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Screening of Pepper (*Capsicum annuum* L.) Genotypes Against Salinity Stress

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Abstract: Soil is one of the most important natural resources for crop production. The World is facing soil salinity problem that adversely affects crop productivity. Use of salt tolerant genotypes is one of the most effective strategies to cope with the problem of salinity. The current study was conducted to evaluate the impact of salinity stress on growth of different pepper genotypes in order to identify salt tolerant genotypes of pepper. A pot experiment was conducted with three salinity levels (control, 1.5, 3.0 and 6.0 dS m^{-1}) and four pepper genotypes (F215, 007F1, Asia and Pusa Jwala). There were three replications. Treatments and different pepper genotypes were arranged according to completely randomized design in factorial fashion. The results showed that salinity stress significantly reduced growth, yield and physiological parameters of the pepper plant. However, at highest salinity level 6 dS m^{-1}cultivar 007F1 showed maximum tolerance as minimum reduction inplant height (40%), root length (39%), shoot dry weight (33%), root dry weight (25%), chlorophyll-a(17%), chlorophyll-b contents (14%), potassium in shoot (37%), relative water contents (24%), and fruit weight (32%) was observed in 007F1 followed by F215 and Asia as compared to normal salinity level (T1: Control 0.6 dS m^{-1}). While Pusajwala showed a maximum increase in Na^+ concentration in shoots (92%) and least performance in all other attributes under all salinity levels. It is concluded that 007F1 showed the best performance andcan be recommended for further genotypic evaluation under salinity stress in field trials to enhance pepper production.

Keywords: Pepper, Salinity, Screening, Stress, Genotypes.

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1 Introduction

Pepper (*Capsicum annuum* L.) is considered as an important vegetable crop in the world. The overall global production of pepper is about 47,000 million tones (FAOSTAT, 2015). Nutritionally, it is a rich source of vitamins, carotenoids and phenolic substances. The addition of these in dietary nutrition helps in protecting several diseases (Marin et al., 2004). Pepper is an important cash crop of arid and semi-arid zones of Pakistan sharing about 1.5% in GDP, but its production is adversely affected by salinity in recent years. Salinity is becoming one of the most drastic limiting factors for crop production (Libutti and Monteleone. 2017). It is the most widespread problem, affecting approximately 20% of the world's cultivated land and nearly half of the area under irrigation (Kaouther et al., 2013; Uddin et al., 2016). The pepper plant is moderately sensitive to salinity but if not managed properly salinity can become a severe limiting factor for its production (Villa-Castorena et al., 2003). Salt stress can directly or indirectly affect the physiological status of plants by disturbing their metabolism, growth, development, and productivity (Das et al., 2015; Farooq et al., 2015; Kere et al., 2016; Acosta-Motos et al., 2017; Formentin, 2017; Hong et al., 2017; Rizzello et al., 2017). It is also known to affect many aspects of anatomy and ultra-structure of plant cells (Zhu, 2007; Muscolo et al., 2015; Park et al., 2016).

Under salinity stress, growth inhibition is primary symptom in plants which leads to cellular disruption, inhibition of photosynthesis and oxidative

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disintegration (Zhu, 2007). Under salt stress, accumulation of toxic ions poses serious problems to plants (Hasegawa et al., 2000). High levels of salt in soil cause injury of roots leading to nutrient deficiencies that severely affect yield potential of the plant (Wahomeet al., 2000; Penella et al., 2016). Salt stress interferes almost every physiological and morphological process in plants. The inhibition of plant growth due to salinity is attributed to salt-induced-ion toxicity, nutrient deficiencies, salt-induced osmotic stress, hormonal imbalance and salt-induced oxidative stress (Munns, 2005). Plants grown in soils having high concentrations of salts tend to uptake and accumulate excessive toxic ions. If accumulated in high concentrations, these ions interfere the normal physiological processes and cause toxicity in plants (Munns and Tester, 2008).

When plants experience the high salt stress conditions, they need to regulate internal physiological status (Tester and Davenport, 2003). To do so, plants naturally accumulate low-molecular-weight compounds which are known as the compatible solutes or secondary metabolites (Zhifang and Loescher 2003). These substances help plants to withstand stress conditions without interfering the biochemical reactions (Hasegawa et al. 2000). These secondary metabolites include proteins, carbohydrates and quaternary ammonia compounds (Ashraf, 2004). These compounds get accumulated in the cytoplasm and protect plant cells from the adverse effect of high salt concentrations by regulating their osmotic relations, preventing the excessive accumulation of toxic ions and scavenging the reactive oxygen species (ROS) (Ashraf and Foolad, 2007).

However, not all plants have the tendency to synthesize these secondary metabolites under stress and vary in their stress tolerance from cultivar to cultivar (Ashraf and Foolad, 2007). The comparison of different cultivars have varying ability to withstand stress conditions is very useful to evaluate the degree of stress tolerance of different cultivars/varieties of a single species, not only enhance our understanding ofbasic mechanisms involved in salinity tolerance but also enable us to recognize the best cultivar that has the increased tolerance against salinity. Keeping in view the economic and dietary importance of red pepper, the present study was designed to screen out the best cultivar of pepper that has maximum ability to tolerate salinity stress which will serve as a specimen to be studied and evaluated further for its genotypic characteristic for enhancing salinity tolerance in pepper.

2. Materials and Methods

2.1 Pot Experiment

Seeds of four different pepper cultivars (Pusajwala, 007F1, Asia, F215) were obtained from local market and nursery was raised. When plants reached four leaves stage, these were transferred to pots with 12 kg of soil and each set of pots had pre-developed salinity levels (Control (0.6 dS m^{-1}), 1.5 dS m^{-1}, 3 dS m^{-1} and 6 dS m^{-1}) by adding calculated amounts of mixed salts (NaCl, $MgSO_4$, $CaCl_2$ and Na_2SO_4). Salts were calculated by using quadratic equation for all salinity levels. Each treatment has three replications. Pots were arranged in completely randomized design (CRD) in factorial arrangement in the wire house at ambient light and temperature. Recommended dose of N, P_2O, K_2O fertilizers (100: 40: 40 kg ha^{-1}) was applied in each pot as urea, diammonium phosphate, and sulphate of potash, respectively. At the time of sowing, a full dose of P, K, and 1/4th of N was applied. The remaining N was applied in three splits at 15 days interval. Pots were irrigated with canal water. After the establishment of seedlings, thinning was done for uniform plant population.

2.2 Plant Analysis

The fresh plant leaves were sampled after 50 days of transplanting for the determination of physio-biochemical attributes and yield parameters were recorded at harvesting of the crop. Data regarding physiological parameters in pepper was measured following the method of Hiscox and Israelstam (1979) for chlorophyll "a" and Arnon (1949) for the chlorophyll "b".

Table 1: Comparative effect of salinity stress on plant height and root length of different pepper genotypes

Salinity level	Plant height (cm)				Root length (cm)			
	Pusa Jwala	007F1	Asia	F215	Pusa Jwala	007F1	Asia	F215
T_1	33.30 a	33.01 a	32.23ab	33.30 a	18.90 b	21.40 a	18.00bc	18.73 b
T_2	30.43 b-d	31.47 a-c	28.54 d	29.93 cd	18.20bc	21.17 a	16.27 d	17.93 b-d
T_3	22.23 f	26.31 e	20.13 g	25.37 e	11.33ef	18.03bc	12.87 e	16.63 cd
T_4	13.80 h	19.67 g	11.33i	14.87 h	9.80fg	12.90 e	8.83 g	10.57 f
LSD ≤ 0.05	**1.9895**				**1.6891**			

Means sharing same letters are statistically at par at 5% level of probability (n=3); T_1; Control (0.6 dS m^{-1}), T_2; 1.5 dS m^{-1}, T_3; 3 dS m^{-1}, T_4; 6 dS m^{-1}.

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Table 2: Comparative effect of salinity stress on shoot dry weight and root dry weight of different pepper genotypes

Salinity level	Shoot dry weight (g plant^{-1})				Root dry weight (g plant^{-1})			
	Pusa Jwala	007F1	Asia	F215	Pusa Jwala	007F1	Asia	F215
T_1	9.30 cd	12.50 a	9.30 cd	10.70 b	6.63 ab	6.70 a	6.63 ab	6.77 a
T_2	7.47 ef	10.40 bc	7.30 ef	8.70 d	5.30 c-f	6.37 a-c	5.97 a-d	5.77 a-e
T_3	5.63 gh	9.33 cd	5.83 gh	6.57 fg	4.63 e-g	6.03 a-d	4.97 d-f	5.43 b-f
T_4	4.77 hi	8.33 de	4.50 i	5.80 gh	3.63 g	5.03 d-f	4.30 fg	4.43 fg
LSD ≤ 0.05	**1.2185**				**1.2369**			

Means sharing same letters are statistically at par at 5% level of probability (n=3); T_1; Control (0.6 dS m^{-1}), T_2; 1.5 dS m^{-1}, T_3; 3 dS m^{-1}, T_4; 6 dS m^{-1}.

Relative water contents (RWC) were determined as described by Barrs and Weatherly (1962), while, nitrogen was determined by Kjeldahl method (Ryan et al., 2001). The P contents were determined by spectrophotometer described by Chapman and Prat (1961) and potassium concentration was measured by flame photometer (Ryan et al., 2001).

2.3 Statistical Analysis

Analysis of variance techniques (ANOVA) was applied to analyze the data (Steel et al., 1997) using completely randomized design (CRD) in factorial fashion, and means were compared by LSD to find the significance of the data.

3. Results

3.1 Comparative effect of salinity stress on growth of different pepper genotypes

The results regarding plant height and root length (Table 1) showed that salinity stress significantly decreased the plant height and root length of different pepper genotypes. The data showed that minimum plant height and root length was observed at the highest salinity level i.e. 6 dS m^{-1} in Asia (11.33 cm and 8.30 cm, respectively) followed by Pusa Jwala (13.80 cm and 9.8 cm, respectively). The results also revealed that minimum decrease in plant height and root length was found in cultivar 007F1 (40% and 39%, respectively) at the highest salinity level, followed by cultivars F215 (55% and 43%, respectively), Pusa Jwala (59% and 48%, respectively) and Asia (64% and 51%, respectively as comparison to normal salinity level (T1: Control 0.6 dS m^{-1}). The results revealed that pepper variety 007F1 possess highest salinity tolerance regarding plant height and root length at salinity level T_4; 6 dS m^{-1} as comparison to other varieties.

3.2 Comparative effect of salinity stress on biomass production of different pepper genotypes

The data regarding the effect of different salinity on shoot dry weight and root dry weight presented in Table 2 indicates that salinity stress statistically significantly decreased the biomass production (shoot dry weight and root dry weight per plant) in all pepper genotypes. However, all genotypes differed significantly in their salinity tolerance, as cultivar 007F1 showed a minimum decrease in shoot dry weight (33%) and root dry weight (25%) as compared to normal salinity level (T1: Control 0.6 dS m^{-1}). Cultivars Pusa Jwala and Asia showed maximum decrease in shoot dry weight (49% and 51%, respectively) and root dry weight (45% and 35%, respectively) as compared to normal salinity level (T1: Control 0.6 dS m^{-1}). Both varieties were sensitivity to salinity stress.

Table 3: Comparative effect of salinity stress on chlorophylla and chlorophyll-b contents in leaves of different pepper genotypes

Salinity level	Chlorophyll-a (µg g^{-1})				Chlorophyll-b (µg g^{-1})			
	Pusa Jwala	007F1	Asia	F215	Pusa Jwala	007F1	Asia	F215
T_1	0.706 cd	0.749 a	0.729 a-c	0.735 a-c	0.356 a-c	0.378 a	0.368 ab	0.371 ab
T_2	0.693 de	0.742 ab	0.716 b-d	0.723 a-d	0.340 c-e	0.364 ab	0.351 b-d	0.355 a-c
T_3	0.626 gh	0.669 ef	0.605 h	0.650 fg	0.306 fg	0.328 d-f	0.296 gh	0.318 e-g
T_4	0.569 i	0.638 fg	0.550 i	0.619 gh	0.278 hi	0.312 fg	0.268 i	0.303 g
LSD ≤ 0.05	**0.0321**				**0.0246**			

Means sharing same letters are statistically at par at 5% level of probability (n=3); T_1; Control (0.6 dS m^{-1}), T_2; 1.5 dS m^{-1}, T_3; 3 dS m^{-1}, T_4; 6 dS m^{-1}.

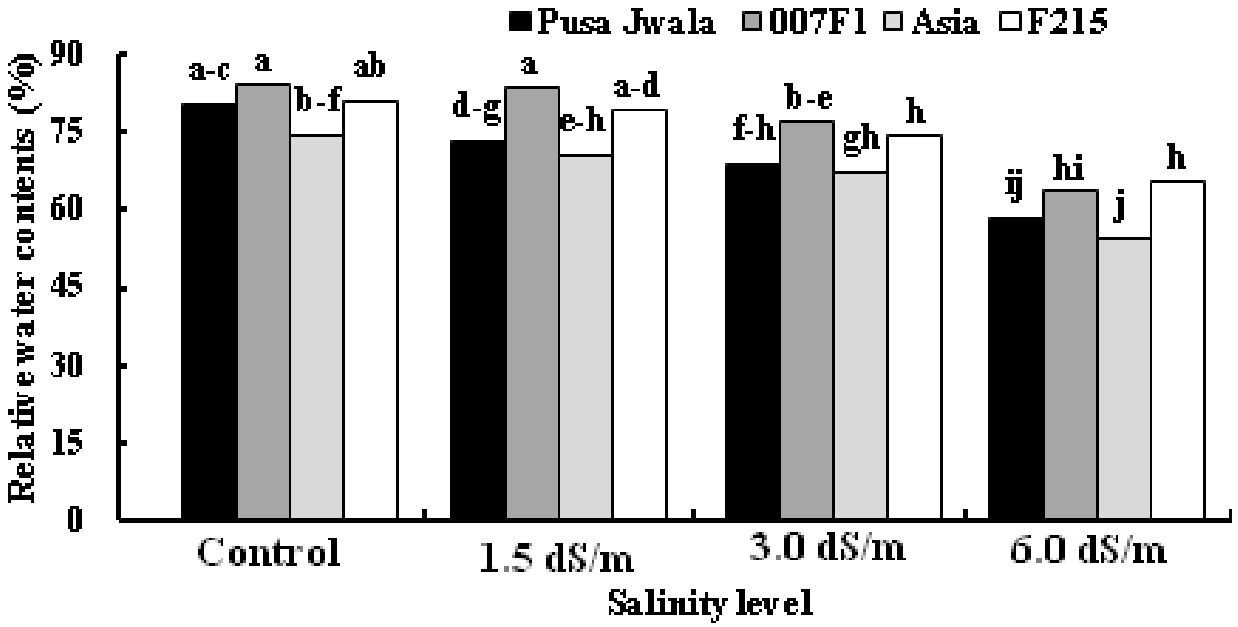

Figure 1: Comparative effect of salinity stress on relative water contents in leaves of different pepper genotypes; Bars sharing same letters are statistically at par at 5% level of probability (n=3); T_1; Control (0.6 dS m^{-1}), T_2; 1.5 dS m^{-1}, T_3; 3 dS m^{-1}, T_4; 6 dS m^{-1}.

3.3 Comparative effect of salinity stress on physiological attributes of different pepper genotypes

Chlorophyll-a contents were decreased significantly with increasing level of salinity in all the genotypes of pepper. The maximum decrease in chlorophyll-a contents was observed at highest salinity level 6 dS m^{-1} in all genotypes as compared to normal salinity (T1: Control 0.6 dS m^{-1}) (Table 3). However, cultivar 007F1 showed maximum tolerance to highest salinity level 6 dS $m^{-1}$and showed a minimum decrease in chlorophyll-a contents in leaves of 007F1 (14%) followed by F215 (16%), Pusa Jwala (19%) and Asia (25%) as compared to normal salinity level (T1: Control 0.6 dS m^{-1}).

Similar results were in the case of chlorophyll-b contents in leaves (Table 3), as salinity stress significantly decreased chlorophyll-b contents in leaves of all pepper genotypes with a maximum decrease in Asia (27%) and Pusa Jwala (22%) as compared normal salinity level (T1: Control 0.6 dS m^{-1}) were found . Whileminimumat highest salinity level 6 dS m^{-1}decrease in chlorophyll-b contents in leaves was observed in cultivar 007F1 (17%) as compared normal salinity level (T1: Control 0.6 dS m^{-1}).

The data regarding relative water contents (RWC) in leaves (Fig. 1) showed that salinity stress significantly disrupted the water relations of all pepper genotypes as significant decrease in RWC was observed with increasing salinity level. The results showed that at highest salinity level 6 dS m^{-1}minimum decrease in RWC was observed in cultivar F215 (19%) followed by 007F1 (24%), Asia (27%) and Pusa Jwala (28%) as compared normal salinity level (T1: Control 0.6 dS m^{-1}).

Table 4. Comparative effect of salinity stress on Na^+ and K^+ concentration in leaves of different pepper genotypes

Salinity level	Na^+ (%)				K^+ (%)			
	Pusa Jwala	007F1	Asia	F215	Pusa Jwala	007F1	Asia	F215
T_1	0.57 f	0.57 f	0.60 ef	0.59 f	2.30 de	2.53 a	2.41 a-d	2.43 a-c
T_2	0.63 d-f	0.60 ef	0.65 d-f	0.65 d-f	2.23 e	2.47 ab	2.31 c-e	2.40 b-d
T_3	0.83 bc	0.67 d-f	0.85 b	0.72 c-e	1.76 g	1.96 f	1.77 g	1.83 g
T_4	1.09 a	0.75 b-d	1.06 a	0.85 b	1.40 i	1.60 h	1.36 i	1.48 i
LSD ≤ 0.05		0.1260				0.1210		

Means sharing same letters are statistically at par at 5% level of probability (n=3); T_1; Control (0.6 dS m^{-1}), T_2; 1.5 dS m^{-1}, T_3; 3 dS m^{-1}, T_4; 6 dS m^{-1}.

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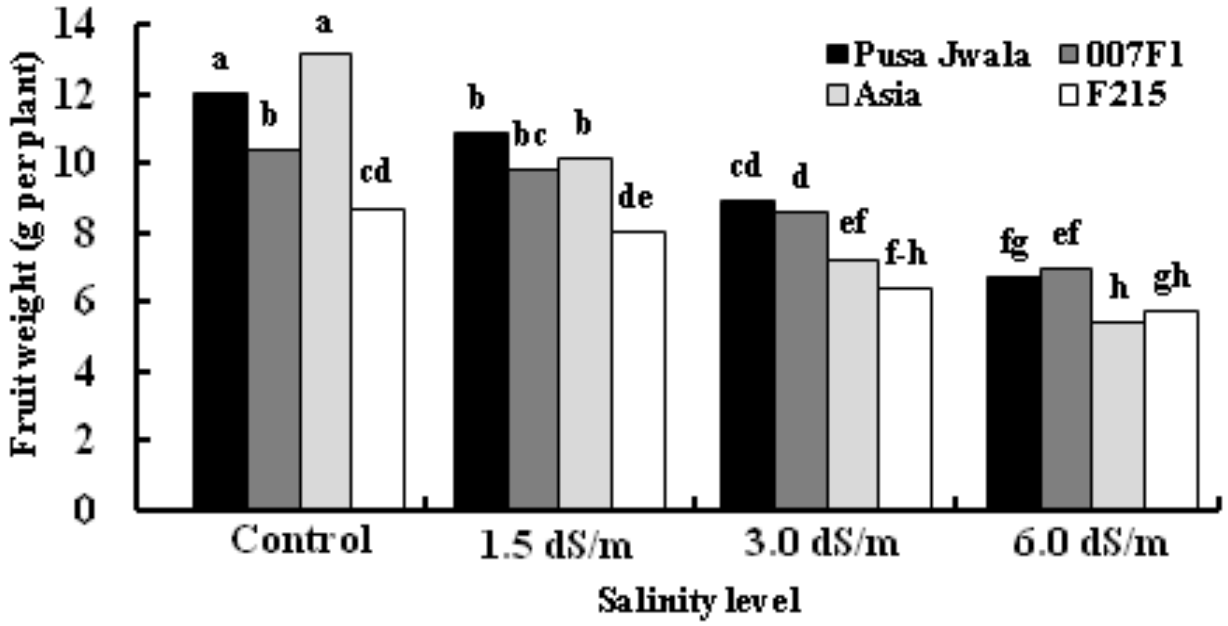

Figure 2: Comparative effect of salinity stress on fruit weight of different pepper genotypes; Bars sharing same letters are statistically at par at 5% level of probability (n=3); T_1; Control (0.6 dS m^{-1}), T_2; 1.5 dS m^{-1}, T_3; 3 dS m^{-1}, T_4; 6 dS m^{-1}.

3.3 Comparative effect of salinity stress on fruit weight of different pepper genotypes

The results (Fig. 2) showed that salinity stress adversely affected the fruit development of all pepper genotypes. However, genotypes showed the varied level of tolerance towards salinity stress. The results showed that at highest salinity level 6 dS m^{-1} minimum decrease in fruit weight was observed in cultivar 007F1 (32%) as compared normal salinity level (T1: Control 0.6 dS m^{-1}).

3.3 Comparative effect of salinity stress on Na^+ and K^+ concentration in leaves of different pepper genotypes

The results showed that Na^+ concentration in shoots (Table 4) of different pepper genotypes was significantly increased with increasing salinity level. The data showed that at highest salinity level 6 dS m^{-1}maximum increase in Na^+ concentration in shoots was observed in Pusa Jwala (92%) as compared normal salinity level (T1: Control 0.6 dS m^{-1}).While, theminimum increase was observed in cultivar 007F1 (31%) followed by F215 (45%). The data presented in Table 4 also showed that K^+ concentration in shoots of different pepper genotypes was significantly decreased with increasing salinity level. Maximum tolerance to at highest salinity level 6 dS m^{-1} was demonstrated by cultivar 007F1 as a minimum decrease in K^+ concentration in shoots (37%) was observed in 007F1 as compared normal salinity level (T1: Control 0.6 dS m^{-1}).

4. Discussion

The findings of the present study showed that salinity stress significantly reduced the shoot length, root length, dry biomass, fruit weight as well as relative water contents, and K^+ concentration in leaves of different pepper genotypes. Reduction in growth, physiology and fruit development is attributed to the adverse effects of salinity inducing salt-induced osmotic stress, ion toxicities, disturbance in ion balance and combination these salt-induced effects (Nirmala et al., 2015; Nahar et al. 2016). The most drastic effect of salinity stress is the lowering of intracellular osmotic potential in roots due to higher uptake of Na^+ (as in our findings),which prevents the uptake of ample moisture required for plant growth decreasing relative water contents in plant tissues leading to poor cell division and elongation (Nirmala et al., 2015). The reduction in cell division significantly affects the vegetative and reproductive growth by decreasing the biomass production that ultimately results in poorshoot, root and fruit development (Sehrawat et al., 2013a; Sehrawat et al., 2013b).

The accumulation of toxic ions (Na^+) alters the membrane permeability which is also a key factor for growth inhibition (Kaouther et al., 2013). Researchers also reported that these toxic ions have an antagonistic effect on several essential ions involved in vital physiological functions e.g. the higher uptake of Na^+ reduces the K^+ uptake. The K^+ is an essential

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component of regulatory machinery of several physiological processes including photosynthesis, stomatal conductance, cell division and osmotic regulation.

The reduction in K^+ uptake adversely affects all these fundamental processes thus reducing plant performance (Roy and Sengupta, 2014). In response to salt stress, plants produce different organic compounds, such as proline glycine, and accumulate minerals for osmoregulation and the production of these moleculesis an energy consuming process (Serraj and Sinclair, 2002; Negrão et al., 2017). Greenway and Gibbs (2003) proposed that consumption of a higher amount of energy for the maintenance rather than growth is also responsible for reduced biomass production under salinity stress. Increasing salt concentration also restricts root and shoot growth due to decreased uptake of nutrients or non-transferability of these nutrients from the soil to plant (Hashemi et al., 2010).

Though, salt stress adversely affected the growth of all pepper genotypes. However, all genotypes differed in their tolerance level towards salinity stress. The results showed that maximum tolerance was showed by cultivar 007F1 and F215, while most sensitive cultivars are PusaJwala and Asia. These results are in line with the reports on different crops indicating the difference in the salinity tolerance of different genotypes of a single crop species including corn, soybean, sorghum, rice, wheat, peanut canola, chickpea and melon (Kausar et al., 2012;Sudharani et al., 2012; Shaheenuzzamn, 2014). The difference in the ability to tolerate thesalt stress of different genotypes is correlated to the efficient regulation of Na^+ and K^+in plants cells as salt tolerant cultivars (007F1 and F215) showed lower uptake of Na^+ and higher uptake of K^+ (Khayat et al., 2010). Selective uptake of ions and exclusion of Na^+at cellular level is a chief salinity tolerance mechanism in plants (Akhtar et al., 2003). Roy and Sengupta (2014) suggested that under high salt concentrations, the tolerant plants protect themselves by efficiently regulating their cellular ionic balance through maintaining high K^+/Na^+ ratio in tissues while less tolerant plants show higher uptake of Na^+ and lower uptake of K^+, consequently low in productivity under salinity stress.

5. Conclusion

The assessment of the effects of salinity stress on the growth, yield and physiological attributes of different pepper genotypes led us to conclude that all parameters were adverselyaffected by salt stress specifically at the highest salinity level (6.0 dS m^{-1}). However, cultivars 007F1 and F215 were classified as the salt tolerant ones, whereas, Asia and PusaJwala as susceptible ones. The 007F1 and F215 showed improved regulation of Na^+ and K^+ concentrations in tissues. Thus, itis suggested that cultivars 007F1 and F215 should be further studied and evaluated for their genotypic characteristicsin field trials to develop more efficient pepper cultivars.

Author Contribution: MFZA and ML initiated and designed the research, FA and MAK performed the experiment. MFZA and IK wrote the first draft of manuscripts. MUJ performed statistical analysis. AH reviewed and made final draft of the manuscript. All the authors discussed the result and assisted in manuscript preparation and revision.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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