: Highest number of leaves was recorded in case of non saline conditions in almost all the genotypes (Figs. 4a and 4b). From the statistical comparison of mean table it was found that Super Shimla showed maximum mean value of number of leaves, followed by Kaka-01 and Orable which were statistically at par while lower mean value for the same attribute was noted in Capistrano, Crusadar and Colossal. Maximum reduction in leaf area of seedlings was observed under 8.0 dS m-1 salt stress (62.81%), followed by 6.0 dS m-1 (43.92%), 4.0 dS m-1 (27.64%) and 2.0 dS m-1 (10.44%).

Table 2, shows the overall ranking of bell pepper genotypes subjected to various salinity levels. On the basis of ranking table bell pepper genotypes can be grouped into comparatively salt tolerant (Zard, Tasty, Super Shimla and Aristotle), moderately tolerant (Crusadar, Capistrano, Yolo Wonder, Orable, CW-03 and Kaka-01) and sensitive genotypes (Colossal, Admiral, PEP-311 and Lafayette).

Table 1.Overall comparison of means of different attributes of 14 bell pepper genotypes at different salinity levels.

Genotypes	FGP (%)	GI	MGT (days)	T_{50} (days)	EAL (cm)	SFW (g)	SDW (g)	SSL (cm)	SRL (cm)	NOL	LA (cm^2)
Crusadar	56.00e	2.30ef	11.58a	10.08a	4.92hi	33.25cd	3.31de	11.69g	8.33f	6.64fg	20.97h
Capistrano	65.33cd	2.51de	11.55ab	8.64de	6.61e	33.24cd	3.29de	12.01fg	13.28b	5.71h	24.66g
Aristotle	65.33cd	2.98c	11.05ef	8.91d	5.42g	34.71c	3.42d	12.15fg	9.00ef	8.11de	31.37d
Admiral	46.00g	1.96fg	11.51abc	10.21a	5.26g	27.13fg	2.70g	9.29h	9.56de	8.27de	29.90def
PEP-311	47.33fg	2.32ef	11.36bcd	9.63b	5.99f	30.76f	3.06f	12.53def	11.29c	7.71e	29.74def
Yolo Wonder	56.67e	2.41de	11.20de	9.54b	5.84f	25.93g	2.59g	12.48ef	9.99d	7.44ef	30.29de
CW-03	52.00efg	2.53de	11.02ef	8.85de	5.85f	31.53de	3.15ef	13.11cd	8.87ef	8.09de	29.42ef
Zard	90.67a	4.89a	10.40hi	7.91f	7.98b	45.73a	4.56a	16.01b	12.85b	8.69cd	33.77c
Tasty	80.00b	3.91b	10.66g	8.61e	5.16gh	39.54b	3.96c	9.64g	10.05d	9.49c	36.16b
Kaka-01	68.67c	2.70cde	10.61g	8.87de	4.68i	28.03f	2.80g	9.22h	9.91d	10.69b	28.41f
Orable	59.33de	2.76cd	10.59gh	9.76b	7.59c	34.39c	3.46d	9.42h	6.56g	10.96b	39.61a
Super Shimla	80.67b	4.59a	10.34i	7.91f	8.49a	45.06a	4.31b	18.11a	15.06a	12.20a	39.37a
Colossal	34.67h	1.77g	11.31cd	9.58b	7.27d	31.56de	3.16d	12.84cde	6.74g	6.33gh	24.03g
Lafayette	54.67ef	2.45de	10.90f	9.22c	7.03d	34.83c	3.27def	13.28c	10.04d	8.75cd	30.49de

FGP: Final germination percentage, GI: Germination index, MGT: Mean germination time, T_{50}: Time to 50% seed germination, EAL: Embryo axis length, SFW: Seedling fresh weight, SDW: Seedling dry weight, SSL: Seedling shoot length, SRL: Seedling root length, NOL: Number of leaves, LA: Leaf area

Table 2. Overall ranking of bell pepper genotypes at different salinity levels on the basis of percentage variation in different attributes.

Genotypes	FGP (%)	GI	MGT (days)	T_{50} (days)	EAL (cm)	SFW (g)	SDW (g)	SSL (cm)	SRL (cm)	NOL	LA (cm^2)	Cumulative	Ranks*	Category
Zard	1	1	2	1	1	1	4	1	1	2	1	16	1	Tolerant
Tasty	2	1	6	3	5	4	2	8	3	1	3	38	2	
Super Shimla	3	3	5	6	4	3	8	3	2	3	2	42	3	
Aristotle	8	7	3	2	2	2	11	2	2	6	8	53	4	
Capistrano	5	6	5	1	3	7	9	5	4	4	6	55	5	Moderately tolerant
Yolo Wonder	4	2	2	10	6	10	7	4	4	9	8	66	6	
CW-03	11	4	4	4	9	6	1	7	7	9	4	66	6	
Crusadar	6	5	1	5	7	5	5	12	8	8	9	71	7	Moderately sensitive
Kaka-01	7	6	8	11	3	8	8	9	6	7	5	78	8	
Orable	9	7	7	7	8	9	3	10	5	5	10	80	9	
Colossal	12	8	9	13	10	11	8	6	10	10	7	104	10	Sensitive
Lafayette	10	8	10	12	12	9	6	11	11	11	11	111	11	
Admiral	13	9	9	8	11	12	12	13	9	12	4	112	12	
PEP-311	14	10	11	9	12	13	10	14	9	13	12	127	13	

FGP: Final germination percentage, GI: Germination index, MGT: Mean germination time, T_{50}: Time to 50% seed germination, EAL: Embryo axis length, SFW: Seedling fresh weight, SDW: Seedling dry weight, SSL: Seedling shoot length, SRL: Seedling root length, NOL: Number of leaves, LA: Leaf area, *Rankings were based on evaluation of each parameter with number 1 being the most tolerant genotype

Discussion

Various screening techniques are being used to classify the available germplasm of plants for salt tolerance. Due to salt stress, plants show characteristic changing pattern in growth, physiological, ionic, biochemical and enzymatic attributes. But, initial screening at seedling stage can be carried out by using mean germination time, time to 50% seed germination, germination index, embryo axis length, seedling fresh and dry biomass, seedling shoot and root length as screening tools for evaluating salt tolerance potential. Among these tools, plant biomass (fresh and dry) is highly significant because it is associated with a number of physiological (photosynthesis, transpiration, stomatal conductance and water use efficiency), biochemical (proline, glycinebetaine, protein contents and sugars accumulation), antioxidant enzyme activities (SOD, POD, CAT, GPX) and ionic attributes (Na, K, Ca and Cl) (Abbas *et al*., 2014). If all these above mentioned attributes would work positively then efficient amounts of plant biomass will be produced, consequently an efficient growth and development of the plant will be observed. Screening for salt tolerance at germination or early seedling stage is less laborious, quick responsive, cost effective and trustworthy than at mature stages. The preference of plants at early stage of growth under saline conditions has been considered highly predictive of the response of adult plants to salinity (Munns & James, 2003; Mahmood, 2009; Abbas *et al*., 2014). Thus, plants at early stage of growth screened for salinity tolerance could show considerable salinity tolerance at the later stages of growth. So, on the basis of germination and emergence tests, available germplasm can be categorized into salt tolerant and sensitive groups.

In this research work, salinity significantly reduced the germination and emergence percentage. In this way salinity inhibited plant growth at early seedling stage. Reduction in germination of plants by increasing salinity levels has been described by Mguis *et al*. (2014); Abbas *et al*. (2014); Hoque *et al*. (2014); Riffat & Ahmad (2016). As in present study, noticeable reduction in germination under different saline treatments had been observed in investigated bell pepper genotypes that may be due to lower osmotic potential of germinating media that altered the rate of seed water imbibition (Khan & Weber, 2008) and might cause the toxicity of Na and Cl ions within seeds, which in turn changed the enzymatic activities of nucleic acid (Gomes-Filho *et al*., 2008) and protein metabolism of germinating seeds (Dantas *et al*., 2007) and disturbed the hormonal balance (Khan & Rizvi, 1994) that may resulted into diminution in seed reserves (endosperm) (Promila & Kumar, 2000; Othman *et al*., 2006). Moreover, it had been reported that salinity has negative effect on cells, tissues and organs ultra-structure of plants (Rasheed, 2009). Hence, all these factors ultimately may become reason of abridged germination of tested bell pepper genotypes. The cultivars Zard, Tasty and Super Shimla showed the high salt tolerance potential regarding the germination, which indicates that these cultivars may accumulated less ratios of toxic ions (Na and Cl) in their tissue, thus absorbed the maximum beneficial ions and moisture contents which facilitated the enzymatic and protein activities necessary for growth and development. Whereas, the remaining bell pepper genotypes specially, PEP-311, Admiral and Lafayette could not hinder the entry of Na^+ and Cl^- in their tissues and these toxic ions might reduce the water up-taking potential of seeds and disturbed the enzymatic activities essential for growth related metabolic processes.

Salt stress also reduced other growth attributes like seedling shoot and root length, seedling fresh and dry weight in tested bell pepper genotypes, which showed that a strong negative correlation existed between these attributes and salinity. Since, plant biomass is a reflection of plant responses to salt stress and thus biomass measurement is an important scale index for evaluating tolerance to salt stress conditions (Phenodays, 2014). There are various plant growth regulators (PGRs) i.e. auxins, gibberellins, cytokinins and antioxidants which are produced in seed and young seedling tissues that regulate the growth and growth related metabolic processes (Ren *et al*., 2011). These hormones activate the synthesis of specific growth promoting enzymes by stimulating the special genes within plant body (Droual *et al*., 1998). Under saline conditions these PGRs can't work properly due to the injurious effect of toxic ions (Na and Cl), ultimately seedling growth and development get disturbed. Since, in present study salinity inhibited various growth aspects, so it could have been due to the inhibiting effect of salt stress on growth related hormones or PGRs. The best performance of Zard, Tasty and Super Shimla indicated that these cultivars had well maintained their PGRs activity due to less accumulation of toxic ions, therefore they showed less reduction in growth attributes in response to salt stress. Whereas, the genotypes PEP-311, Lafayette and Admiral presented the highest reduction in growth attributes which might be linked with high negative effect of salinity on the functioning of PGRs, stimulating the growth and development. In addition, cell turgor also plays a significant role in regulating growth. Actually, the plant growth is carried out by expansion of cell vacuole by the entry of water into cell cytoplasm and vacuole, consequently cell becomes enlarged due to high turgor potential (Ray, 1972). The turgor pressure causes the cell differentiation and cell elongation, so resultantly plant biomass is produced (Hessini *et al*., 2009). Likewise, in current study, high salinity in root zone may be involved in reducing the water potential, which indirectly resulted in reduced cell turgidity. Due to this reduced cell turgidity the cell differentiation and cell elongation get decreased, which lead to inhibition of growth in tested bell pepper genotypes grown under saline conditions. Since, all the investigated pepper genotypes, exposed to salt stress showed the significant reduction in growth attributes as compared to those grown under non-saline control conditions, therefore these parameters can be used as a screening criteria for salt tolerance. On the basis of this experiment it can be concluded that reduction in germination percentage, seedling shoot and root length and seedling biomass was highly associated with salt stress and these can serve as an effective tool for the assessment of salt tolerance potential of pepper genotypes. Literature also depicts that these attributes can be used as screening tools for salinity tolerance potential (Tlig *et al*., 2008**,** Guan *et al*., 2009). The screened salt-tolerant bell pepper genotypes like Tasty, Super Shimla and Zard having comparatively better genetic capacity to tolerate the excessive salts in root zone, can be cultivated on marginal saline lands.

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