

Germination and seedling growth of *Zea mays* L. under different levels of sodium chloride stress

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ABSTRACT

Salt stress is one of the most severe environmental factors that reduces and limits growth and development of plants. Abiotic stresses such as heavy metals, salinity, drought, temperature, UV-radiation, ozone causes drastic yield reduction in most of the crops. Especially salt stress affects around 20 % of the world's cultivated areas. This study was conducted in order to evaluate the effect of NaCl on germination and seedling growth of *Zea mays* L. Seeds of *Zea mays* were germinated in glass Petri-dishes of 100 × 15 mm diameter lined with blotting paper. Ten seeds were placed in each petri-dish. Petridishes were irrigated with 25, 50, 75, 100, 125, 150, 175 and 200 mM concentrations of NaCl. A control was moistened with ten milliliters of distilled water. The germination percentage, water absorption of the seeds, water uptake percentage and the growth parameters were observed. The results obtained showed that the inhibition of the germination percentage, germination rate, water uptake, growth and biomass accumulation of the seedlings were observed to decrease with increasing NaCl concentrations. At the highest level of stress both plumule and radical decreased significantly. The salt stress decreased seed germination, biomass and growth of maize seedlings due to ion toxicity, decrease osmotic potential and oxidative stress.

Keywords: *Zea mays*; salt stress; inhibition; water content; germination; seedling growth

1. INTRODUCTION

Germination is the first stage and one of the important and sensitive stages of the plant life cycle. It is an important process in seedling growth (De Villiers, et al, 1994). This stage of growth is strictly influenced by environmental factors, especially, temperature and humidity (Soltani et al., 2008). Researchers have proposed seed priming as a technique for germination rate improvement. In this method, seeds will enter in the second stage of germination i.e. imbibitions, but they don't enter to the third stage of germination process. In fact, this operation is one of the most important seed treatments before seedling. After this act, primed seeds, just like the untreated seeds, will be stored (McDonald et al., 1999). Seed germination is a major factor limiting the establishment of plants under saline conditions. Salinity may cause significant reductions in the rate and percentage of germination, which in turn may lead to uneven stand establishment and reduced crop yields (Foolad et al., 1999). According to Steppuhn and Wall (1999), salinity could be defined as a water property that indicates the concentration of dissolved solutes. Soil salinity refers to the state in which dissolved constituents concentrate beyond the needs of plant roots. Salinity is estimated that

about 20 % of the irrigated land in the present world is affected by salinity that is exclusively classified as arid and desert lands comprising 25 % of the total land of our planet (Rasool et al., 2013). Salinity is responsible for delayed seed germination and establishments of seedlings (Bybordi and Tabatabaei, 2009). Lower levels of salinity delayed germination, whereas higher levels reduce the final percentage of seed germination (Ghoulam and Fares, 2001). Salts of different nature and concentration increase water potential, restricting the movement of water towards the seed surface (Tester and Devenport, 2003; Poleskaya et al., 2006; Houimli et al., 2008). Under increasing salinity stress, water is not imbibed which delays the germination percentage (Almansouri et al., 2001). High soil salt content reduces the rate of germination and total seed germination percentage (Sinha et al., 2004).

Germination in *Zea mays* (*Z.mays* L.) decreases linearly with rising salinity. Salt stress creates both ionic as well as osmotic stress in plants (Parvaiz and Satyawati, 2008). These stresses can be distinguished at several levels such as shoot, root and tissues (Tester and Davenport, 2003). Salt stress is an abiotic stress that can effects the plant growth and physiological and biochemical activities such as photosynthetic activity and chlorophyll content (Hajer et al., 2006; Saleh, 2012).

Osmotic stress is caused due to the excess of Na^+ and Cl^- in the environment that decrease the osmotic potential of the soil solution and hence water uptake by plant root (Rasool et al., 2013). According to Mahajan and Tuteja (2005), increased salinity of agricultural and is expected to have destructive global effects. The salt tolerance of plants varies with the type of salt and osmotic potential of the medium (Kayani and Rahman, 1988). It is reported that plants growing under saline conditions are affected in three ways: reduced water potential in root zone causing water deficit, phytotoxicity of ions such as Na^+ and Cl^- and nutrient imbalance depressing uptake and transport of nutrients (Munns, 2002). Maize (*Zea mays* L.) is the third most important cereal in the world after wheat and rice, and grows under a wide range of climatic conditions (Khodarahmpour, 2011). It is used as food for human consumption as well as food grain for animals (Moussa, 2001). It is moderately sensitive to salinity and considered as the most salt-sensitive of the cereals (Ashraf and McNeally, 1990).

Among the stages of the plant life cycle, seed germination and seedling emergence and establishment are key processes in the survival and growth of plants (Hadas, 2004). It is well established that salt stress has negative correlation with seed germination and vigor (Rehman et al., 1996). Maize, which belongs to the plants with C4 metabolism, is also classified as moderately sensitive to salinity (Mass and Hoffmann, 1977; Ouda et al., 2008). Seeds contribute as a vital component of world's diet. The embryos present in the seed act as a miniature plant and mode of dispersal and provide food reserve to the growing seedlings. Seed germination and early seedling growth are critical events for plant development (Kitajima and Fenner, 2000).

Germination becomes visible by emergence of the structures surrounding the embryo by the radicle. The shoot and root length are the most important parameters for salt stress because roots are in direct contact with soil and absorb water from soil and shoot supply it to the rest of the plant. For this reason, root and shoot length provides an important clue to the response of plants to salt stress (Jamil and Rha, 2004). High salinity may inhibit root and shoot elongation due to the lower water uptake by the plant (Werner and Finkelstein, 1995). Demir and Arif (2003) state that the root growth was more adversely affected as compared to shoot growth by salinity. According to McKensie and Leshen (1994), the effect of salinity on plant water relations, nutritional imbalance and ion toxicity are responsible for the inhibition of growth and as a consequence decrease in plant yield. Rehman et al (1996) state that

intolerance to salinity may result in physiological and biochemical disorders which prevent or delay germination or cause abnormal seedlings.

Generally the growth of plant is reduced by salinity but may vary from species to species in their tolerance (Munns and Termaat, 1986). Therefore, the objective of this study is to examine the effect of salt stress on the germination, water content and seedling growth of *Zea mays* L.



Photo 1. *Zea mays* L.

2. MATERIALS AND METHODS

The seeds of maize (*Zea mays* L. Va. NK6240) were procured from Rasi seed company Attur. The seeds were selected randomly on the basis of uniformity of size. The selected seeds were surface sterilized by dipping in 5 % sodium hypochlorite solution for five minutes to prevent fungal infection and they were rinsed for about five minutes in running tap water and air-dried at an ambient temperature of 32 °C in the laboratory. Ten seeds were put in each Petri-dish which was lined with blotting paper.

The blotting paper in each of the Petri-dishes allocated to the control was moistened with ten millilitres of distilled water while that of the Petri-dishes allocated to the other treatments were moistened with ten millilitres of the appropriate concentrations of the salt solution. The Petri-dishes were incubated at room temperature for two weeks. Emergence of one millimeter of the radicle was used as the criterion for germination. Water uptake per cent was recorded for 12 hours.

2. 1. Water uptake percentage

Water uptake per cent was calculated by the formula according to Mujeeb-ur-Rahman et al., (2008).

$$\frac{WU = W_2 - W_1}{W_1} \times 100$$

where,

W_1 = Initial weight of seed

W_2 = Weight of seed after absorbing water in a particular time.

2. 2. Germination percentage

Germination percentage (GP) was calculated according to the International Seed Testing Association (ISTA) method

$$GP = \frac{\text{Number of normally germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

2. 3. Tissue water content

The tissue water content (TWC) was calculated according to the formula of Black and Pritchard (2002)

$$TWC = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Fresh weight}} \times 100$$

2. 4. Vigour Index

Seedling vigour index (SVI) was calculated following modified formula of Abdul-Baki and Anderson (1973).

$$SVI = \text{Germination percentage} \times \text{Seedling length}$$

2. 5. Plumule length

Plant height was recorded by measuring the height of the seedling from the surface of the soil to the tip of the top most leaf. This was recorded on 14th day after treatment and expressed in cm plant⁻¹.

2. 6. Radicle length

The radicle length was measured from the point of first cotyledonary node to the tip of longest root and expressed in cm plant⁻¹.

2. 7. Measurement of biomass accumulation

To record the fresh weight, plumule, radicle portions were separated and weighed. They were dried in a hot air oven at 80 °C for 24 hours. Then, the dry weight was taken by using an electronic balance.

2. 8. Data analysis

The experiment was set up as a completely randomized design. Statistically significance was assessed at the $P < 0.05$ level using one way ANOVA and means were separated by Duncan's multiple range test ($P < 0.05$) With the help of SPSS 16.0 software package. Means and standard deviation were calculation from three replications.

3. RESULTS AND DISCUSSION

Salinity had highly significant effect on growth attributes of maize in Petri-dishes under different levels of NaCl stress. The results are given below.

The percentage germination of the control was 89 % while that the 25 mM, 50 mM, 75 mM, 100 mM, 125 mM 150 mM, 175 mM and 200 mM NaCl regimes 74 %, 66 %, 58 %, 54 %, 46 %, 33 %, 24 % and 13 % respectively. The salt concentration increased as the germination percentage decreased. There was reduction in % germination with increasing salt stress.

Studying the length of the maize seedlings obtained from the germination of the maize seeds under laboratory conditions, after 14th days of germination, we observed that the salt treatments significantly reduces growth in length, and the dry weight content of the maize plantlets was very significantly reduced.

Root lengths decreased with increasing NaCl concentrations. Maximum reduction in root length was noted at 200 mM NaCl. The shoot length decreased with increasing levels of salt stress compared with non-hardened seeds. High level of salinity has more drastic in reducing shoot length. With increase in salinity levels, both fresh weight and dry matter of seedlings declined.

The effect of the saline conditions was more on the number of days to accomplish germination and percentage seedling emergence than percentage germination. The gradual decrease in % seed germination with increasing concentration of NaCl is evident from the present results where both percentage germination and the rate of germination declined with salinity. Several chemicals and salts are used to accomplish better germination and accomplish healthy seedlings under stress (Ashraf et al., 2008). Our results are in line with the findings of Rahman et al., (2000), in which germination was directly related to the amount of water absorbed and delay in germination due to the salt concentration of the medium. Decrease and delay in germination in saline medium was also reported by Rahman et al. (2000) and Mirza and Mahmood (1986). The present results agree with the reports of Francois et al. (1984) and Francois (1985) in sorghum and squash, respectively. These workers observed decrease in percentage germination and seedling emergence with enhanced salinity. High salt stress is known to disturb osmotic and ionic homeostasis that limit the availability of water to the seeds and ultimately cause slow and poor germination. Abiotic stresses are reported to alter levels of plant growth hormones leading to decrease in plant growth (Gupta et al., 1993). Seeds in the control dishes (0 mM NaCl) had the highest germination percentage (100 %), and as the salt concentration increased, germination percentage decreased up to 200 mM NaCl concentration. The higher germination percentage

of *Zea mays* in the control (0 mM NaCl) was due to lack of salt in the medium. High concentration of NaCl in the salt solution increases its osmotic potential. In addition, high absorption of Na⁺ and Cl⁻ ions during seed germination can be due to cell toxicity that finally inhibits or slows the rate of germination and thus decreases germination percentage.

Salinity affects the seedling growth of plants (Tezara et al., 2003) by slow or less mobilization of reserve foods, suspending the cell division, enlargement (Meiri and Poljakoff-Mayber, 1970) and injuring hypocotyls. Other researchers (Francois, 1994), demonstrated that plants exhibit different sensitivities to salinity at different stages of growth. The root and shoot growth reduces abruptly in salt sensitive plants (Parvaiz and Satyawati, 2008). Giaveno et al. (2007) reported that salt treatments affected root and shoot fresh weight. Application of salinity levels decreased stem length of maize. Results of Savvas et al., (2007) showed that the increase of the NaCl concentration in the root zone restricted stem length of roses in soilless culture in greenhouse. Growth processes are particularly sensitive to salinity; biomass yield and growth rate are considered reliable criteria for evaluating the degree of salt sensitivity (Larcher, 1995).

Table 1. Effect of NaCl on germination percentage, water uptake percentage, tissue water content and growth of *Zea mays*.

NaCl (mM)	Germination %	Water uptake %	Tissue Water Content	Plumule length	Radical length
Control	89.9±3.35	7.66±0.029	8.42±0.637	9.76±0.305	10.3±0.208
25	74.4±5.09	7.05±0.214	8.72±0.232	9.06±0.208	9.46±0.208
50	66.6±3.35	5.63±0.598	9.09±0.212	8.10±0.264	8.26±0.251
75	58.8±1.96	4.23±0.385	9.10±0.192	6.33±0.351	7.36±0.305
100	54.4±1.90	3.13±0.303	8.57±0.232	5.70±0.200	6.36±0.208
125	46.6±3.35	1.79±0.592	8.61±0.232	5.00±0.200	5.43±0.251
150	33.3±3.30	-0.51±0.224	8.84±0.034	4.26±0.208	4.40±0.264
175	24.4±5.09	-2.42±0.218	8.69±0.231	3.03±0.152	3.26±0.208
200	13.3±3.30	-3.54±0.756	9.23±0.035	1.33±0.152	2.66±0.152

± Denote Standard deviation

These phenomena were closely related with the extensibility of the cell wall, affecting cell growth and cell division process (Giaveno et al., 2007).

The radicle and plumule length were reduced by the application of the salt solution. This was consistent with the finding of Munns and Termaat (1986) who state that the growth of a plant is generally reduced by salinity. The result showed that the NaCl salt solutions reduced the water content of the test crop. Water content of the seedlings decreased with increasing salt concentration. Osmotic effects of salt on plants are the result of lowering of the soil water potential due to increase in solute concentration in the root zone. In the present investigation there is regular decline in the root and shoot length, with increase in salinity

levels. The variability in the plant root and shoot length was measured after 14 days of growth subjected to 25, 50, 75, 100, 125, 150, 175 and 200 mM NaCl stressed maize seedlings. In our studies, 200 mM NaCl induced severe stress in plant growth. The inhibition in root and in particular shoot growth with NaCl treatments for sugar beet, rice and cotton seedlings while a decrease in length of shoots at high salt concentration (20mM) was observed by Osorio et al. (1998). Some workers have reported that leaf elongation was immediately inhibited in maize. The accumulation of biomass in the control test crop was significantly higher than that of the seedlings in the NaCl regimes. This was consistent with the finding of Gururaja-Rao et al. (2005) who state that salinity decreased biomass production. Salinity affects both water absorption and biochemical processes resulting in reduction of plant growth (Parida and Das, 2005) and a decline in the rate of photosynthesis significantly by negatively affecting CO₂ assimilation and leads to decrease largely nutrient uptake and finally growth of plants is getting reduced (Lauchli, 1984; Seeman and Sharkey, 1986; Cha-Um and Kirdmanee, 2009).

Table 2. Biomass accumulation, vigour index and tolerance index in *Zea mays* as affected by the different concentrations of NaCl.

NaCl (mM)	Plumule Fresh Weight	Radical Fresh Weight	Plumule Dry Weight	Radical Dry Weight	Vigor Index
Control	2.84±0.015	1.64±0.020	0.36±0.360	0.16±0.015	8.78±25.19
25	2.73±0.030	1.48±0.015	0.29±0.020	0.11±0.010	6.74±32.99
50	2.63±0.030	1.23±0.036	0.25±0.005	0.05±0.010	5.39±34.46
75	2.56±0.321	1.10±0.020	0.21±0.015	0.01±0.005	3.72±25.31
100	2.50±0.005	1.05±0.030	0.16±0.015	-1.16±0.036	3.10±21.15
125	2.48±0.010	0.91±0.036	0.12±0.020	-2.30±0.085	2.33±22.47
150	2.40±0.015	0.59±0.020	0.08±0.030	-2.76±0.305	1.42±16.71
175	2.14±0.140	0.45±0.040	0.05±0.020	-2.95±0.500	-2.54±29.5
200	1.55±0.097	0.29±0.025	0.01±0.005	-3.06±0.040	-17.5±3.55

± Denote Standard deviation

4. CONCLUSION

In conclusion, the study showed that salt stress decrease seed germination, biomass and growth of maize seedlings due to ionic toxicity, decrease osmotic potential and oxidative stress. Considering the retardatory effects of NaCl on the germination and growth of *Zea mays*, important features like increased germination capacity have to be explored in programs to select and /or develop tolerant cultivars that are able to remain productive at saline soil to make possible the utilization of waste saline water. Future research should be directed towards elucidating the physiological and biochemical studies during salinity stress.

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