Pak. j. sci. ind. res., vol. 32, no. 1, January 1989

EFFECTS OF PHENYLMERCURIC ACETATE AND WATERLOGGING ON *LEPTOCHLOA FUSCA* (L.) KUNTH (KALLAR GRASS)
Part II. Salt Relations

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(Received October 4, 1987; revised December 28, 1988)

Studies were conducted to characterise Na^+ and Cl^- extrusion by the leaves of 'Kallar' grass subjected to treatment with phenylmercuric acetate (PMA) and to various moisture levels in the soil. Application of PMA did not have any substantial effect on the extrusion of Na^+ and Cl^- by the 'Kallar' grass leaves. The amounts of Na^+ and Cl^- extruded by the leaves substantially increased 72 hours after treatment with increasing NaCl concentration in the medium both at flooding and at field capacity. However, this trend was reversed at water deficit. Low availability of water seems to have affected transport of salts to the leaves and their subsequent extrusion.

Key words: Phenylmercuric acetate, Waterlogging, Kallar grass, Salt relations.

INTRODUCTION

In time sequence studies in solution cultures, net uptake of Na^+ and Cl^- by 'Kallar' grass over the NaCl concentration range from 10–200 mM was not proportional to the NaCl concentration [1]. However, Na^+ and Cl^- concentrations in the tissue increased progressively with time over the period from 6 to 144 hours at each concentration of NaCl. The net transfer rates for Na^+ and Cl^- in 10 mM NaCl were reduced from 6 hours onwards [1]. At high salinity (100 mM NaCl), transfer rates of both Na^+ and Cl^- increased slightly from 6–24 hours, and thereafter remained fairly constant. Leaves of the plants grown in low NaCl extruded Na^+ and Cl^- in the same way as those in high NaCl and the extrusion had already started after 6 hours of root exposure to low salinity. Extrusion in 'Kallar' grass seemed, therefore, not to be specific to high concentrations of Na^+ and Cl^- in the leaf tissue.

The present experiment was designed to define the nature of extrusion by following salt absorption by 'Kallar' grass and the extrusion of salts by the leaves in relation to the effects of phenylmercuric acetate (PMA), an antitranspirant, on the leaves and the effect of waterlogging and water deficit on plants grown at two concentrations of NaCl in the soil.

MATERIALS AND METHODS

(a) *Plant culture.* Sets of plants were raised from nodes of 'Kallar' grass stem, grown in 1500 g Faisalabad sandy loam soil (Clay = 6.8%, silt = 10.5%, sand = 82.7, EC_e = 2.2 $mScm^{-1}$) and given nitrogen and phosphorus as nutrient solution at the rate of 60 kg/ha as urea and 18 kg/ha phosphorus as $(NH_4)H_2PO_4$. Three moisture levels used were: field capacity (F.C. 25% moisture), flooding (2 x F.C.) and water deficit (1/2 F.C.). PMA and NaCl were also applied as described elsewhere [2–12]. In one set of plants, rates of transpiration and relative water contents of 'Kallar' grass leaves were determined, with these results [2]. In another set of plants treated with PMA and NaCl, the leaves were washed with equal quantities of distilled water at 24, 72 and 144 hours after the NaCl treatment were imposed. Washing of the leaves (6 ml/plant) was done while they were intact and the plants grew in the pots [3]. The leaf washes were analysed for Na^+ and K^+ on a Corning 400 flame photometer and for Cl^- on a Corning 920 Chloride meter.

The plants were harvested and fresh weights of the above ground parts were taken. The roots were removed from the soil, washed in tap water and distilled water, dried between folds of tissue papers and weighed. The roots and tops were then dried in an oven at 70°. The leaf blades were separated from leaf sheaths and dry weights of all plants parts were obtained.

(b) *Analysis.* Plant material, to which CaO had been added at 25% of the weight of plant material [4] was dry ashed in a muffle furnace at 500° for 3 hours. The dry ashed plant material was dissolved in 0.5 M HNO_3 [5] and analysed for Na^+, K^+ and Cl^-.

RESULTS

(a) *Extrusion of sodium, chloride and potassium by leaves.* The amounts of Na^+, K^+ and Cl^- extruded by

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'Kallar' grasss leaves at 24 hours following treatment with 300 mM NaCl were more or less similar at all moisture levels i.e., flooding, field capacity and water deficit (Table 1).

Table 1. Effect of soil water supply treatments on total amounts of Na^+, K^+ and Cl^- (m-moles/plant) in leaf washings of Kallar grass at 24 hours after treatment with 300 mM NaCl.

Treatments	Total amount (m-moles/plant) Na^+	K^+	Cl^-
Flooding	5.84	2.34	5.22
Field capacity	6.86	2.78	5.36
Water deficit	5.98	2.67	6.85

The amounts of Na^+ and Cl^- extruded as a percentage of the total plant contents in leaves treated with PMA, were, in general, similar to those in the leaves which were not treated with PMA both at 72 and 144 hours following treatment (Tables 2, 3). The amounts extruded at 72 and 144 hours were, however, significantly higher than those at 24 hours (Table 2).

At 10 mM NaCl, absolute amounts of Na^+ and Cl^- extruded by the leaves were generally similar at field capacity and water deficit, but they were substantially lower at flooding. The amounts of Na^+ and Cl^- extruded by the leaves during leaf washing increased at 72 hours with increase in salinity of the root medium from 10 to 300 mM NaCl at flooding and field capacity (Tables 2, 3). However, this trend was reversed in the water deficit treatment. Low

Table 2. Effect of soil water supply treatments and PMA on the total amount of Na^+ (m-moles/plant) in the leaf washings, leaf blades, leaf sheaths and roots of Kallar grass grown in pots at NaCl concentrations of 10 and 300 mM.

Treatments	NaCl (mM)	– PMA Leaf washing	Tops Blades	Tops Sheaths	Roots	Total	Extrusion as% total contents	+ PMA Leaf washing	Tops Blades	Tops Sheaths	Root	Total	Extrusion as% total contents
At 72 hours													
Flooding	10	7.4	25.9	14.3	25.5	73.0	10.1	6.8	24.0	16.4	14.9	62.0	10.9
	300	15.2	32.9	18.0	24.2	75.2	20.2	9.6	21.7	13.4	21.2	65.9	14.6
Field capacity	10	19.0	33.0	22.3	14.9	89.1	21.3	22.4	35.7	17.8	14.0	89.9	24.9
	300	29.2	40.5	24.9	28.4	93.7	31.1	39.0	32.4	21.9	26.7	120.0	32.5
Water deficit	10	13.7	24.2	16.9	12.2	66.9	20.5	11.5	13.7	12.9	9.0	47.0	24.4
	300	5.5	25.0	21.7	24.5	75.8	7.2	8.2	22.7	25.5	23.8	80.2	10.2
At 144 hours													
Flooding	10	12.7	27.2	11.2	11.3	62.4	20.3	10.8	30.2	14.6	16.7	72.1	15.0
	300	27.4	47.0	21.5	16.5	112.4	24.5	32.0	48.7	23.5	18.7	123.7	26.5

Table 3. Effect of soil water supply treatments and PMA on the total amount of Cl^- (m-moles/plant) in the leaf washings, leaf blades, leaf sheaths and roots of Kallar grass grown in pots at NaCl concentrations of 10 and 300 mM.

Treatments	NaCl (mM)	– PMA Leaf washing	Tops Blades	Tops Sheaths	Roots	Total	Extrusion as% total contents	+ PMA Leaf washing	Tops Blades	Tops Sheaths	Root	Total	Extrusion as% total contents
At 72 hours													
Flooding	10	10.4	34.9	16.4	26.4	88.0	11.8	8.4	26.0	32.0	17.3	83.8	10.0
	300	15.8	48.8	39.4	15.5	119.5	13.0	15.5	27.1	35.3	17.5	95.3	16.3
Field capacity	10	18.4	31.7	27.1	9.0	86.2	21.3	20.1	20.0	36.1	18.9	95.1	21.2
	300	22.4	50.0	36.4	31.3	140.0	16.0	43.9	51.6	58.2	22.8	176.4	24.8
Water deficit	10	13.4	38.6	26.2	11.2	89.4	15.0	12.3	27.4	24.0	15.5	79.2	15.6
	300	4.8	28.3	41.4	20.9	95.4	5.0	7.4	34.2	40.0	16.7	98.4	7.6
At 144 hours													
Flooding	10	15.0	19.2	34.4	26.2	94.8	15.8	14.4	27.6	32.7	11.4	86.0	16.7
	300	27.1	91.7	37.3	14.1	170.2	15.9	34.0	99.6	29.3	20.9	183.9	18.5

availability of water may have affected the transport of salt to the leaves, thus affecting its extrusion.

An increase in time of exposure to the flooding treatment from 72 to 144 hours increased the extrusion during leaf washing of Na^+ (Table 2), Cl^- (Table 3) and K^+ (Table 4). The amounts of extruded ions were higher at field capacity than at flooding and water deficit both under low and high salt levels (Tables 2, 3).

(b) *Tissue concentrations.* The concentration of Na^+, Cl^- and K^+ in various parts of PMA-treated plants were similar to those in plants which were not treated with PMA (Tables 2, 3, 4).

Sodium and chloride. The amounts of Na^+ and Cl^- in the leaf blades increased with the increase in salinity of the root medium from 10 to 300 mM at flooding and field capacity (Table 2, 3). However, in the water deficit treatment Na^+ contents remained similar at both low and high NaCl concentration but the amount of Cl^- was less at 300 mM NaCl than at 10 mM NaCl in the water deficit treatment (Without application of PMA).

In the leaf sheath, Na^+ and Cl^- content increased with the increased salinity of the root medium at all moisture levels. The amount of Na^+ in the roots remained similar at low and high NaCl concentration in the flooding treatment, but was greater at high NaCl concentration in the field capacity and water deficit treatments (Table 2). The pattern of Cl^- content in the roots was similar to that of Na^+, except for a lower content at high than at low NaCl in the flooding treatment. Total amount of Na^+ per plant was higher at field capacity than in the flooding and water deficit treatments, both at 10 and 300 mM NaCl. At 10 mM NaCl, the total Cl^- content in the plant remained similar in the three water treatments.

At flooding, the total Na^+ and Cl^- contents per plant increased substantially with time (from 72 to 144 hours) in the 300 mM NaCl the treatment, but not at 10 mM NaCl (Tables 2, 3).

Potassium. In leaf blades and leaf sheaths, increasing the NaCl concentration from 10 to 300 mM slightly increased the K^+ content at 72 hours after imposition of the flooding treatment (Table 4). However, the K^+ contents in these tissues remained similar at both NaCl concentrations in the field capacity and water deficit treatments. At 144 hours, the K^+ content had decreased in the flooded plants grown at 300 mM NaCl. The K^+ content of the root was decreased at the high salt concentration in the flooding treatment, but not in the field capacity and water deficit treatments. Similar effects of treatments were observed on the total K^+ content of the whole plant.

DISCUSSION

Salt relations. Although the growth of 'Kallar' grass plants treated with PMA was decreased at 72 hours after its application [2], there was no effect on the absorption of Na^+ and Cl^- by the plant, or on extrusion of Na^+ and Cl^- by the leaf blade (Tables 2, 3). Data on rates of transpiration and relative water contents of the leaves reported in an earlier paper [2] suggested that the effect of PMA on these parameters was confined only to the first 24 hours after its application. If the effects of PMA had continued beyond 24 hours, a substantial suppression of Na^+ and Cl^- uptake, in addition to suppression of growth [6, 7, 8] might have been expected. In its absorption of Na^+ and Cl^-, however, 'Kallar' grass resembled *Armeria maritima* [9] in which application of NaCl increased the uptake and extrusion of Na^+ and Cl^-. Since the effect of increased application of NaCl on uptake

Table 4. Effect of soil water supply treatments and PMA on the total amount of K^+ (m-moles/plant) in the leaf washings, leaf blades, leaf sheaths and roots of Kallar grass grown in pots at NaCl concentrations of 10 and 300 mM.

Treatments	NaCl (mM)	−PMA Leaf washing	−PMA Tops Blades	−PMA Tops Sheaths	−PMA Roots	−PMA Total	−PMA Extrusion as% total contents	+PMA Leaf washing	+PMA Tops Blades	+PMA Tops Sheaths	+PMA Root	+PMA Total	+PMA Extrusion as% total contents
At 72 hours													
Flooding	10	3.0	48.0	17.3	70.2	138.6	2.2	3.2	36.7	19.6	35.7	95.2	3.32
	300	3.6	58.2	30.9	31.7	124.6	2.9	3.3	32.9	18.7	34.4	89.4	3.71
Field Capacity	10	6.2	40.1	21.1	24.7	92.2	6.8	6.1	37.9	21.4	27..3	92.6	6.6
	300	4.1	38.0	18.0	27.7	87.9	4.7	6.6	30.3	16.4	21.8	75.0	8.8
Water deficit	10	4.3	43.1	21.5	27.1	96.0	4.5	3.7	30.6	19.4	16.5	70.3	5.3
	300	3.3	39.3	19.8	28.6	91.0	3.6	1.3	38.3	19.5	27.6	86.7	1.5
At 144 hours													
Flooding	10	4.7	54.1	19.8	31.3	109.9	4.3	4.4	59.1	23.1	31.6	118.2	3.7
	300	4.6	41.6	14.9	22.9	84.0	5.5	5.9	53.4	17.0	31.1	107.5	5.5

and extrusion of Na^+ and Cl^- by 'Kallar' grass was obvious only in the flooding and field capacity treatments, it would appear that the treatments were complementary in their effects with a non-limiting moisture regime necessary for effects on uptake and extrusion to occur.

In other studies [10, 11] in which the concentration of Na^+ and Cl^- exceeded that of K^+ in the leaf washings, it was suggested that Na^+ and Cl^- were selectively extruded. Although, Na^+ predominated over other ions viz. K^+, Ca^{2+}, Mg^{2+} in various studies [1, 12] including the present one, the decreased extrusion of Na^+, K^+ and Cl^- by the leaves of plants grown at limiting moisture supply [1] does not support the view [10, 11] that Na^+ and Cl^- were selectively extruded. On the other hand, in those plants such as *Atriplex,* in which high amounts of Na^+ and Cl^- were excreted, a suggestion was made that active extrusion processes were involved [13]. This suggestion was based on measurements of electro-chemical potential difference. In 'Kallar' grass, direct evidence that extrusion of Na^+ and Cl^- is an active process remains to be produced.

In addition, more elaborate studies involving compartmental analysis of Na^+ and Cl^- in the leaf blade tissue and in the salt glands are also needed to understand the distribution of Na^+ and Cl^- at cellular level. In our laboratories, however, precise experiments are being carried out using excised leaves and intact plants to define the nature of salt extrusion in 'Kallar' grass.

Acknowledgement. The authors are thankful to Dr. S.H.M. Naqvi, Director, NIAB, Faisalabad, for encouragement and support of the project and to Dr. Zainul Abedin, Dept. of Botany, Punjab University, Lahore for useful discussions.

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