
THE SALT SECRETION PHYSIOLOGY OF A CHLORIDOID GRASS, *CYNODON DACTYLON* (L.) PERS., AND ITS IMPLICATIONS

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ABSTRACT: Leaves and branches of bermuda grass, *Cynodon dactylon* (L.) Pers., were exposed to various concentrations of NaCl and KCl salt solutions and were examined using light microscopy, electron microscopy and atomic absorption spectrophotometer. Salt secretion was observed first as droplets and later on as salt crystals. Even though salt was secreted at both sides of the leaf, most of the secretion occurred at the abaxial side of the leaf. The salt was secreted by salt glands which are distributed on the adaxial and abaxial leaf surfaces. Each gland consists of two cells: the basal cell and the cap cell. This plant bears salt glands with a mean density of 765 cm⁻². Amount of secretion was influenced by the external salt concentration. The amount of sodium secretion increased following an increase in the external NaCl concentration of up to 0.2 M. Leaves secreted more than 50 percent more under a higher relative humidity than under a lower relative humidity at higher NaCl concentrations. This suggests the influence of water stress on secretion. The secretion mechanism was found to be selective for sodium against potassium. Mixed treatment of NaCl and KCl markedly reduced secretion of sodium while the effect on potassium was less significant. Salt secretion of *C. dactylon* has an ecological significance in maintaining low salt levels in the shoot. It could play perhaps an important role in the rehabilitation of saline soils.

Key words/phrases: *Cynodon dactylon*, salinity, salt glands, salt secretion

INTRODUCTION

The presence of an excessive amount of salts in irrigation soil water has been considered a major threat to the permanency of irrigation agriculture throughout

the world (Allison, 1964). Despite the historic record of salinity problems subsequent to intensive irrigation, the expansion of irrigation in the arid and semi-arid regions of the world is considered by some as one of the most important possibilities for increasing world food production (Rains and Epstein, 1967). Moreover as water resources suitable for irrigation become less abundant throughout the world, water currently considered too saline will have to be used to meet agricultural need (Francois *et al.*, 1994). Again, the valuable crop land resource is shrinking annually by a variety of reasons including salinization which necessitates to make marginal lands more productive (Hinman and Hinman, 1992). These conditions call for a better understanding of adaptation of plants to saline environments and its application to agricultural production

Most higher plants can not tolerate saline environments. Their growth is affected from a combination of several factors. These include an osmotically reduced water availability in the soil, an excessive ion build up in the tissue and a reduced absorption of essential elements. In plants growing in a saline habitat, salt is continuously transported into the shoot which may accumulate to a level toxic enough to interfere with normal functioning. The ability of the plant to survive under such environments may depend on reduction of salt content in its shoot. Secretion of ions by special glands is the best known mechanism for regulating mineral content of plant shoots (Waisel, 1972).

Salt glands are epidermal structures specialized for the secretion of excess salt from the shoot. They are known to occur in genera of at least thirteen plant families (Waisel, 1972). Thomson (1975) classified salt glands in three groups based on their structural organization. These are: the two celled glands of the grass family, the multicellular glands of some dicotyledonous families and the bladder cells of Chenopodiaceae. The simplest type is the two celled gland found in grasses.

The study by Lisphschitz and Waisel (1974) revealed that salt glands appear in many genera especially in the Chloridoideae sub family of the Gramineae. The Chloridoideae are probably the most adapted of the grasses to saline environments. This seems to indicate the adaptive value of salt glands in a saline habitat.

Analysis of the secretion products of salt glands has shown that a variety of mineral elements are secreted (Thomson, 1975). These include cations Na, Ca, Mg, K and anions Cl, SO₄, PO₄ and CO₃. The large number of mitochondria associated with salt glands, sensitivity of secretion to temperature and metabolic inhibitors (Thomson, 1975) and the higher concentration of secreted fluid than the treatment solution (Pollak and Waisel, 1979) were evidence indicating that an active process is involved in the secretion process.

Although salt glands have been discovered in many plant species, detailed studies have been made only in a few cases. Especially investigation of the eco-physiological aspects of salt secretion in most of the studied species is lacking. The purpose of this study was to examine the distribution and morphology of salt glands in *Cynodon dactylon* and to investigate the effect of certain environmental factors on the salt secretion physiology of its salt glands.

MATERIALS AND METHODS

Cynodon dactylon plants were used in this study. The plants were propagated by cuttings and grown in a greenhouse irrigated with tap water as often as required.

Light microscopy: salt secretion and distribution of salt glands

To observe the secretion of salt by salt glands, branches were immersed in different concentrations of NaCl salt solution. Deionized water was used as a control treatment. At intervals the leaves were examined with a Zeiss binocular microscope.

Distribution of salt glands was studied by immersing leaves and branches in a 0.2 M sodium chloride (NaCl) solution in small specimen tubes. The treatments were kept in a growth chamber with 25° C and under continuous light for two days. After two days the leaves and branches were observed with a binocular microscope to determine the number and distribution of salt glands on different parts of the plant. Salt crystal count was used to determine the number of salt glands on the adaxial and abaxial leaf surface.

Electron microscopy: structure and distribution of salt glands

For electron microscopy examination, leaf sections of about 4 mm² were taken from branches previously treated with 0.2 M NaCl solution. The salt treatment was applied on the assumption of activating the glands and hence making observation easier. The specimens were prepared in three stages.

1. The specimens were fixed by immersion in cold 2.5% glutaraldehyde buffered in 0.1 M phosphate for 16 hours. This was followed by washing of the specimens with a 0.1 M phosphate buffer.
2. The specimens were dehydrated in a graded acetone series, i.e. 50%, 60%, 70%, 80%, 90%, and 100%. Each immersion lasted 20 minutes.
3. Critical point drying of samples was done in a polaron E 300 critical point drier for 2 hours. Some of the samples were mounted the adaxial side facing down while some were mounted the abaxial side facing down. The prepared samples were observed with an Hitachi 5430 scanning electron microscope. Photographs were taken during observation.

Leaf sections of about 4 mm², taken from a branch previously treated with 0.2 M NaCl solution, were fixed with glue on metal discs for cryo-observation. The mounted samples were immersed in liquid nitrogen for 5 minutes. The samples were observed with a scanning electron microscope mentioned above.

Physiological aspects of salt secretion

Experiments on the physiology of salt secretion were carried out on mature detached leaves. Pollak and Waisel (1979) reported that detached leaves secreted ions without apparent changes during 10 to 12 hrs after excision.

To examine the effect of external NaCl concentration on salt secretion, leaf sheaths of detached leaves were immersed in the treatment solutions while the leaf lamina was freely exposed. The treatment solutions were 0.0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 M NaCl. The experiment was conducted in a growth chamber (60% RH) at 25° C with continuous light. The leaves were thoroughly washed with deionized water before they were used for the experiment. After

10 hrs, the leaf blades were cut off and the secreted salt was rinsed off the blade surface with 5 ml of deionized water for 15 seconds.

To investigate the effect of humidity on salt secretion at different NaCl concentrations, leaf sheaths were immersed in a test tube containing the treatment solutions (0.2, 0.4 and 0.6 M NaCl) at the bottom. Then the test tubes were sealed to create a humid condition. The same set of treatments were set up and kept at the relative humidity of the growth chamber (60%) to represent the drier condition. The secreted salt was removed from leaves in the same way as mentioned above.

NaCl and KCl solutions were mixed at different proportions, with a fixed 0.3 M final concentration, to compare the relative amount of sodium and potassium secretions. The NaCl/KCl proportions were 1:1, 1:2, 1:3 and 1:4.

Mature leaves were also exposed to mixed NaCl and KCl solutions of equal concentration and to separate NaCl and KCl solutions to determine the effect of mixed salt treatment.

In all the experiments sodium and potassium were determined by an SP9 atomic absorption spectrophotometer.

RESULTS

Distribution and structure of salt glands

When leaves or branches were immersed in various concentrations of NaCl solution, salt secretion was observed first as droplets at the base of each gland and later as large salt crystals. Visible droplets were observed with a binocular microscope after about four hours of exposure to a 0.2 M NaCl solution. Salt crystals appeared on all the leaves of the salt treated branches including the young and mature ones. Secreted salt crystals were also observed with a scanning electron microscope (Figs 1 and 2).

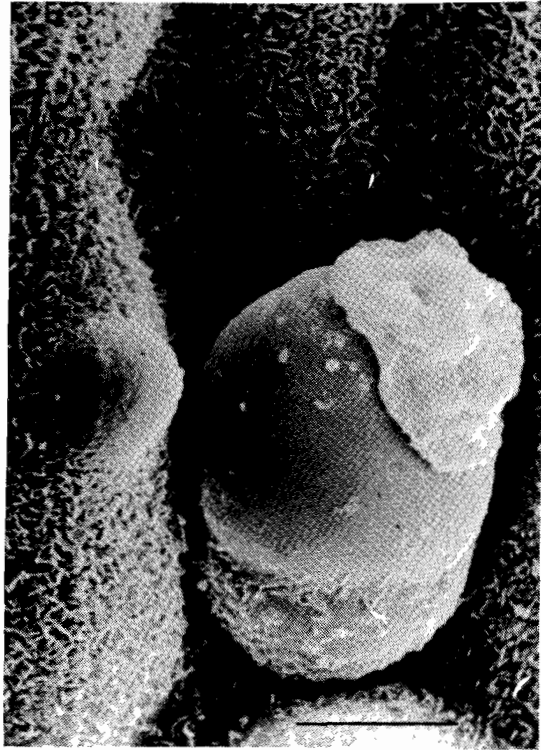


Fig. 1. Scanning electron micrograph of a salt gland showing secreted salt on the cap cell. Mag. = X3800, scale bar = 5μ .

Salt crystals appeared only on the adaxial and abaxial sides of the leaf lamina. Other parts of the plant do not possess salt glands. Distribution of salt crystals was examined on twelve leaf samples. The results of the salt crystal count were 2.1 ± 1.1 and 13.2 ± 2.0 for the adaxial and abaxial leaf surfaces, respectively. This indicates that salt glands are much more abundant on the abaxial side of the leaf. It was also observed that the density of salt glands was higher at the centre of the leaf in comparison to the base and the tip portion.



Fig. 2. A cryo-scanning electron micrograph showing fractures of salt crystals around and on the salt glands. S, salt crystals; G, salt gland; Mag. = X1900, scale bar = 5μ .

Scanning electron micrographs illustrated that salt glands of *Cynodon dactylon* appeared on both sides of the leaves parallel to the veins (Figs 3 and 4). Each gland is comprised of two parts, *i.e.* the cap cell and the basal cell. The cap cell is implanted on the basal cell which is attached to the epidermal cells (Fig. 5). The cap cell and the neck of the basal cell are located above the leaf surface. A noticeable structure on the cap cell is the cavity which occupies the upper most section of the cap cell (Fig. 5).

During the experiment, it was also observed that branches immersed in NaCl solutions showed wilting response especially at higher NaCl concentrations (0.4, 0.5 and 0.6 M).

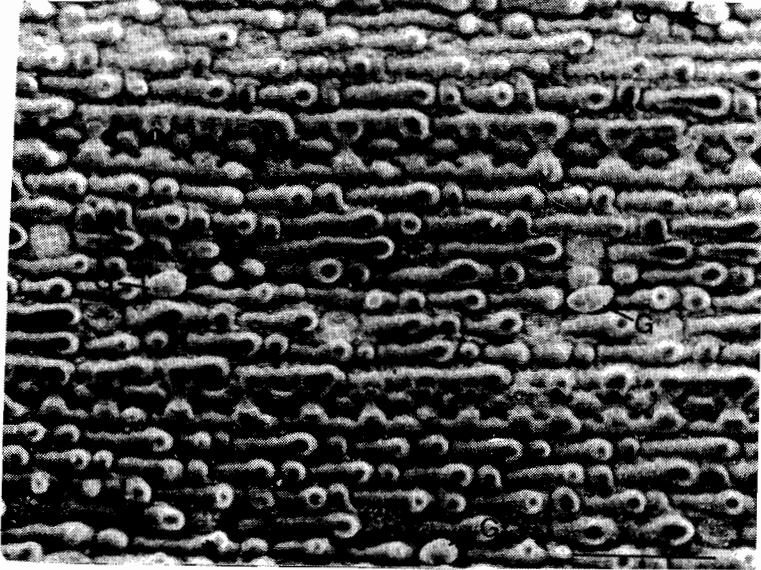


Fig. 3. Scanning electron micrograph showing distribution of salt glands on the upper leaf surface. G, salt gland; Magnification = X400; scale bar = 50 μ .

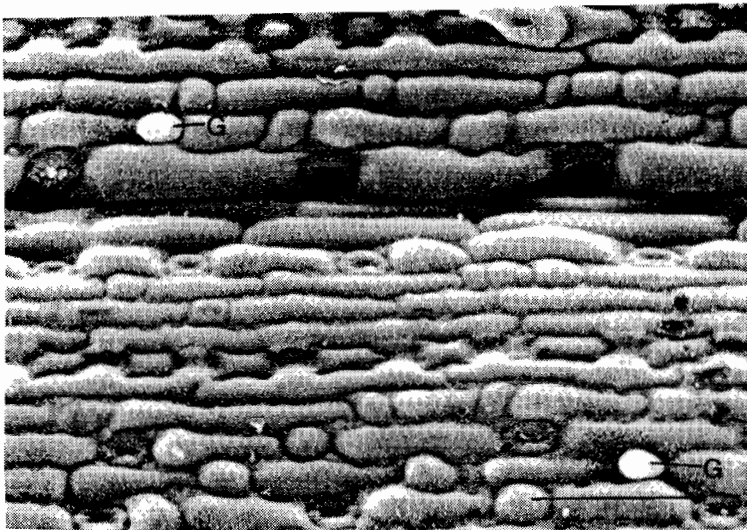


Fig. 4. Scanning electron micrograph showing distribution of salt glands on the lower leaf surface. G, salt gland; P, papillae; Mag. = X310, scale bar = 50 μ .

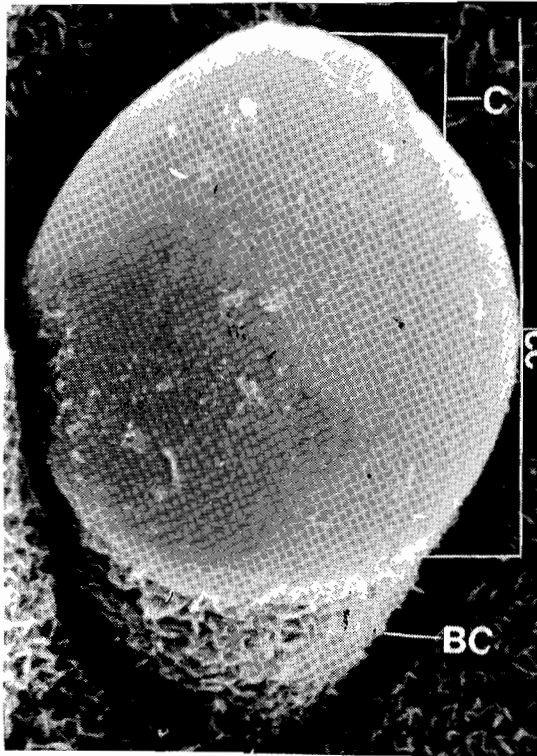


Fig. 5. Scanning electron micrograph of a salt gland showing the cap cell (CC), the basal cell (BC) and the cavity (C). Mag. = X5500; scale bar = 5μ .

The effect of external NaCl concentration on secretion

An increase in salt secretion was obtained up to the external NaCl concentration of 0.2M. Beyond this, salt secretion was reduced (Fig. 6). The highest secretion was obtained at 0.2 M NaCl concentration. The statistical analysis made did not show a significant correlation between amount of secretion and the external NaCl concentration.

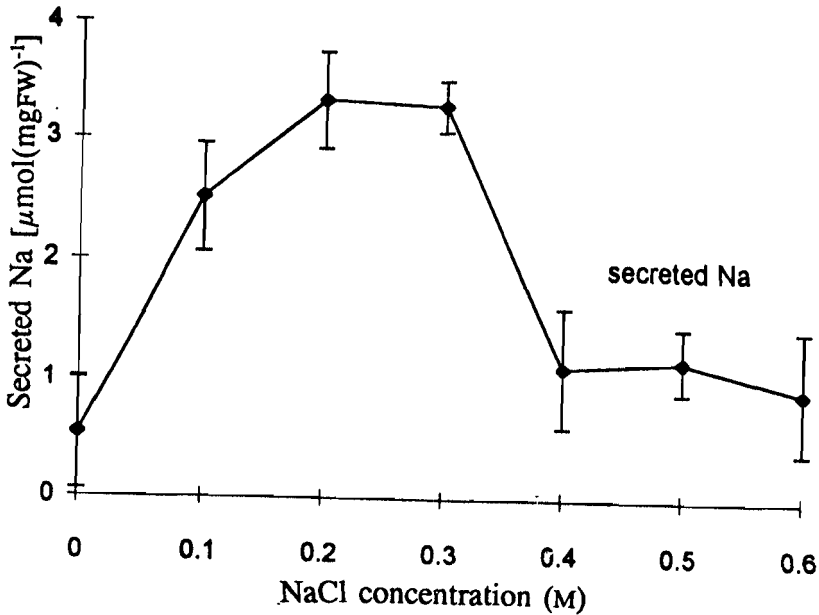


Fig. 6. The effect of external NaCl concentration on salt secretion.

The effect of humidity on salt secretion

In a previous observation, branches placed in a salt solution showed wilting which is a sign of water stress. The water stress which was severe especially at higher concentrations may influence the capacity of leaves to secrete salt. To investigate this situation, detached mature leaves were treated with NaCl at different regimes of relative humidities. At lower NaCl concentration, there is almost no difference in secretion between the high and low relative humidities (Fig. 7). However, differences in secretion were observed at higher NaCl concentrations. Treatments under a low relative humidity, 60%, showed a reduction in secretion as the external concentration of NaCl increased. On the other hand, treatments under a high relative humidity, 99%, showed an increase in secretion when the external concentration of NaCl increased.

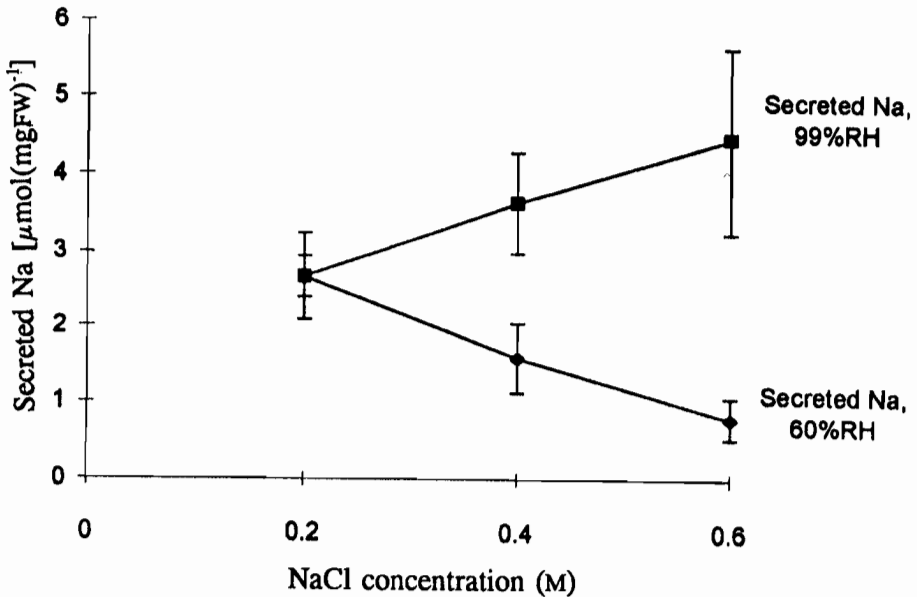


Fig. 7. The effect of relative humidity on salt secretion.

Comparison of sodium and potassium at various Na/K ratios

When the Na/K ratio in the treatment solution was 1:1, the amount of secreted sodium was much higher than potassium (Table 1). It was only when the amount of KCl in the treatment solution was three times more than NaCl that secreted potassium became nearly equal to that of sodium. Generally, it may be said that as the ratio of Na/K in the treatment solution decreased, secretion of sodium decreased while secretion of potassium increased.

The effect of mixed salt treatment on secretion of sodium and potassium

The mixed salt treatment caused a marked reduction in the secretion of sodium while only a slight drop in the secretion of potassium was observed (Table 2). Sodium was secreted better when the leaves were treated with the separate salt solutions.

Table 1. The effect of different Na/K ratios in the treatment solution on the secretion of sodium and potassium.

Na/K ratio in treatment soln. ^a	Secreted Na ^b [$\mu\text{mol}(\text{mgFW})^{-1}$]	Secreted K ^b [$\mu\text{mol}(\text{mgFW})^{-1}$]	Na/K ratio in secretion
1.00	1.60 \pm 0.90	0.40 \pm 0.07	3.96
0.50	1.00 \pm 0.40	0.89 \pm 0.11	1.11
0.33	0.92 \pm 0.37	0.90 \pm 0.12	1.01
0.25	0.72 \pm 0.34	1.07 \pm 0.25	0.67

a, final concentration of NaCl + KCl is 0.30 M; b, mean of three samples \pm SE.

Table 2. The effect of mixed NaCl and KCl treatments on the secretion of sodium and potassium.

Treatment	Secreted sodium ^a [$\mu\text{mol}(\text{mgFW})^{-1}$]	Secreted potassium ^a [$\mu\text{mol}(\text{mgFW})^{-1}$]
0.1 M NaCl	2.62 \pm 0.81	—
0.1 M KCl	—	0.74 \pm 0.14
0.1 M NaCl + 0.1 M KCl	0.51 \pm 0.48	0.72 \pm 0.40
0.2 M NaCl	3.45 \pm 0.59	—
0.2 M KCl	—	0.95 \pm 0.12

a, mean of three samples \pm SE.

DISCUSSION

As a member of the Gramineae, *Cynodon dactylon* possesses salt glands comprised of two cells: the basal cell and the cap cell. The glands are located above the epidermal cell layer. Structurally the gland cells differ from the surrounding epidermal cells by their shape, size and cutinized cell walls. The cells contain dense cytoplasm and a prominent nucleus but lack a central vacuole (Lipshitz and Waisel, 1974). Similar salt gland structures were

reported by Oross and Thomson (1982) in a hybrid bermuda grass, *Cynodon dactylon* x *C. transvalensis* and in a desert salt grass *Distichlis stricta*.

All aerial parts of halophytes may bear salt glands in their epidermis though the typical location of salt glands is in the leaves (Lüttge, 1975). *C. dactylon* bears salt glands on the adaxial and abaxial side of the leaf with a mean density of 765 cm⁻². The density is much higher on the abaxial (1320 cm⁻²) leaf surface than on the adaxial surface (210 cm⁻²). Density of salt glands vary in different plants. In the Gramineae, *Spartina* sp. and *Aeluropus litoralis* posses 1400 and 4800 cm⁻² salt glands, respectively (Waisel, 1972).

In the present expermint, salt secretion took place on both young and mature leaves though the efficiency may not be the same. This substantiates the suggestion put forward by Liphshitz *et al.* (1974) that salt glands are initiated at an early stage in the development of leaves and are differentiated earlier than many other leaf tissues.

In *Cynodon dactylon*, the presence of salt glands which are capable of secreting salt from the shoot is established. The amount of secretion depends on the concentration of the salt to which the plant is exposed. The relationship between amount of secretion and external salt concentration varies in different plant species. For instance, in *Aeluropus litoralis* (Pollak and Waisel, 1979), a positive correlation up to an external concentration range of 0.4 M was obtained. In *C. dactylon*, though correlation was non significant, salt secretion increased up to 0.2 M NaCl. At higher concentration secretion started to drop in both species. This may signify the limit of salt concentration the plant can tolerate. According to the classification of halophytes (Chapman, 1960; Mudie, 1975) *C. dactylon* belongs to miohalophtes which is in agreement with the present result. This implies that the plant can tolerate moderate salinity though it grows best under non saline conditions. Miohalophytes seem adapted to occupy the niche between highly saline and non saline conditions. Physiological flexibility to cope with both non saline and moderately saline conditions may be more important than the fixation of genes for extreme salt tolerance (Mudie, 1975).

Under salt stress conditions dissolved ions in the medium create a negative water potential resulting in a condition similar to water stress. The lower relative humidity causes a rapid transpiration rate in excess of absorption from

a medium of reduced water potential. This causes dehydration of the plant and a subsequent reduction in secretion. At a higher relative humidity desiccation is prevented by a reduced transpiration rate. This enables the plant to secrete more even under high salt concentrations, an additional evidence substantiating the idea that secretion is an active process directly involved with the physiological functions of the plant.

Salt glands of *C. dactylon* exhibit a preferential secretion of sodium to potassium. When sodium and potassium ions are given in equal amounts much more sodium was secreted than potassium. Selective secretion of sodium against potassium was also reported in *Glaux maritima* (Rozema *et al.*, 1977) and in *Aeluropus litoralis* (Pollak and Waisel, 1970; 1979). It was suggested that this may be in accordance with the maintenance of a favourable proportion of ions in the plant. If secretion is non discriminating, it may disrupt the proper balance of ions in the plant and hence of normal functioning. It is known that plants contain and require much higher amount of potassium than sodium during their life cycle. The selectivity of the secretion mechanism could also have a certain adaptive value. It can be advantageous, for instance, for efficient exclusion of excess salt as most saline soils predominantly contain sodium.

The mixed salt treatment markedly inhibited the secretion of sodium while secretion of potassium was found to be fairly constant. This might seem to indicate that the plant disposes limited energy for the secretion mechanism regardless of the number of salts involved. On the other hand, Pollak and Waisel (1979) observed that in *Aeluropus litoralis*, mixed treatment of NaCl and KCl inhibited secretion of sodium while it slightly stimulated the secretion of potassium. The amount of salt secretion depends on the concentration and composition of the external solution to which the plant is exposed. It appears that presence of various salts in the root medium substantially lowers the coping ability of the plant against the more harmful sodium salts. Additional work is required to verify these observations at higher concentrations.

Salt secretion of *C. dactylon* evidently has an ecological significance in maintaining low salt levels in the shoot. The results from the experiments of Kelly (1951) support this fact. He found that the growth of *C. dactylon* was encouraged on an area that was highly toxic to barley and alfalfa because of high exchangeable Na^+ in the soil. Moreover, its growth for two years brought a reduction of excess sodium in the soil to a level sufficient to allow the growth

of barley and alfalfa with good yields. *C. dactylon* has perhaps an important role to play in reclamation of saline and alkaline soils. It is extremely easy to propagate by cuttings of branches and runners. It is shallow rooted as most of its roots are distributed on the upper 25 cm soil layer. It is relatively drought tolerant. On the other hand, it is worth mentioning that there are many salt tolerant grasses possibly more efficient than *Cynodon dactylon* but which as yet have not been subjected to experimental investigation.

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