

NUTRITIONAL EFFECT OF CALCIUM ON GROWTH AND IONIC CONCENTRATION OF WHEAT UNDER SALINE CONDITIONS

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The effects of calcium (6 and 12 mM) supply on plant growth, Na, K and Ca uptake of unstressed (0 mM NaCl) and stressed (75 mM NaCl) wheat seedlings (*Triticum aestivum* cv. Sarsabz) were investigated. Shoot dry matter production was significantly ($p < 0.05$) lower (58%) at 75 mM NaCl level than that at control. Elevated calcium (12 mM) supply to the root medium enhanced shoot growth by 36% as compared to control. Similarly 53 % reduction in root dry matter yield was recorded at 75 mM NaCl and 43 % yield increase was noted at 12 mM Ca level. At 75 mM NaCl + 12 mM Ca level, there was 17 % less sodium accumulation in shoot tissues as compared to 75 mM NaCl + 6 mM Ca level while 27% reduction in Na concentration in root tissue was observed at 12 mM Ca as compared to 6 mM Ca level. Relatively higher calcium supply (12 mM) to the root medium resulted in 20 % more K concentration. At 75 mM NaCl, 40% reduction in K concentration was found in shoot tissue as compared with control. In root tissue, 12 mM calcium supply enhanced K concentration by 58 % as compared to 6 mM calcium level at both salinity levels. A significant positive correlation ($r=0.99$) between shoot calcium content and corresponding dry matter yield indicated a positive role of calcium for increasing dry matter yield. Similarly, significant positive correlation ($r=0.99$) between shoot K and shoot dry matter yield was noticed exhibiting high K accumulation with the increasing dry matter yield. However, a negative correlation ($r= - 0.95$) between shoot dry matter yield and sodium content in shoot, is indicative of growth inhibition due to increasing sodium contents in shoots.

INTRODUCTION

The role of calcium in increasing salt tolerance of plants is well documented. External Ca^{2+} supply in the saline root medium can improve plant growth (Coale, *et al.*, 1984) by its dual effects: (a) improving soil structure and soil aeration and (b) increasing Ca^{2+}/Na^{+} ratio and enhancing Na^{+} exclusion ability of plant roots to suppress Na^{+} and Cl^{-} transport to leaves (Banuls, *et al.*, 1991). Elevated levels of external Ca^{2+} also frequently regulate and maintain K^{+} concentration in plants (Läuchli, 1990). These ameliorating effects of Ca^{2+} are in accordance with its functions in membrane integrity and control of selectivity in ion uptake and transport. On the other hand, high Na^{+} concentration in root medium may induce Ca^{2+} deficiency in plants grown with low Ca^{2+} or high Na^{+}/Ca^{2+} ratio by suppressing Ca^{2+} uptake and transport (Lynch and Läuchli, 1985). High Na^{+} concentration may also result in leakiness of leaf tissue membrane (Leopold and Willing, 1984). However, substitution of Ca^{2+} for Na^{+} may result in an impressive protective effect on membranes against leakiness induced by NaCl. Under saline-sodic conditions, plant yields may be reduced mainly due to ion imbalance of high Na^{+}/Ca^{2+} ratio. Gypsum, phosphogypsum, pressmud and other Ca^{2+} sources can improve plant growth by amelioration and management of sodic waters/soils (Chhabra, 1996). This paper describes the growth behavior of wheat for nutritional effects of Ca^{2+} under varied NaCl salinity. The seedling performance was evaluated for Ca^{2+} nutrition with respect to dry matter production and ionic concentrations in root and shoot under saline conditions.

MATERIALS AND METHODS

The hydroponic study was conducted to investigate the nutritional role of calcium on growth and ionic concentration of wheat (*Triticum aestivum* cv. Sarsabz) at two levels of NaCl salinity (0 and 75 mM). Two 7 days old pre-germinated uniform seedlings were foam plugged per hole in lids (with four holes) of the plastic pots containing 2.5 L of continuously aerated half strength modified Hoagland's nutrient solution (Epstein, 1972), without calcium salts. Two salinity levels (0 and 75 mM) with NaCl and two calcium levels (6 and 12 mM) with $CaCl_2$ were developed. The salinity levels were imposed a week after transplanting by incremental additions of 25mM NaCl on alternate days while Ca levels were maintained two days after transplantation. The pH of nutrient solution was maintained at 5.9 (± 0.05) and monitored daily. The nutrient solution was changed after ten days interval during the growth period. The pots were kept in growth chamber under controlled conditions having light intensity of 450 $\mu mol\ m^{-2}\ s^{-1}$, temperature at 30 (± 2) °C and photoperiod was adjusted 14 hours light period. The pots were arranged in factorial CRD fashion with three replications. The plants harvested 36 days after transplanting, were immediately separated into root and shoot and rinsed with deionized water. The plant samples were oven dried at 60 °C to a constant weight. The dried plant samples were ground to pass through a 40-mesh screen in a Wiley Mill. Ground samples of root and shoot were digested in di-acid mixture of nitric acid and perchloric acid (2:1) to estimate Na, K, Ca and Mg in the digest by atomic absorption spectroscopy. The data were statistically analyzed according to CRD and treatment means were compared using Duncan's Multiple Range (DMR) test (Gomez and Gomez, 1976).

RESULTS AND DISCUSSION

There was significant ($P < 0.05$) effect of NaCl salinity and calcium supply on shoot fresh weight of wheat cv. Sarsabz (Table 1). Decrease in total biomass production with increasing NaCl salinity at both levels of Ca^{2+} supply was significant ($P < 0.05$). Reduction in fresh shoot weight at 75 mM NaCl was 78 % over control. Adverse effect of increasing root medium salinity on plant growth has been reported by Hussain and Ilahi (1995), Ali *et al.* (1999) and Ali *et al.* (2001). Significant increase in shoot fresh weight was recorded due to enhanced Ca^{2+} supply in root medium at both the salinity levels. At elevated Ca^{2+} level (12 mM) in root medium, 65% and 28% increase in fresh shoot weight was recorded at 0 and 75 mM NaCl, respectively as compared to 6 mM Ca^{2+} level. Calcium supply significantly alleviated the adverse effects of NaCl salinity on biomass production. Application of external Ca + has been known to ameliorate salinity stress symptoms in many plant species (Leopold and Willing, 1984, Banuls, *et al.*, 1991). Although this effect is based on several phenomena (Kinraide, 1998), a clear physiological basis is now available from many studies on the impact of Ca^{2+} on ion channel. The results of this study are in accordance with these findings. Sixty five Percent increase in fresh shoot weight at 12 mM Ca + 0 mM NaCl as against 6 mM Ca + 0 mM NaCl which is indicative of relatively high Ca requirement of wheat variety "Sarsabz".

Significant ($P < 0.05$) increase in root fresh weight was recorded at elevated level of Ca supply (12 mM) (Table 1). However, the extent of increase in fresh weight was significantly less as compared to shoot fresh weight. Seven and twenty five percent increase in root fresh weight was recorded at 12 mM Ca + 0 mM NaCl and 12 mM Ca + 75 mM NaCl, respectively as against 6 mM Ca supply.

Table 1. Effect of salinity and calcium levels on shoot and root weights (g pot⁻¹) of wheat..

NaCl (mM)	Calcium (mM)		Means
	6	12	
shoot fresh weight			
0	5.33	8.83	7.18 A
75	1.37	1.75	1.56 B
Means	3.45 B	5.29 A	
Root fresh weight			
0	2.51	2.69	2.60A
75	0.91	1.22	1.06 B
Means	1.71 B	1.95 A	
Shoot dry weight			
0	1.12	1.52	1.32 A
75	0.52	0.58	0.55 B
Means	0.82 B	1.06 A	
Root dry weight			
0	0.50	0.65	0.57 A
75	0.20	0.34	0.27 B
Means	0.35 B	0.50A	

Significant ($P < 0.05$) effect on shoot dry matter yield was noticed due to NaCl salinity and Ca levels (Table 1). At 75 mM NaCl level, 58 % reduction in shoot dry matter yield was exhibited as against 0 mM NaCl. Grattan and Grieve (1992; 1999) reported nutritional differences between salt stressed and non-stressed plants. There are three major plant constraints for plant growth on saline substrates as described by Greenway and Munns (1980): (i) "drought stress" related to the low water potential of the rooting medium; (ii) ion toxicity associated with excessive uptake particularly of Na+ and Cl; (iii) nutritional imbalance as a result of depressed uptake, shoot transport and impaired internal distribution of minerals principally of Ca. Calcium levels significantly ($P < 0.05$) affected shoot dry matter yield. Twenty nine percent increase in shoot dry matter yield was recorded at 12 mM Ca supply as against 6 mM Ca supply. At 0 mM NaCl + 12 mM Ca level, 36 % yield increase was registered and at 75 mM NaCl + 12 mM Calcium level, 12 % increase in yield was recorded as compared to 6 mM calcium at both the salinity levels (Table 1). Calcium improves K+/Na+ selectivity of membranes (Marschner, 1995) and prevents the soil from invasion of toxic ions (Cramer *et al.*, 1987~. However, cation relations in the shoot tissue such Ca +/Na+ have been shown to possess a strong influence on salt tolerant than absolute Na+ levels (Ebert *et al.*, 2002).

Root dry matter yield was significantly ($P < 0.05$) affected by NaCl salinity levels (Table 1). Fifty three percent less root dry matter yield was noticed at 75 mM as against 0 mM NaCl. Calcium levels also significantly affected the root dry matter yield. Forty three percent yield increase was noticed at elevated supply of Ca (12mM). At 0 mM NaCl+ 12 mM Ca, 30 % yield increase was noticed as against 0 mM NaCl + 6 mM Ca level. Seventy percent yield increase was exhibited at 75 mM NaCl + 12 mM Ca level against 75 mM NaCl + 6 mM Ca level (Table 1).

Sodium chloride levels (Table 2) significantly affected Na+ concentration in shoot tissue. At 75 mM NaCl level, 750 % higher Na+ concentration was accumulated in plant tissue as against 0 mM NaCl. Calcium levels also significantly affected Na+ concentration in plants. There was 17 % less accumulation in shoot tissue grown at elevated supply of 12 mM Ca^{2+} supply as against 6 mM Ca^{2+} level.

In root tissues, same pattern of sodium accumulation was observed. At 75 mM NaCl level, 100 % more sodium accumulation was noticed in root tissues as against 0 mM NaCl level (Table 2). However, 27 % reduction in sodium concentration was observed where elevated level of Ca^{2+} (12 mM) was supplied as against normal Ca^{2+} supply (6 mM). Application of external Ca^{2+} has long been known to ameliorate salinity stress symptoms in many species. Although this effect is based on several phenomena (Kinraide, 1998), a clear

physiological base is now available from many studies on impact of Ca^{2+} on ion channel.

Table 2. Effect of salinity and calcium levels on ionic concentration (mg g⁻¹) in shoot and root of wheat.

NaCl (mM)	Calcium (mM)		Means
	6	12	
Sodium concentration in shoot			
0	4.6	2.5	3.55 B
75	32.3	28.1	30.2 A
Means	18.45 A	15.30 B	
Sodium concentration in root			
0	8.77	6.00	7.38 B
75	17.0	12.70	14.80 A
Means	12.88 A	9.35 B	
Calcium concentration in shoot			
0	1.42	1.80	1.61 A
75	0.92	1.20	1.06 B
Means	1.17 B	1.50 A	
Calcium concentration in root			
0	3.05	4.40	3.72 A
75	1.83	2.40	2.10 B
Means	2.44 B	2.75 A	
Magnesium concentration in shoot			
0	24.20	20.60	22.40 B
75	26.82	23.37	25.09 A
Means	25.51 A	21.98 B	
Magnesium concentration in root			
0	25.5	21.4	23.45 B
75	27.1	25.6	26.35 A
Means	26.3 A	23.5 B	
Potassium concentration in shoot			
0	69.43	72.65	71.04 A
75	34.03	51.80	42.91 B
Means	51.73 B	62.22 A	
Potassium concentration in root			
0	36.80	53.57	45.18 A
75	5.60	13.40	9.50 B
Means	21.20 B	33.48 A	

Salinity and Ca^{2+} levels in root medium significantly ($P < 0.05$) affected the Ca^{2+} concentration in shoot tissue (Table 2). There was 34 % reduction in shoot Ca^{2+} concentration at 75 mM NaCl level as against 0 mM NaCl. At 12 mM Ca supply, 28 % higher Ca^{2+} concentration was registered as against 6 mM Ca^{2+} supply in root medium.

Sodium chloride stress and Ca^{2+} levels significantly ($P < 0.05$) affected the root Ca^{2+} concentration (Table 2). Forty three percent decrease of Ca^{2+} concentration

in root was noticed at 75 mM NaCl level while 42 % more Ca^{2+} concentration was found where 12 mM Ca^{2+} was supplied in root medium as against 6 mM Ca^{2+} supply. Kinraide (1998) reported that main effect of Ca^{2+} on Na^{+} uptake at high Ca^{2+} concentration was the electrostatic displacement of Na^{+} from plasma membrane surface. Maathuis and Amtmaan (1999) concluded that the direct effect of Ca^{2+} on the movement of Na^{+} is manifested in voltage independent channels (VICs). The VIC-mediated Na^{+} currents in wheat and maize root protoplasts were strongly reduced by external Ca^{2+} . Robert and Tester (1997) and Tyer *et al.* (1997) reported Na^{+} inhibition by Ca in intact wheat roots. Similarly Jacobson *et al.* (1961), Rains and Epstein (1967), Elzam and Epstein (1969) and LaHaye and Epstein (1969) demonstrated that Ca^{2+} in the root medium depress the absorption of sodium.

Potassium concentration of shoot tissues was significantly affected by Ca supply and root medium salinity (Table 2). Elevated Ca (12 mM) supply to the root medium resulted in 20 % more K concentration in tissue as against 6 mM Ca supply. At 75 mM NaCl, 40 % less K concentration was found in shoot tissue. In root tissue, 12 mM Ca supply enhanced K concentration by 58 % as against 6 mM Ca level (Table 2). Pitman (1966) reported that Ca may alter the selectivity of K^{+} in certain plants. A part of Ca may have a simple physical effect on root permeability. In addition, Ca^{2+} may act on metabolism, in view of its own effects on enzyme systems and in particular on ATPase system. Moreover, Kinraide (1999) in his study concluded that Ca^{2+} prevents the leakiness of intercellular K^{+} . Severe reduction (79 %) was recorded in root K concentration at 75 mM NaCl level in root medium as compared to non-saline medium.

Magnesium concentration of shoot tissue was significantly ($P < 0.05$) affected by the levels of Ca^{2+} and salinity (Table 2). At 12 mM Ca^{2+} level, 13 % less magnesium was accumulated in shoot tissue as compared to 6mM Ca supply. There was 12 % more Mg accumulation at 75 mM NaCl as compared to non-saline medium. Similar pattern of Mg accumulation was noticed in root tissue (Table 2). About 11% less Mg accumulation was noticed at 12 mM Ca^{2+} as against 6 mM Ca^{2+} . However, 12 % more Mg concentration was recorded in root tissue at 75 mM NaCl.

Root medium salinity resulted in more Mg and Na accumulation in root and shoot tissue that inhibited plant growth (Table 1). While addition of Ca (12mM) in the root medium significantly reduced the concentration of Na and Mg in plant tissues and increased the Ca and K concentration in plant tissues which had a positive effect on plant growth.

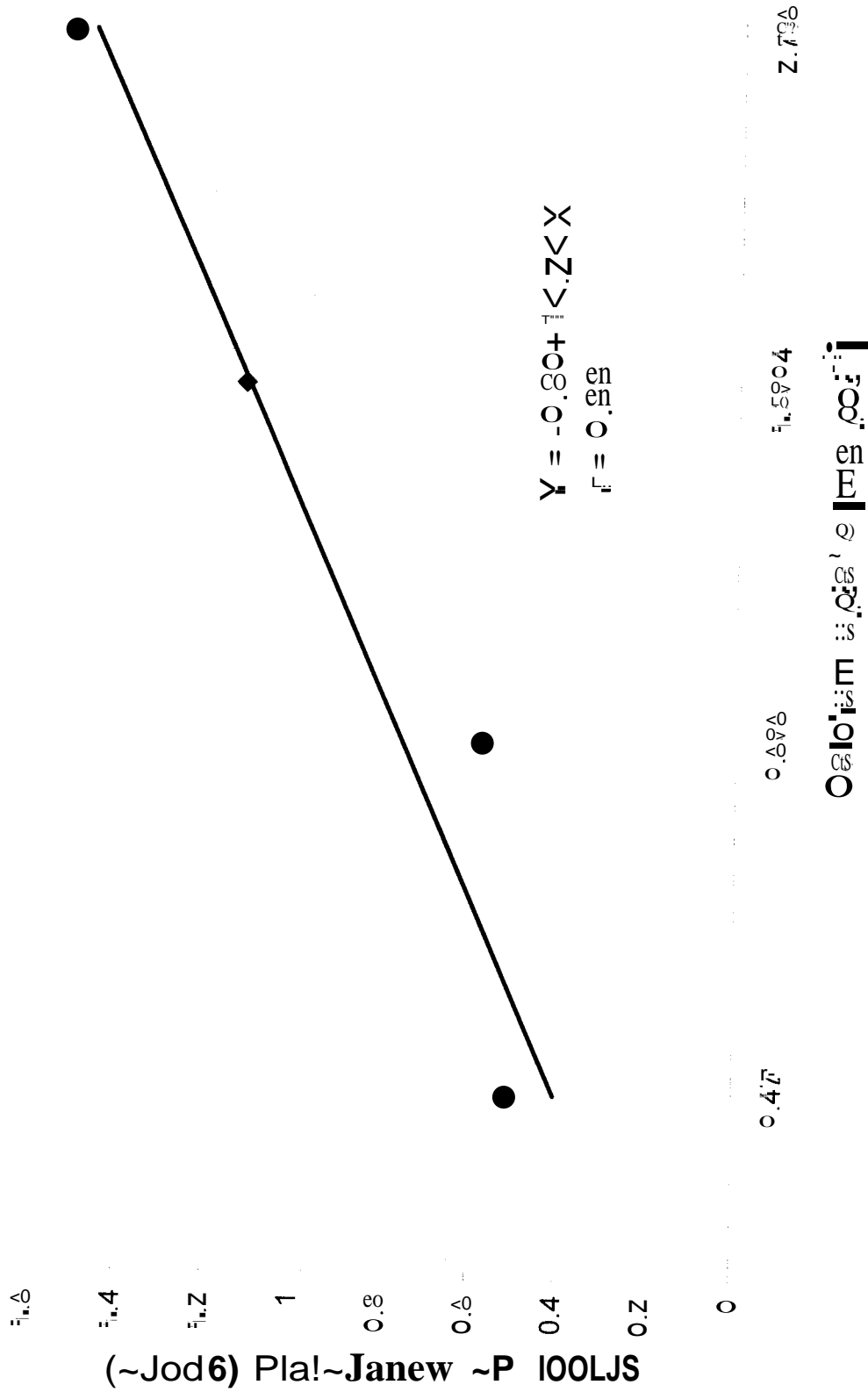


Fig. 1. Relationship between calcium and weight gain in growing rats.

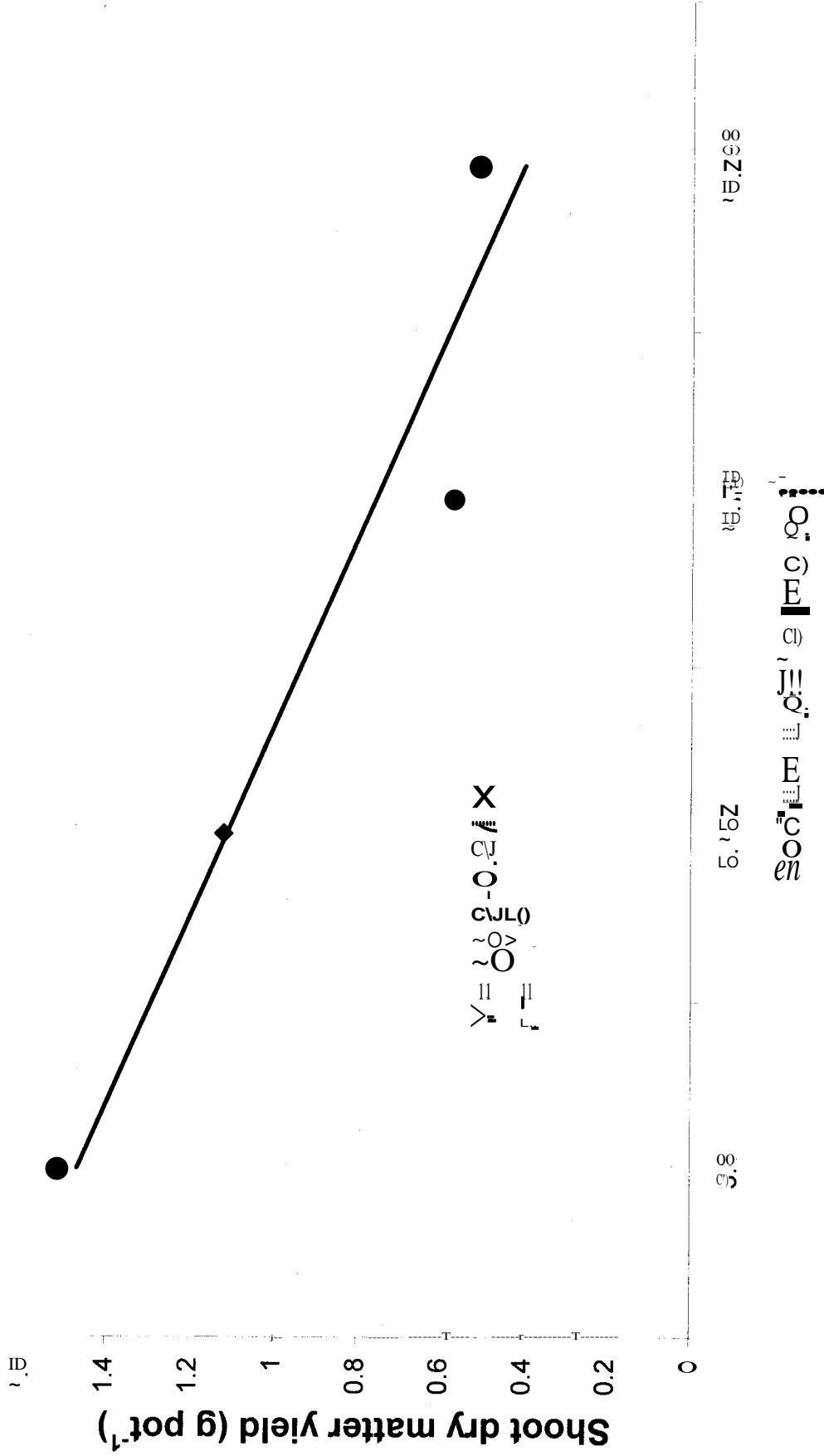


FIG. 2. Relationship between shoot dry matter yield and the variable on the x-axis.

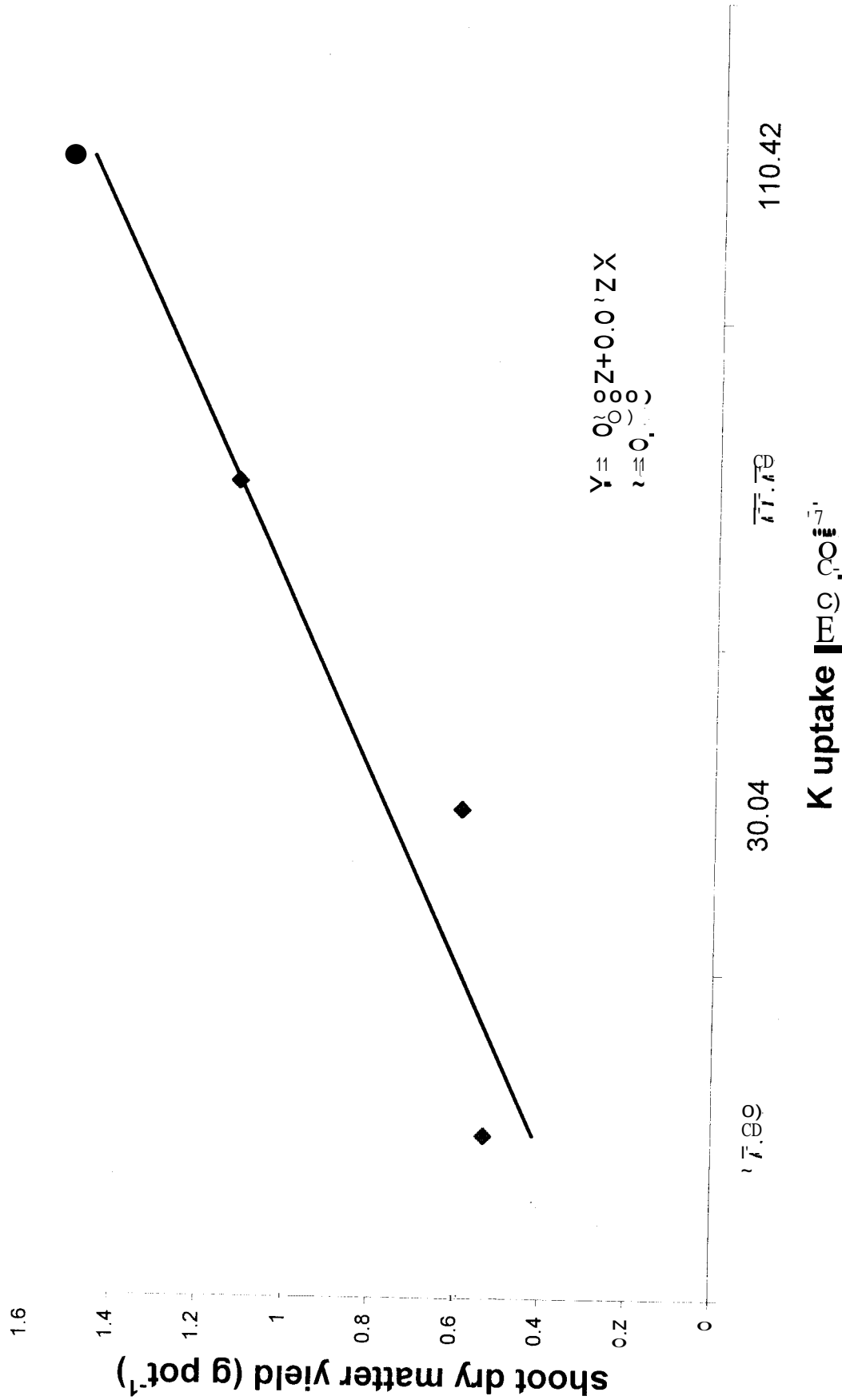


Fig. 3. Relationship between shoot dry matter yield and K uptake

Data in Fig. 1 indicate a significant positive correlation ($r = 0.99$) between shoot Ca^{2+} uptake and shoot dry matter yield. However, data in Fig 2 exhibit negative correlation ($r = - 0.95$) between shoot dry matter yield (g pot⁻¹) and Na uptake in shoot, which is indicative of growth inhibition due to increasing Na contents in shoots. The accumulation of Na in shoot decreases plant growth as explained by Ali *et al.* (2001) and Salim and Pitman (1983). Relationship between K uptake and shoot dry matter was similar to Ca^{2+} vs dry matter yield (Fig.3). There was significant positive correlation ($r=0.99$) between shoot K and shoot dry matter yield. It is concluded that NaCl presence in root medium decreased plant growth, K and Ca uptake and increased Na uptake. However, elevated supply of Ca in the presence of NaCl improved plant growth and uptake of K and Ca.

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