Effect of NaCl stress on seed germination and seedling growth in Wheat

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***Abstract*—Effects of NaCl stress on seed germination and seedling growth in wheat were studied in this paper. The results showed that the germination rate of the seeds treated with NaCl concentration at <100 mM, germination rate of the seeds was not significantly comparing with control, however the germination of those seeds treated with NaCl at higher concentration (≥200 mM) was inhibited and at 400 mM NaCl concentration no emergence of germination was noticed. The root length, shoot length and fresh weight of seedlings also decreased with an increase in NaCl concentration and treatment time. Activity of POD tended towards descenting after ascenting. MDA contents tended to go up steadily within lower concentration of NaCl, then sharply within higher concentration of NaCl.**

Keywords- NaCl stress; wheat; seed germination; seedling growth; peroxidase(POD); malondialdehyde (MDA)

I. INTRODUCTION

Salinity is one of the most important problems in the agriculture areas of the world[1]. In fact this is a worldwide problem, particularly in arid and semi arid areas[2]. Plants respond to salinity stress through morphological, physiological and metabolic modifications occurring in all plant organs[3,4]. NaCl is the most important constituent of a saline environment. NaCl salinity is known to decrease seed germination, shoot and root length, hydrolytic enzyme activity during germination and also affect other metabolic processes[5,6]. Considering the aforesaid, in the present work effect of salinity (NaCl) stress was studied on seed germination and growth in wheat during early seedling stages.

II. MATERIALS AND METHODS

A. The seeds, NaCl concentrations used

In this study, wheat (xinmai 208 cultivar) seeds were used. Seeds of wheat were surface sterilized in 0.1% $HgCl_2$ for 5 minutes and thoroughly washed in distilled water. Salt(NaCl) concentrations used in the experiment were 0, 25, 50, 100, 200, 400 mM.

B. Seed germination

To evaluate the inhibition of germination by NaCl stress, seeds of wheat were germinated in 9cm-diam Petri dishes covered with two flat Whatman filter papers containing 10 ml distilled water(control) or 25, 50, 100, 200, 400 mM NaCl solution and kept in dark at 25℃.From every application, 10 seeds were arranged "3-4-3" in Petri dishes. It was assumed that the radicle should be 2 mm long for germination to take place. Seeds were geminated upto 5 days in dark. Every 1 day, germination percentage was calculated from every application.

C. Growth conditions of seedlings from seeds and morphological observations

To evaluate how NaCl stress inhibits seedling growth, the seedlings coming out of seeds within 5 days in dark were transfered into a controlled growth environmental conditions (16h photoperiod, temperature of 28/18℃ day/night, relative humidity of 60-70%) upto the 8th day. The root and shoot length of the seedlings in mm and their fresh weights in g were measured. At the same time, inhibition was calculated by the formula as follows. All experiments were repeated three times. Statistical evaluation was realized by using SPSS program according to Duncan's multiple range test.

Inhibition(%): [(control－treatment)/control]×100

D. Extraction of enzyme and determination of enzyme activities

For enzyme analysis, the fully expanded leaves (the 8th day) were sampled and frozen in liquid nitrogen immediately and then stored at -40 °C until analysis. Samples for Peroxidase(POD), lipid peroxidation (MDA content) were prepared by homogenizing 0.5 g of frozen leaf material in 3 ml of cold 50 mM Na phosphate buffer (pH 7.8) containing 1 mM EDTA and 2 % (m/v) PVPP. The homogenate was centrifuged at 13 000 g for 40 min at 0 °C. All spectrophotometric analyses were conducted on a UV-1600 spectrophotometer.

Peroxidase activity was measured by recording changes in absorbance at 470 nm (ΔA 470) using guaiacol as hydrogen donor in presence of H_2O_2[7]. The assay mixture consisted of 12 mM K-phosphate buffer (pH 6.4), 4 mM guaiacol, enzyme and 1 mM H_2O_2. The activity is expressed as ΔA470 g-1of fresh weight*min -1.

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content by the thiobarbituric acid reaction[8]. The concentration of MDA was calculated from the absorbance at 532 nm .

The experiment was arranged in a randomized block design with three replications. Statistical evaluation was realized by using SPSS program according to Duncan's multiple range test.

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III. RESULTS

A. Inhibition of germination by NaCl

Seed germination percentage decreased with increase in NaCl concentrations(Figure 1). The germination rate of the seeds treated With NaCl concentration at <100 mM, germination rate of the seeds was not significantly comparing with control, however the germination of those seeds treated with NaCl at higher concentration (≥200 mM) was inhibited and at 400 mM NaCl concentration no emergence of germination was noticed.

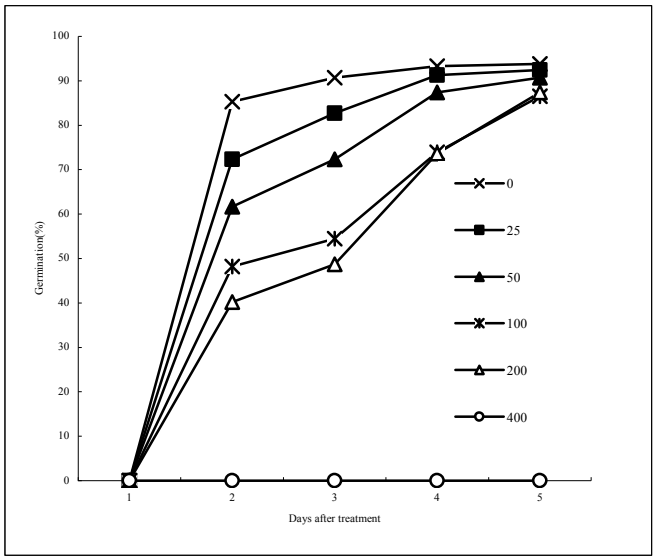

Figure 1. Effect of different NaCl concentrations on cumulative germination of wheat seeds. Data represent means±SE of three replicates.

B. Growth inhibition by NaCl

Growth ratios were calculated for randomly selected NaCl treated wheat seedlings. Root length, shoot length and fresh weight were determined by measuring their increments from beginning to end of the NaCl treatment(Table 1). The root length, shoot length and fresh weight of seedlings decreased and inhibition rate increased with an increase in NaCl concentration and treatment time(Figure 2).

Table 1. Effect of different NaCl concentrations on root length, shoot length and fresh weight in germinating wheat seeds measured 8 days after treatment. Data represent means±SE of three replicates.

Treatment (mM)	Root length(mm)	Shoot length(mm)	Fresh weight(g)
Control	95.3±7.23	69.8±6.67	1.5476±0.1345
NaCl 25	87.4±5.25	57.6±5.68	1.3627±0.1568
50	32.1±3.12	46.8±5.34	1.1215±0.1438
100	13.6±1.34	15.4±3.13	0.9470±0.0956
200	7.8±1.12	6.5±2.17	0.8023±0.1013
400	-	-	-

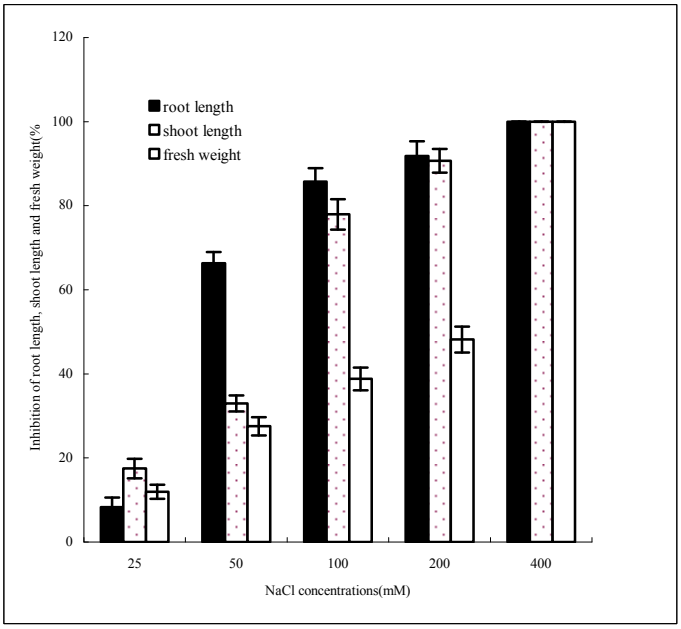

Figure 2. Effect of different NaCl concentrations on the inhibition of root length, shoot length and fresh weight in germinating wheat seeds. Data represent means±SE of three replicates.

C. Peroxidase activity and malondialdehyde content

POD activity gradually increased firstly and then decreased in leaves of wheat seedlings subjected to NaCl concentrations throughout the experimental period (Figure 3). Under 25 mM and 50 mM NaCl stress, these increases were 166.2 %, 91.2 % over the controls in leaves of wheat respectively. When NaCl concentration is over 100 mM, POD activity decreased to the level less than controls.

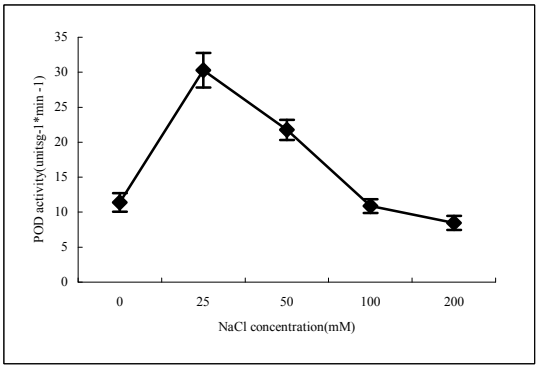

Figure 3. Peroxidase(POD) activity in leaves of wheat in response to different NaCl concentrations. Data represent means±SE of three replicates.

Lipid peroxidation (MDA content) in leaves of wheat showed a significant concentration-dependent increase under control conditions (Figure 4). MDA contents tended to go up steadily within lower concentration of NaCl, then sharply within higher concentration of NaCl.

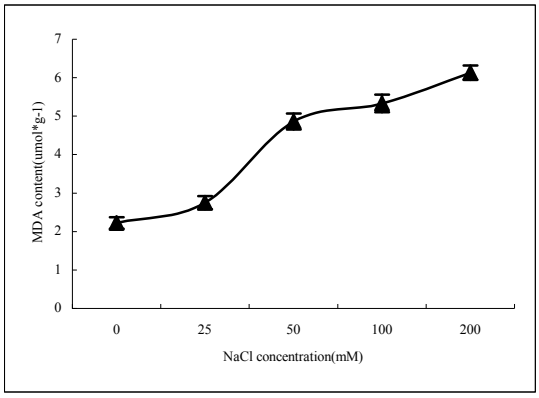

Figure 4. Lipid peroxidation (MDA content) in leaves of wheat in response to different NaCl concentrations. Data represent means±SE of three replicates.

IV. DISCUSSION

There is little difference in germination rate, root length, shoot length and fresh weight between lower sodium content and controls, however high concentration is showing strong inhibition. Similar results have been reported by Dash and Panda[5], Young Geol Sohn et al[9], Kursat Cavusoglu et al[10] and X. Zhao et al[11]. In certain condition, seed germination rate unchanged or speed because of possible genetic resistance, or specific physiological mechanism[12]. Higher sodium content disrupts the nutrient balance and osmotic regulation, thereby causing specific ion toxicity[13].

The results also showed that activity of POD tended towards descenting after ascenting and MDA contents tended to go up steadily within lower concentration of NaCl, then sharply within higher concentration of NaCl. It showed that low concentration NaCl stimulated the enzyme activities to protect plants from harming, however enzyme activities reduced drastically with the higher NaCl content. Although the enzyme activities strengthened, the ability was limited. MDA contents in wheat(xinmai 208) were always higher than controls under salt treatments. A higher level of lipid peroxidation, hence a higher degree of membrance damage might be resulted from the lower POD activities in wheat. In conclusion, salinity tolerance of xinmai 208 is weak, in other words, xinmai 208 was sensitive to NaCl stress.

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REFERENCES

[1] Ergene A , Toprak Bilgisi. Atatu¨rk U¨ niversitesi Ziraat Faku¨ltesi Yayınları No:267, Ders Kitapları Serisi No: 42, Erzurum,1982.

[2] SNEHAL JOSHI* ,J. D. NIMBALKAR, “Effect of salt stress on growth and yield in *Cajanus ca* jan L”, Plant and Soil,1983,74, pp.291-294 .

[3] Shennan C, Hunt R, Macrobbie EAC, “The effect of salinity on growth”,Plant Cell Environ, ,1987,10,pp.59–65

[4] Yeo AR, Lee KS, Izard P, Boursier PJ, Flowers TJ , “Short-term and long-term effects of salinity on leaf growth in rice (Oryza sativa L.)”, J Exp Bot, 1991,42,pp.881–889.

[5] Dash M, Panda SK, “Salt stress induced changes in growth and enzyme activities in germinating Phaseolus mungo seeds”, Biol Plant,2001,44,pp.587–589.

[6] Ghoulam C, Fores K, “Effect of salinity on seed germination and early seedling growth of sugar beet (Beta vulgaris L.)”, Seed Sci Technol ,2001, 29,pp.357–364.

[7] Reuveni R.M., Shimoni M., Karchi Z., Kue J..Peroxidase activity as a biochemical marker for resistance of musk melon (Cucumis melo) to Pseudoperonospora cubensis. Phytopathology, 1992,82,pp 749-753.

[8] Madhavara Rao, K.V., Sresty, T.V.S. Antioxidative parameters in the seedlings of pigeonpea (Cajanus cajan (L). Millspaugh) in response to Zn and Ni stresses. Plant Sci, 2000,157, pp113-128.

[9] Young Geol Sohn , Byung Hyun Lee , Kyu Young Kang , and leung Joo Lee, “Effects of NaCI Stress on Germination, Antioxidant Responses, and Proline Content in Two Rice Cultivars”, *Journal of Plant Biology,* 2005,48(2),pp.201-208.

[10] Kursat Cavusog˘lu, Semra Kılıc¸, Kudret Kabar, “Some morphological and anatomical observations during alleviation of salinity (NaCI) stress on seed germination and seedling growth of barley by polyamines”,Acta Physiol Plant ,2007, 29,pp.551–557.

[11] X. Zhao , H. J. Tan , Y. B. Liu, X. R. Li,G. X. Chen, “Effect of salt stress on growth and osmotic regulation in *Thellungiella* and *Arabidopsis callus”*, Plant Cell Tiss Organ Cult, 2009, 98,pp.97–103.

[12] Foolad M R, Lin G Y. Relationships cold-and salt-tolerance during seed germination in tomato: Germplasm evaluation[J]. Plant Breeding, 1999, 118,pp45-48.

[13] Alan SM. Nutrient uptake by plants under stress conditions.In M Pessarakli, ed, Handbook of Plant andCrop Stress. Ed 2, Marcel Dekker, New York, 1999, pp 285-313.