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Variations in Proline, Chlorophyll and Mineral Elements Contents of Wheat Plants Grown under Salinity Stress

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Abstract: Variations in proline, chlorophyll, Na, Cl, N, P, K, Fe, Zn, Cu and Mn concentrations and growth of wheat plants (*Triticum aestivum* L. cv: Cakmak-79) grown under salinity stress were investigated in greenhouse conditions. For this purpose, 0, 25 and 50 mM NaCl were applied to the experimental soil taken from Aridisol lands. The growth of wheat plants was inhibited by salinity. The increased amount of NaCl applied to soil resulted in lower dry weight. Application of NaCl caused an increase on proline, Na, Cl, P, Zn and Mn concentrations, whereas decreases in chlorophyll, N, K, Fe and Cu concentrations were detected.

Key words: Wheat, growth, salt, mineral elements, proline, chlorophyll

INTRODUCTION

Salinity is a serious problem in arid and semi-arid zones (Maas and Grattan, 1999). In these areas, salt tends to accumulate in the upper soil profile and layer soil surface, where precipitation leads to an insufficient leaching (Rhoades *et al.*, 1992). Plantation of salt-tolerant species, distinctly N₂-fixing species, is the advantageous practice in rehabilitating regions with salinity problems (Oba *et al.*, 2001). There are a great number of plant species which are regarded as salt-tolerant, the most competitive being those that are able to become established, grow to maturity and survive until they are able to reproduce (Khan *et al.*, 2000). Salinity and its effects on biomass production have been considered by numerous authors (Khan *et al.*, 2000; Mehari *et al.*, 2005).

Adaptation of plants to salinity is associated with osmoregulation adjustment. Osmotic regulators in plants include potassium, soluble sugar, proline and betaine. These small molecules are important physiological indicators for evaluating osmotic adjustment ability (Zhu, 2000; Conceicao *et al.*, 2002; Yordanov *et al.*, 2003; Hong-Bo *et al.*, 2006) and drought resistance in wheat species and genotypes.

Salt tolerance in plants is a complex phenomenon involving morphological, physiological and biochemical processes. The accumulation of proline is essential for plants under osmotic stress (Nanjo *et al.*, 1999) and salt stress up-regulates the key enzyme, P5CS, for proline biosynthesis in *Arabidopsis* (Hare *et al.*, 1999). Proline accumulation is increased in the plant tissue of crops due

to salinity stress (Irrigoyen *et al.*, 1992; Kundu and Paul, 1997). Soluble sugars are believed to accumulate in plant tissues and cells due to drought stress. This may act as an osmotic adjustment or osmotic-protectant factor (Irrigoyen *et al.*, 1992; Bohnert *et al.*, 1995; Sanchez *et al.*, 1998).

Agricultural soils have many kinds of salt ions; however, NaCl is usually the damaging and predominant salt. Increased sodium chloride-salinity causes decreases on vegetative growth and the rate of photosynthesis (Cusido *et al.*, 1987). In saline soils, water availability and nutrient uptake by plant roots is limited because of high osmotic potential and toxicity of Na and Cl ions (Al-Karaki, 1996). Thus excessive uptake of Na and Cl may lead to ionic disturbance of whole plants. Although most salt-tolerant species control the accumulation of inorganic ions as the basic mechanism to adjust their internal tissue osmotic potential against external salinity, they differ widely in the extent to which they accumulate inorganic ions (Munns, 1993; Glenn *et al.*, 1996).

This research was carried out to determine the effect of salinity on the growth, proline, chlorophyll and mineral elements composition of wheat plants grown in greenhouse conditions.

MATERIALS AND METHODS

The experimental soil taken from Aridisol is calcareous (212 g kg⁻¹ CaCO₃), clay loam in texture, slightly alkaline (pH 8.04, E.C. 0.108 dS cm⁻¹, both in water extract). The soil sample has 42.9 mg kg⁻¹ extractable Na and 6.37 mg kg⁻¹ water extractable Cl.

In the greenhouse, for the pot experiment conducted at under natural light conditions, the soil (2000 g) put into the pots was salinised at the rates of 0, 25 and 50 mM NaCl.

Before sowing the seeds to the pots, as being basal fertilizers, 100 mg N kg⁻¹ as ammonium nitrate, 60 mg P kg⁻¹ as triple super phosphate and 75 mg K kg⁻¹ as potassium sulphate were applied.

Fifteen bread wheat (*Triticum aestivum* L. cv: Cakmak-79) seeds were sown into each pot. After emergence, wheat plants were thinned to ten. Plants were harvested eight weeks after sowing. After weighting, the fresh plants were washed, for chlorophyll and proline determinations 2.0 g samples were taken and the remaining plant samples were dried at 65°C in order for determination of dry matter values with Na, Cl, P, K, Fe, Zn, Cu and Mn concentrations. Total nitrogen was determined by Kjeldahl digestion method according to Bremner (1965). Chloride was analyzed in aqueous extracts by potentiometric titration with AgNO₃ (Lambert and DuBois, 1971). Sodium and potassium were determined by flame photometry (Eppendorf Flex 6361 model). Iron, Zn, Cu and Mn were determined by AAS (Philips model 9200x).

Proline was extracted from 0.5 g of fresh leaf tissue into 10 mL of 3% sulfosalicylic acid and filtered through Whatman No: 2 filter papers. Proline was determined by the ninhydrine method (Bates *et al.*, 1973) in Shimadzu UV-1201 model spectrophotometer, using pure proline as a standard.

Chlorophyll was estimated according to the method described by Withan *et al.* (1971) using spectrophotometer (Shimadzu UV-1201 model) at 652 nm.

The experimental design was a completely randomized design with three replicates, including three different salt concentrations. The data obtained from the different measurements were evaluated by Minitab Package Program (Minitab Release 10.51) and treatment

means were evaluated using the MSTAT Package Program (Version 3.00) for Duncan's Multiple Range Test.

RESULTS

Dry weights of wheat plants: Plant growth was inhibited by soil salinity and dry weights were decreased significantly ($p < 0.01$) on contrary to increased amount of NaCl applied (Table 1). The highest reduction (-27.87%) at both growth and dry weight values were observed at 50 mM NaCl treatment. Growth and dry weight of wheat plants at 25 mM NaCl was not significantly different from non-salinised control plants (Table 1).

Mineral elements concentration of wheat plants: NaCl salinity affected ion concentrations of wheat plants (Table 2 and 3). Except Na, P, Zn, Cl and Mn, other mineral elements concentrations decreased with increasing NaCl salinity. Na and Cl accumulation is increased with increased salinity. The more Na accumulation in the plant, the lower K/Na ratio was observed (Table 2).

Total Chlorophyll and Proline Contents of Wheat Plants: Total chlorophyll and proline contents of wheat plants were affected by salinity ($p < 0.001$) (Table 4). NaCl treatments caused a decrease in total chlorophyll contents as 21.61% at 25 mM NaCl and 45.23% at 50 mM NaCl (Table 4). Proline contents of plants ascended with increased salinity (Table 4). The increases in proline content of plants were 48.00% at 25 mM NaCl and 131.90% at 50 mM NaCl levels.

Table 1: Dry weight (g pot⁻¹) of wheat subject to different NaCl treatments

NaCl (mM)	Dry weight (g pot ⁻¹)	Change (%)
0	2.87±0.45a	-
25	2.54±0.36a	-11.50
50	2.07±0.23b	-27.87
Treatments	**	

** Significant at $p < 0.01$ level; Means followed by the same letter(s) are not significantly different (Duncan's Multiple Range test at $p < 0.01$)

Table 2: Effects of NaCl on Na, Cl, K, N concentrations and K/Na ratio of wheat

NaCl (mM)	Na (g kg ⁻¹)	Cl (g kg ⁻¹)	K (g kg ⁻¹)	N (g kg ⁻¹)	K/Na ratio
0	1.9±0.2a	9.8±0.6a	49.8±1.4a	38.1±3.4a	26.21±0.25a
25	7.5±0.1b	42.0±1.2b	39.0±2.1b	36.7±4.1a	5.20±0.13b
50	11.2±0.8c	49.6±2.3b	29.8±1.7c	34.0±3.8b	2.66±0.06c
Treat.	***	**	***	**	***

Significant at $p < 0.01$ level; *Significant at $p < 0.001$ level; Means followed by the same letter(s) are not significantly different (Duncan's Multiple Range test at $p < 0.01$)

Table 3: Effects of NaCl on P, Fe, Zn, Cu and Mn concentrations of wheat

NaCl (mM)	P (g kg ⁻¹)	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Mn (mg kg ⁻¹)
0	3.2±0.3a	144.5±2.34a	18.07±0.16a	24.37±1.25a	147.3±2.84a
25	3.3±0.2a	120.9±1.67b	19.53±1.23a	19.00±1.96b	181.8±3.45b
50	3.8±0.5b	110.0±1.98b	23.73±0.97b	16.43±1.87c	194.9±4.23c
Treat.	**	**	**	***	***

Significant at $p < 0.01$ level; *Significant at $p < 0.001$ level; Means followed by the same letter(s) are not significantly different (Duncan's Multiple Range test at $p < 0.01$)

Table 4: Effects of NaCl on total chlorophyll and proline contents of wheat

NaCl (mM)	Chlorophyll (mg g ⁻¹ fresh weight)	Change (%)	Proline (μ mol g ⁻¹ fresh weight)	Change (%)
0	3.98±0.25a	-	0.652±0.02a	-
25	3.12±0.67b	-21.61	0.965±0.04b	48
50	2.18±0.34c	-45.23	1.512±0.08c	131.9
Treat.	***		***	

***Significant at p<0.001 level; Means followed by the same letter(s) are not significantly different (Duncan's Multiple Range test at p<0.01)

DISCUSSION

NaCl-salinity is described to cause an inhibition on the growth of wheat plants by affecting water absorption and biochemical processes (Cusido *et al.*, 1987) and increasing energy losses for salt exclusion mechanisms, usually decreasing nutrient uptake that leading to reduced plant growth (Lauchli, 1984). On the other hand, salinity is known to decrease soil structural index, hydraulic conductivity to water in to soil and reduced root zone aeration (De Pascale and Barbieri, 2000).

It was observed in the study, particularly, application of 50 mM NaCl might result in ceased plant growth.

It is known that competition effects between different anions and cations are occurred in saline environment (Bar *et al.*, 1997) and the balance between mineral elements break down to benefit of Na and Cl. These results are in agreement with Chavan and Karadge (1986), Cusido *et al.* (1987) and Taban *et al.* (1999). On account of the antagonism between Cl and N (De Witt *et al.*, 1963), high concentration of Cl was negatively correlated with nitrogen concentration. Moreover, NaCl salinity decreased nitrogen concentration in the plants (Cordovilla *et al.*, 1995) and has a negative interference on the nitrogen acquisition and utilization (Lewis, 1986). As a result, Cl or NaCl may depress the nitrogen uptake by plants. In most cases, salinity decreases the concentration of P in plant leaves (Award *et al.*, 1990; Sharpley *et al.*, 1992). Nevertheless, P concentration of wheat plants was found to increase with high salinity. This may be the result of enhanced availability of P in the soil or synergistic effect of Na, which is involved in P uptake and/or transport to the shoot (Grattan and Maas, 1988). Plant K concentration was decreased as mentioned by Siegel *et al.* (1980) due to antagonism between Na and K (Glenn *et al.*, 1996).

Reports on the effects of NaCl on micronutrients concentration in plants are often contrasting. In fact, unlike the results of Maas *et al.* (1972) and Bhivare and Nimbalkar (1984), it was found that NaCl decreased Fe concentration in plants. Similar results for increased Fe concentration were reported by Shrivastava *et al.* (1993) and Taban *et al.* (1999). On the contrary of the result of Shukla and Mukhi (1985) and Mehrota *et al.* (1986), Zn

concentrations of plant were increased by NaCl application. On the other hand, with increased salinity levels Mn concentrations increased (Khattak and Jarrell, 1989) and Cu concentrations (Rahman *et al.*, 1993).

Salinity decreased the chlorophyll content of wheat leaves. It is attributed to a salt-induced weakening of protein-pigment-lipid complex (Strogonov *et al.*, 1970) or increased chlorophyllase (EC: 3.1.1.14) enzyme activity (Stivsev *et al.*, 1973). Proline contents of wheat plants increased under salt-stress conditions. Proline is defined as an indicator of tolerating or adapting plant to saline conditions (Chowdhury *et al.*, 1993; Madan *et al.*, 1994). Accumulated free proline was correlated with tissue Na ion concentration for many plant species, suggesting playing a possible role for osmoregulation mechanism under salt-stress (Lewitt, 1980).

Consequently, soil salinity significantly inhibited the growth and caused a decrease in dry matter of wheat plants. While NaCl lead to increase proline content, decreased chlorophyll content of wheat plants.

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