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Plant Tissues Analysis of Two Tobacco Varieties Grown at Six Different Levels of NaCl Salinity

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Abstract: Plant tissue, roots and shoots of two tobacco varieties (Nc 2326 and Coker 254) grown in artificial saline soil has been analyzed. The results showed that sodium and chloride contents were greater in the shoots than in the roots in both these varieties at both the harvests. These contents increased as the levels of salinity increased. Sodium and chloride contents of both shoots and roots of variety NC 2326 were higher than that of Coker 254 at the highest levels of salinity. Both of the varieties accumulate low Na and Cl contents in the root and shoot tissues and are salt sensitive but Coker 254 is more salt sensitive than NC 2326.

Key words: Tobacco, varieties, salinity, levels, tissue, analysis

Introduction

Recent estimates put the area of salt affected soils at some 9.5 million square kilometers on a world scale (Szabolcs, 1989). The plants growing in saline ecosystem accumulate high levels of sodium chloride and glycinebetaine (Storey *et al*., 1977). Salt-affected soils have low biological activity both because of osmotic and ionic effects and owing to the limitation of carbonaceous substrates (Rao and Pathak, 1996). High sodium chloride concentrations in the growth medium decreased the fresh and dry weight of shoot and root of Spartina x townsendii and sodium and chloride contents rose in the root and shoot tissues with sodium chloride (Storey and Wyn Jones, 1978). Salt affected soils have excessive amounts of soluble salts as chlorides and sulphates of sodium, calcium, potassium and magnesium. Salinity tolerance varies among plant species and varieties (Allen *et al*., 1985; Ashraf and McNeilly, 1992). Salt tolerance is a complex character and is highly influenced by environmental characters such as soil, temperature, light and type of salinity. Soil salinity is one of the most important problem in the arid and semi arid zones of Pakistan where canal irrigation is practiced (Akbar *et al*., 1972). With the increase in population, effective utilization of these soils has become necessary after reclamation by growing salt tolerant species and varieties (Kumar, 1996; Qadir *et al*., 1996a and 1996b). Chloride salinity has more adverse effects than sulphate salinity (Lauter and Munns, 1986; Sharma *et al*., 1990). Fitter and Hay (1981) advocated that three ions commonly causing toxicity were Cl^- and SO_4^{-2} with Na^+ association. It is true that most of the cultivated crops have been bred for growing on normal soils. However, marked differences with regard to salt tolerance do exist among genera, species and varieties of various field crops (Amber *et al*., 1972). NC 2326 and Coker 254 varieties of tobacco are Flue Cured Virginia (FCV) varieties, which are commonly cultivated in Pakistan. The production and yield of FCV tobacco varieties in Pakistan from 1975 to 1977 have been increased (Khan, 1979). Tobacco is a cash crop, the tissue analysis of which is tested for salt tolerance, economic purpose and also for reclamation. The objective of the experiment is to know the response of these two varieties to the salinity of the medium of their growth and the correlation of dry weights of roots and shoots with the accumulation of sodium and chloride in these tissues.

Materials and Methods

Preparation of growth medium for preliminary experiment: The experiment was carried in Botanical Garden University of the Punjab Lahore. Four week old seedlings of tobacco varieties, NC 2326 and Coker 254, provided by Regional Office, Pakistan Tobacco Board, Lahore were grown at six different levels of artificially salinized soil-T0, T1, T2, T3, T4 and T5 having EC values of soil saturation extracts 0.8, 1.8, , 3.5, 7.5, 15.5 and 31.4 dS/m respectively. A total of 96 earthen pots were filled with artificially salinized soil for two varieties, 4 replicates and 2 harvests. At first three seedlings per pot were grown which were thinned out to two per pot after a week. The pots were then placed in a wire-house in completely randomized block design and their position was randomized once a week. The pots were watered daily with same amount of water (300 ml) which was enough to moisten the soil. The harvest I was taken after 12 weeks and harvest II was taken after 24 weeks.

Plant tissue analysis: Both the roots and shoots of plants grown in soils of various salinity treatments were analyzed for their sodium and chloride contents. The oven-dried material was ground. The shoots of the randomly selected replicates of a treatment were ground together and sub-sample from this ground material was used for analysis. Similar method of sampling was also used for the roots.

Ashing of plant material: To prevent the loss of chloride as chlorine which may otherwise occur, the addition of lime (calcium oxide) before ashing of the plant material has been recommended (Piper, 1942). Therefore the plant material was ashed for chloride and sodium determinations using the following procedure:
One gram dried plant material was transferred to a silica crucible. The sample was mixed with 250 mg of calcium oxide and sufficient quantity of distilled water to make a thin paste. The paste was then placed in a muffle furnace and the temperature was raised gradually up to 550 °C and maintained at this temperature for at least 90 min. The sample was removed from the muffle furnace and cooled. Fifteen ml of hot distilled water was added to it and then placed on a hot plate. Ash was broken into fine powder using a large diameter blunt rod. This ash was filtered into a 50 ml volumetric flask. The

residue on the filter paper was washed in a crucible by the addition of hot distilled water. The volume was made up to 50 ml.

Sodium contents: Sodium content of plant tissues was determined by flame photometry. A calibration curve was prepared using sodium chloride solutions having sodium concentrations of 0.0, 0.2, 0.5, 1, 2, 4, 8, 12, 16 and 20 meq/l. Sodium concentrations of aliquot was determined by using a Korl Kolb flame photometer and the appropriate calibration curve.

$$\text{Na. Meq/g o.d. plant material} = \frac{\text{Na. Meq/l}}{20}$$

Chloride Content: The method used by Richard (1954) was used. 20 ml ashed solution was transferred to a conical flask and 4 drops of potassium chromate indicator were added to it. It was titrated against N/50 silver nitrate.

$$Cl, meq/l = \frac{\text{Normality of } AgNO_3 \times \text{Vol. of } AgNO_3 \text{ used}}{\text{Vol. of aliquot}} \times 1000$$

$$Cl, meq/g \text{ o. d. plant material} = \frac{Cl.\ Meq/l}{20}$$

Results

Variety NC 2326 at harvest I: The amount of sodium and chloride in the shoots and roots of the plants increased with an increase in the salinity of soil. In T0, T1 and T2 treatments, the amounts of sodium and chloride in the shoots were more than their corresponding values in the roots (Table 1). The amount of sodium in the shoots were less than in the roots in T3 and T4 treatments while in T3 treatment that of chloride also was less in the shoots than in the roots. In T5 treatment the amount of sodium and chloride only were again greater in the shoots than in the roots.

Variety NC 2326 at harvest II: The concentration of sodium and chloride in the shoots and roots increased with an increase in the salinity of soil (Table 2). In T0, T1 and T3 treatments the concentration of sodium in the shoots were more than their corresponding concentration in the roots. On the contrary, in T3, T4 treatments content of shoot was, respectively less or nearly equal to its value in the roots. In T5 treatment the amount of sodium in the shoots was again higher than in the roots.

In T0 treatment the amount of chloride in the shoots was more than in the roots. In T1 and T2 treatments the chloride contents of roots and shoots were nearly equal. In T3 treatment the relative position was reversed and the amount of chloride in the roots was more than in the shoots while in T4 and T5, the opposite relationship was observed, i.e. more chloride in the shoots than in the roots.

A comparison of data (Tables 1 and 2) reveals that the relative concentration of sodium and chloride in the roots and shoots were, in general, higher at harvest II than those at harvest I for different salinity treatments. It is further added that both Na and Cl increased with the increase of salinity levels and the Cl contents were higher than that of Na at both the harvest in these treatments.

Variety Coker 254 at harvest I: The amounts of sodium and chloride in the shoots and roots of the plants of the various treatments at the time of harvest I (Table 3). Generally speaking the amounts of sodium and chloride in the shoots

Table 1: Amount of sodium and chloride meq/g o.d.wt. in variety NC 2326 at harvest I.

Treatments	Sodium		Chloride	
	Shoot	Root	Shoot	Root
T0 (0.8 dS/m)	0.16	0.05	0.27	0.08
T1 (1.8 dS/m)	0.17	0.14	0.48	0.35
T2 (3.5 dS/m)	0.25	0.17	0.62	0.58
T3 (7.5 dS/m)	0.34	0.64	0.83	1.01
T4 (15.5 dS/m)	0.38	0.65	1.53	1.22
T5 (31.4 dS/m)	1.20	0.83	1.90	1.57

Table 2: Amount of sodium and chloride meq/g o.d.wt. in variety NC 2326 at harvest II.

Treatments	Sodium		Chloride	
	Shoot	Root	Shoot	Root
T0 (0.8 dS/m)	0.19	0.09	0.37	0.07
T1 (1.8 dS/m)	0.32	0.16	0.39	0.37
T2 (3.5 dS/m)	0.37	0.25	0.54	0.62
T3 (7.5 dS/m)	0.39	0.70	0.90	1.12
T4 (15.5 dS/m)	0.81	0.84	1.60	1.32
T5 (31.4 dS/m)	1.25	0.94	1.95	1.62

Table 3: Amount of sodium and chloride meq/g o.d.wt. in variety Coker 254 at harvest I.

Treatments	Sodium		Chloride	
	Shoot	Root	Shoot	Root
T0 (0.8 dS/m)	0.14	0.04	0.27	0.07
T1 (1.8 dS/m)	0.21	0.13	0.33	0.27
T2 (3.5 dS/m)	0.26	0.18	0.51	0.29
T3 (7.5 dS/m)	0.33	0.39	0.78	0.39
T4 (15.5 dS/m)	0.79	0.45	1.33	0.80
T5 (31.4 dS/m)	0.87	0.52	1.57	0.92

Table 4: Amount of sodium and chloride meq/g o.d.wt. in variety Coker 254 at harvest II.

Treatments	Sodium		Chloride	
	Shoot	Root	Shoot	Root
T0 (0.8 dS/m)	0.15	0.05	0.37	0.07
T1 (1.8 dS/m)	0.32	0.14	0.45	0.30
T2 (3.5 dS/m)	0.40	0.20	0.60	0.35
T3 (7.5 dS/m)	0.41	0.39	0.80	0.41
T4 (15.5 dS/m)	0.93	0.47	1.42	0.88
T5 (31.4 dS/m)	0.93	0.58	1.62	1.02

were greater than in the roots. Both in shoot and root the amount of sodium and chloride increased as the soil salinity increased.

Variety Coker 254 at harvest II: The amounts of sodium and chloride in the shoots were greater than in the roots (Table 4). Both in the shoots and roots the amounts of sodium and chloride increased with the increase in soil salinity levels in different treatments.

Discussion

Growth reduction of plants in a saline medium is the mechanism for osmotic adjustment (Bernstein, 1963; Storey and Wyn Jones, 1978). Pamenter and Smith (1983) found that when the Antarctic grass exposed to NaCl in rooting medium, leaf water potential and osmotic potential was declined. Salinity may affect plant water retention by reducing water conductivity of roots (O'Leory, 1969). Capacity of stem to conduct water decreases in plants upon exposure to saline conditions (Strognov, 1964). Soil salinity limits crop production which arise from intrinsic soil components, use of low quality water for irrigation or excess use of fertilizers (Benzil and Reuveni, 1994).

From this experiment it was found that sodium and chloride contents increased with the increase of salinity levels in the growth medium. Similar results were reported by Storey and Wyn Jones (1978), that with the addition of 50-200 mol/m^3 NaCl, the

Na and Cl levels in the shoots and roots increased markedly with each increment of salt. Wyn Jones and Storey (1978) also reported similar results for barley varieties. The sodium and chloride contents were greater in the shoots than in the roots at both the harvests (Tables 1, 2, 3, 4). Bhatti *et al*. (1976) have found similar results for barley varieties. Mrumaker and Chavan (1989) also proved that stem and leaf tissue Na concentration increased with increasing salinity and Cl contents increased after 5 week treatment. The levels of Cl in the shoot was higher than the Na and these results differed with the results of Storey and Wyn Jones (1978) who found that Cl in the shoots was less than the Na. These contents were generally higher in amount in variety NC 2326 than that of variety Coker 254 and both sodium and chloride increased from harvest I to harvest II in these varieties.

In the light of foregoing, on the basis of salt accumulation in the root and shoot tissues of these tobacco varieties, it is concluded that both these varieties are salt sensitive but Coker 254 is more salt sensitive than NC 2326.

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