RICE GROWTH AND IONIC COMPOSITION UNDER SALINE HYDROPONIC CONDITIONS: II. SUPPLEMENTED WITH Cl^-: SO_4^{2-} RATIOS

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A very important and well established chemical characteristics of salt affected soils relevant to crop growth is their anionic composition i.e. $Cl^-:SO_4^{2-}$ ratio of soil solution. Rice soils of the upper Indus Plain (Punjab) in Pakistan have SO_4^{2-} type of salinity while; the rice soils of lower Indus (Sindh) dominate in Cl^- salinity. It was, therefore, an economically important issue to study effect of different proportions of Cl^- and SO_4^{2-} in the saline medium on the rice growth, and their physiological implication by employing rice lines of known salt tolerance behavior. Therefore, some experiments were conducted to determine whether some appropriate external ratios of ions help to maintain the suitable internal ionic composition and thus improve rice growth in the saline conditions. Hence, different ratios were established in the saline substrate involving two rice lines, i.e. NIAB 6 (salt tolerant) and BG 402-4 (less salt tolerant). The effect of Cl^- : SO_4^{-2} ratios in the saline medium on the shoot and root weight of rice was outstanding; shoot weight was almost twice as high at Cl^- : SO_4^{-2} ratio of 1:100 as compared to ratio of 100:1 while, this difference was more than twice in the case of root weight. On overall basis, the better growth of rice at lower Cl^- : SO_4^{-2} ratio was related to improved K^+ and Ca^{2+}concentrations in the leaf sap (led to high internal Ca^{2+}: Na^+ and K^+:Na^+ ratios), and also a relatively lower P concentrations under saline conditions.

INTRODUCTION

In general, the type of salt in the growth medium is known to affect rice growth and paddy yield. At 2.2 mol m^{-3} salt concentration, rice seedlings were more tolerant of Na_2SO_4 than NaCl (Kaddah, 1963). At the comparable Ec level injury was less in seawater than in solution of common salt (Ponnamperuma, 1984). This may be due to more harmful effect of NaCl than Na_2SO_4, KCl and K_2SO_4 on rice (Rao et al., 1969) Mostly, Cl^- and SO_4^{2+} are the dominant anions in the saline soils of Pakistan (Qureshi, 1986). High level of Cl^- could decrease nitrate and phosphate uptake under saline environment (Akbar, 1975). Chloride induced deficiency was responsible for growth retardation in wheat plants exposed to high NaCl salinity (Torres and Bingham, 1973).

Toxic effects of accumulated Na^+ and Cl^- had been reported by Muralithran et al. (1992) in leaves of blue berries. Usually, accumulation of chloride is parallel to that of sodium (Yeo and Flowers, 1985). Aslam (1987) pointed out that differences between moderately salt tolerant and tolerant plants of rice cultivar NIAB 6 were related to the low concentrations of chloride ions apart from maintenance of high K^+:Na^+ ratio. The role of ionic composition of saline growth medium on growth of various crops had been studied by many workers (Wyn Jones, 1981; Kuiper, 1984; Aslam et al., 1993). However, no such study has been reported on the role of anionic ratios in the medium on the growth of rice whereas, there exists a great variation in this respect in many rice growing areas. For example, under saline soils on the rice tract of the Punjab in Pakistan, SO_4^{2-} dominates while, in the lower Indus (Sindh) Cl^- and SO_4^{2-} dominates in the salt effected rice lands. Different ions are reported to be responsible for the inhibition in seed germination of sorghum (Paliwal and Gandhi, 1968) alkali scaton (*Sporobolus airoids torr.*) (Hyder and Yasmin, 1972). Salts of common anions i.e. $MgCl_2$, KCl and NaCl affected Alakili scatons, a highly salt tolerant plant differently. Though it is difficult to separate the effect of cations from anions, but the final effect on the Alfalfa depended on the particular combination of cation and anions (Redmann, 1974). Types of salts in growth medium also have pronounced effect on the growth of rice (Ponnamperuma, 1984). Rice is considered more sensitive to Cl^- as compared to SO_4^{2-} but detailed information aspects regarding more toxicity of chloride than the sulphate is still needed. In the present study, the effect of varying external Cl^-:SO_4^{2-} ratios on growth and chemical composition of the leaves of two rice genotypes has been determined. In this study, an outstanding role of the relative proportion of Cl^- and SO_4^{2-} in the saline medium in the fresh and dry weights of the rice shoot and root was observed.

MATERIALS AND METHODS

Seedlings (15 days old) of two rice lines i.e. NIAB 6 and BG 402-4 were transferred in 1 cm plugged holes in the thermo pole sheets floating over 20 liters of Yoshida nutrient solution (Yoshida et al., 1976) in plastic tubs. Three holes were used for each line, each hole having one seedling. There were three replications in the experiment. Nutrient solutions were prepared in the distilled water. Various ratios and concentrations of Cl^- and SO_4^{2-} used in different treatments were as follows.

NaCl and Na_2SO_4 were used in the equivalent basis to develop different Cl^-: SO_4^{2-} ratios in saline medium. The total salinity was expressed in terms of mol m^{-3} NaCl for convenience. The ratios developed were as under.

Ratio Cl^-: SO_4^{2-}	The total salinity me L^{-1} Cl^-+SO_4^{2-} (Equivalent basis)	Cl^- contents mol m^{-3}	Na^+ Contents mol m^{-3}
Control (1:0.025)	Nutrient solution		
100:1	100	106.6	110.0
75:25	100	83.0	112.0
50:50	100	57.9	104.0
25:75	100	34.2	110.0
1:100	100	5.2	104.0

Sodium chloride and Sodium sulphate was dissolved in equivalent basis to develop the required salinity is expressed in mol m^{-3} of NaCl for convenience. An equal level of sodium was maintained in all treatments. Third leaf from the top was sampled at two stages i.e. 15 days and 30 days after salt stress, processed for the analysis. The plants were harvested after thirty days and physical data was collected. Potassium and sodium selectivity ($S_{K+,Na+}$) and coefficient of shoot elongation (CSE) were calculated.

$$S_{K+,\,Na+} = \frac{K^+:Na^+ \text{ in plant tissue}}{K^+:Na^+ \text{ in external solution}} \qquad \text{(Pitman, 1965)}$$

$$CSE = \frac{\text{Rate of shoot elongation of treatment plants in cm day}^{-1}}{\text{Rate of shoot elongation of control plants in cm day}^{-1}} \qquad \text{(Devitt et al., 1984)}$$

Plant leaf cell sap was extracted and it was analyzed for Na^+, K^+, Ca^{2+} and Cl^- while P and Zn were determined from wet digestion extract. (Richard, 1954; method 54a). The data was analyzed statistically (Steel and Torrie, 1980).

RESULTS AND DISCUSSIONS

Almost all physical characteristics of both the rice lines; shoot and root length, tillering capacity, shoot and root weight were severely affected by 100 mol m^{-3} salinity (NaCl+Na_2SO_4). However the effect was much pronounced in higher Cl^-:SO_4^{2-} ratio than lower ratios in the growth medium (Table 1&2). Shoot weight was almost twice as high at the Cl^-: SO_4^{2-} ratio of 1:100 while this difference was more than two times in case of root weight.

In many crops, salinity tolerance depends on the efficiency with which the root system can limit excess of Na^+ and Cl^- to above ground parts of the plants (see Yeo et al., 1977). In addition, Na^+ is generally accumulated in leaves to lesser extent than Cl^- due to apparently separate mechanism which regulate the transport of Na^+ and Cl^- to leaf after exposure of plants to salt (Grieve and Walker, 1983). Higher toxicity of NaCl as compared to Na_2SO_4 is documented by many researchers (Curtin, 1993). Rice crop is especially more tolerant to Na_2SO_4 than NaCl (Aslam et al,. 1993). One obvious reason to this trend could be the difference in the relative uptake rates of the two ions. Also, SO_4^{2-} is biologically metabolized in the plant and becomes a part if various plant organs and thus its accumulation as a free ion is much less as compared to Cl^- to cause toxicity. Moreover S is an essential macro element while Cl^- is not. Some authors have reported depressive effect of SO_4^{2-} on plant growth (Lone, 1988). Leaf Cl^- was increased in response to NaCl and leaf Na^+ and Cl^- concentrations were manifold higher compared with the nutrient solution (Table 3). These measurements suggest the rice is a poor excluder of both Na^+ and Cl^-. One of the most striking effects of increasing proportion of SO_4^{2-} vis-à-vis Cl^- was the substantial increases in K^+ (> 1.5 times at extremes) and Ca^{2+} (> 3 times at the extremes) concentration (Table 3&4) which also lead to increased K^+: Na^+ and Ca^{2+}: Na^+ ratios in the leaf sap (Fig. 1). This trend, however, is not easy to explain. Probably, a

Table 1. Effect of external chloride sulphate ratio at 100 mol m^{-3} ($NaCl+Na_2SO_4$) on growth of two rice lines (Average of three replicates).

Varieties/lines	Control (Nutrient Soln)	100 mol m^{-3} ($NaCl+Na_2SO_4$) Cl:SO_4^{2-} ratio				
		100:1	75:25	50:50	25:75	1:100
Shoot length in (cm)						
NIAB 6	68.7 a	55.0 c-d	54.7 c-d	55.7 b-e	57.3 b-d	60.5 b
BG 402-4	68.5 a	51.7 e	52.0 d-e	54.2 c-e	55.2 c-e	58.5 c
Coefficient of Shoot elongation						
NIAB 6	1.0	0.8	0.8	0.8	0.8	0.9
BG 402-4	1.0	0.7	0.8	0.8	0.8	0.8
Root Length in (cm)						
NIAB 6	20.2 a	15.8 cd	17.0 b-d	17.2 bd	17.8 bc	17.8 bc
BG 402-4	20.2 a	15.2 d	16.0 cd	16.8 b-d	16.5 b-d	17.0 b-d
Tillering Capacity (no. $Plant^{-1}$)						
NIAB 6	42 a	22 de	23 d	26 c	27 c	30 b
BG 402-4	40 a	18 f	20 e	21 de	24 d	27 c

Means followed by the same letters in rows or columns are statistically non-significant with each other.

Table 2. Effect of external chloride Sulphate ratio at 100 mol m^{-3} ($NaCl+Na_2SO_4$) on shoot and root weight of two rice lines (Average of three replicates)

Varieties/lines	Control (Nutrient Soln)	100 mol m^{-3} ($NaCl+Na_2SO_4$) Cl:SO_4^{2-} ratio				
		100:1	75:25	50:50	25:75	1:100
Shoot fresh weight (g $plant^{-1}$)						
NIAB 6	79.3 a	31.5 g	32.5 f	41.0 e	49.1 d	61.7 b
		(60.2)	(59.0)	(48.3)	(38.0)	(22.2)
BG 402-4	77.0 a	28.0 h	30.0 gh	36.2 f	42.8 e	54.4 c
		(63.5)	(61.0)	(53.0)	(44.5)	(32.0)
Shoot dry weight (g $plant^{-1}$						
NIAB 6	18.1 a	6.2 fg	7.0 ef	7.8 de	8.8 cd	11.5 b
		(56.6)	(61.3)	(56.4)	(51.2)	(35.2)
BG 402-4	16.0 a	5.2 g	5.7 fg	6.2 fg	7.0 ef	9.1 c
Root fresh weight (g $plant^{-1}$)						
NIAB 6	31.2 a	8.4 fg	12.2 de	14.0 cd	16.5 c	20.1 b
BG 402-4	29.7 a	6.7 g	10. 1 ef	12.6 de	14.0 cd	16.4 c
Root dry weight (g $plant^{-1}$)						
NIAB 6	3.0 a	0.8 g	1.0 f	1.2 e	1.4 d	2.1 b
BG 402-4	2.9a	0.37 h	0.8 g	1.0 f	1.2 e	1.8 c

Means followed by the same letters in rows or columns are statistically non-significant with each other.

Table 3. Effect of external chloride sulphate ratio at 100 mol m^{-3} ($NaCl+Na_2SO_4$) on ionic composition (mol m^{-3}) of third leaf of two rice lines sampled at fifteen days (D_{15}) and thirty days (D_{30}) of salts stress (Average of three replicates)

Varieties/ lines	Control		100 mol m^{-3} ($NaCl+Na_2SO_4$) $Cl^-:SO_4^{2-}$ ratio									
	(Nutrient Soln)		100:1		75:25		50:50		25:75		1:100	
	D_{15}	D_{30}	D_{15}	D_{30}	D_{15}	D_{30}	D_{15}	D_{30}	D_{15}	D_{30}	D_{15}	D_{30}
NA^+ in leaf sap												
NIAB 6	18.3 i	20.2 hi	94.8g	98.6 b	95.1g	102.8 e	95.8 fg	104.8 ce	96.5 fg	105.2 d-e	96.5 fg	105.5 a-e
BG 402-4	21.2 h	22.8 h	105.0 b-e	107.0 a-d	105.0 b-e	107.5 a-c	104.8 ce	108.2 a	106.8 a-d	108.0 a-b	106.5 a-d	108.5 a
K^+ in leaf sap												
NIAB 6	330.2 g	425.7 a	164.8 s	289.2 j	180.0 r	310.6 i	200.2 p	335.5 f	225.0 n	360.5 e	260.2 l	391.0 o
BG 402-4	33.0 g	419.0 b	138.3 u	250.7 m	159.7 t	281.0 k	185.0 q	315.0 h	200.0 p	330.8 g	220.0 c	364.5 d
Cl^- in leaf sap												
NIAB 6	32.8 v	50.0 t	210.6 f	260.0 c	185.0 h	240.2 d	144.8 l	179.8 i	110.2 p	134.8 m	60.2 s	106.7 q
BG 402-4	39.3 u	59.2 s	232.2 e	292.3 a	200.0 g	265. 0 c	164.8 j	200.2 g	125.3 o	160.0 k	80.3 r	130.3 n

Means followed by the same letters in rows or columns are statistically non-significant with each other.

Table 4. Effect of external chloride sulphate ratio at 100 mol m^{-3} ($NaCl+Na_2SO_4$) on chemical composition of third leaf sampled at fifteen days (D_{15}) and thirty days (D_{30}) of salts stress of two rice lines (Average of three replicates)

Varieties/ lines	Control		100 mol m^{-3} ($NaCl+Na_2SO_4$) $Cl^-:SO_4^{2-}$ ratio									
	(Nutrient Soln)		100:1		75:25		50:50		25:75		1:100	
	D_{15}	D_{30}	D_{15}	D_{30}	D_{15}	D_{30}	D_{15}	D_{30}	D_{15}	D_{30}	D_{15}	D_{30}
Ca^+ in leaf sap (mol m^{-3})												
NIAB 6	16.8 q	20.0 op	20.5 n-p	25.0 m	27.5 l	31.0 k	35.2 j	39.7 i	44.8 h	49.2 g	58.5 d	61.3 c
BG 402-4	19.3 p	22.3 n	22.0 no	27.3 l	32.2 k	36.0 j	40.0 i	43.0 h	51.7 f	55.0 e	65.5 b	68.3 a
Zn in dry leaf ($\mu g\ g^{-1}$)												
NIAB 6	38.2 q	48.2 o	99.3 g	134.8 a	95.0 h	128.2 b	86.5 j	115.7 d	80.7 k	113.2 e	74.0 l	99.8 g
BG 402-4	33.0 r	45.7 p	85.2 j	121.0 c	80.3k	113.0e	74.8l	105.2f	62.8m	98.2g	57.8n	90.7i
P in dry leaf ($\mu g\ g^{-1}$)												
NIAB 6	2029 l	2206 k	3053 e	3412 c	2747 fg	3212 d	2502 hi	2770 fg	2248 k	2525 h	1974 l	2203 k
BG 402-4	2461 hi	2503hi	3498 bc	3840 a	3195 d	3549 b	2831f	3104d e	2301 jk	2690 g	2055 l	2390 ij

Means followed by the same letters in rows or columns are statistically non-significant with each other.

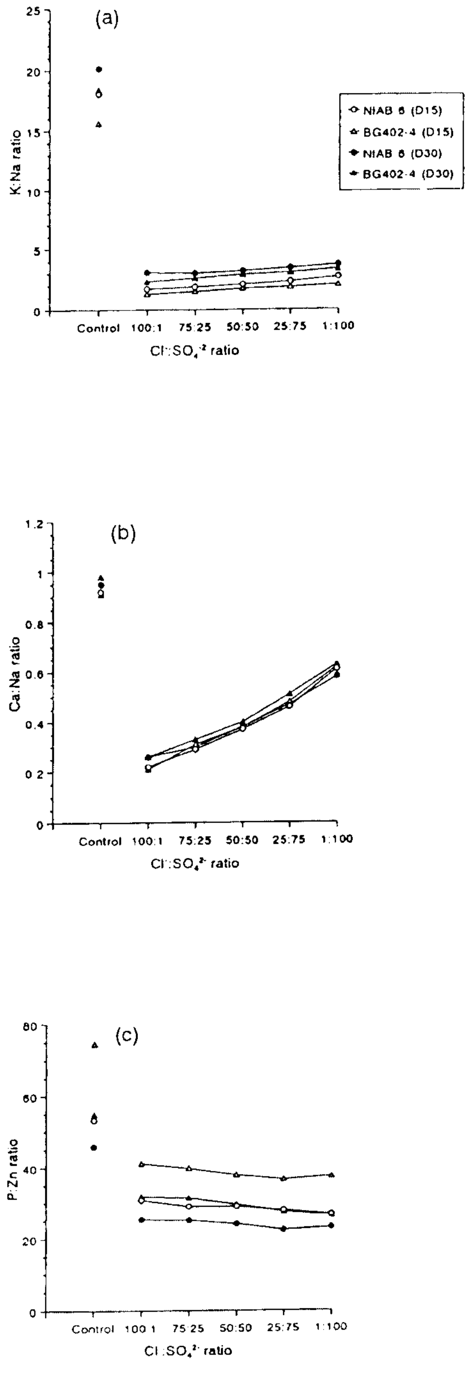

Fig. 1. Effect of external $Cl^-:SO_4^{2-}$ ratio at 100 mol m^{-3} ($Cl^-+SO_4^{2-}$) on leaf $K^+:Na^+$ ratio (a), $Ca^{2+}:Na^+$ ratio (b) and P:Zn ratio (c) of two rice lines

greater uptake of SO_4^{2-} resulted in a concomitant higher uptake of the divalent cation i.e. Ca^{2+} for a better charge balance at concentrations where $CaSO_4$ did not precipitate. The severe reduction in leaf K^+ under NaCl salinity may inhibit photosynthesis of the plants.

Increasing proportion of SO_4^{2-} in the saline medium reduced Zn and P concentrations in the leaf sap (Table 4) which seems to be important as lower P:Zn ratio was related to better salt tolerance (Aslam et al., 1993) and present results also verify these observations(Fig. 1). Overall, better growth of rice at lower Cl^-: SO_4^{2-} ratios seems to mediated through improved K^+ and Ca^{2+} concentrations in the leaf sap (Tobe et al., 2002 and James et al., 2005). It might lead to high Ca^{2+}:Na^+ and K^+:Na^+ ratios better utilization of NO_3 and also relatively lower p concentrations as these parameters were reported to be related with rice growth under saline conditions (Aslam et al., 1993). Our results confirm that rice is a poor Na^+ and Cl^- excluder, accumulating ions in leaves (See Hussain et al., 2003). Chloride and sodium toxicity, together with a severe reduction in K^+, ultimately resulted in leaf necrosis. This parameter was much pronounced in BG 402-4 indicating it s greater sensitivity as compared to NIAB 6.

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