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https://doi.org/10.5109/4581

出版情報：九州大学大学院農学研究院紀要. 49 (2), pp.217-224, 2004-10-01. Faculty of Agriculture, Kyushu University
バージョン：

J. Fac. Agr., Kyushu Univ., **49** (2), 217–224 (2004)

Responses of Soybean and Cucumber Plants to NaCl and $CaCl_2$ Salinity in Nitrate and Enhanced Ammonium Nutrition

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(*Received June 15, 2004 and accepted July 13, 2004*)

The effect of nitrate (4 mM NO_3^-) and enhanced ammonium (3.5 mM NH_4^+ + 0.5 mM NO_3^-) nutrition on the growth of soybean and cucumber plants grown hydroponically at two levels (25 and 50 mM Cl^-) of NaCl and $CaCl_2$ salinity stress was investigated. The growth of both plants was better in nitrate nutrition than in enhanced ammonium nutrition. The pH values for enhanced ammonium solution decreased similarly for both plants regardless of salinity stress. In nitrate nutrition, lower salinity did not affect soybean growth, whereas higher salinity clearly suppressed its growth. In the case of nitrate–fed cucumber plants, dry matter production was not decreased by the addition of $CaCl_2$ but was by the addition of NaCl. In enhanced ammonium nutrition, the extent of growth suppression due to salinity was only small in soybean and cucumber plants. In both plants, trends in increases in the concentrations of Na, Ca and Cl in leaf parts caused by NaCl and $CaCl_2$ salinity were similar in both nitrate nutrition and enhanced ammonium nutrition. Leaf Na concentrations did not increase in soybean plants but did in cucumber plants. Leaf N concentrations that were higher in enhanced ammonium nutrition than in nitrate nutrition were not affected by salinity treatments. These results suggest that the acidity of the solution rather than the high levels of added salts affects plant growth in enhanced ammonium nutrition, and therefore, the effect of salinity on plant growth is less discernable in enhanced ammonium nutrition.

INTRODUCTION

Tolerance to salinity considerably varies among plant species and depends on the plant's ability to control salt uptake from media, such as soil, and to translocate salt from root to shoot and to effectively compartmentalize the salt at the cellular levels (Munns, 2002). In our previous study, we investigated the sensitivity of soybean and cucumber plants that were grown using nitrate as the source of N (Dabuxilatu and Ikeda, 2003). Soybean was shown to have a higher sensitivity to Cl than cucumber. Soybean also demonstrated a greater ability to restrict translocation of Na from root to leaf. As a result, Na toxicity is unlikely to be observed in the leaf of soybean. In contrast, cucumber was found to be tolerant to Cl and sensitive to Na (Dabuxilatu and Ikeda, 2003).

It is well recognized that ammonium nutrition results in lower concentrations of cationic minerals in each organ of various plants compared to nitrate nutrition (Van

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Beusichem *et al.,* 1988). Several researchers reported the effect of nitrogen sources on the growth of various plant species grown under saline conditions (Silberbush and Lips 1988; Bourgeais–Chaillow *et al.*, 1992; Leidi *et al.,* 1991; Lewis *et al.,* 1989; Hawkins and Lips, 1989; Speer *et al.,* 1994; Speer and Kaiser, 1994; Ashraf, 1999; Ashraf and Sultana, 2000; Al–Mutawa and El–Katony, 2001). The supply of ammonium nitrogen increased the sensitivity of wheat, maize and pea plants to salinity (Lewis *et al.*, 1989; Speer *et al.*, 1994). Shoot dry weight of wheat plants was not affected by nitrogen sources under salinity stress (Leidi *et al.*, 1991). In soybean, root and stem biomass were not decreased by NaCl but leaf biomass was in both nitrate and ammonium media (Bourgeais–Chaillow *et al.*, 1992). Peanuts grew better in ammonium media than nitrate media under saline conditions (Silberbush and Lips, 1988). The biomass production of nitrate–supplied wheat was reduced by NaCl stress to a greater extent than that of ammonium–supplied wheat, and increasing Ca concentration in nutrient media resulted in an increase in biomass production of NaCl–stressed, nitrate–supplied plants but this was not the case for ammonium–supplied plants (Hawkins and Lewis, 1993).

In this study, we compared the growth and mineral accumulation in soybean and cucumber plants grown in nitrate and enhanced ammonium nutrition with high concentrations of NaCl and $CaCl_2$.

MATERIALS AND METHODS

Soybean (*Glycine max* L. Merrill cv. Fukuyutaka) seeds that had been sterilized with NaClO solution (0.5% effective chlorine) and cucumber (*Cucumis sativus* L. cv. Chojitsuochiai Nigou) seeds sterilized with a fungicide by the producer were germinated for 2 days on filter paper in petri dishes at 25 °C in the dark. The germinated seeds were transplanted into vermiculite moistened with tap water and grown for 13 days. Seedlings of both plants were then transferred individually to 3–L pots containing one–fourth strength aerated Hoagland nutrient solution (pH 6.0) (Downs and Hellmers, 1975; Ikeda *et al.,* 1992). At this stage, the soybean seedlings had fully expanded primary leaves and the cucumber the first leaf. Both plants were grown under natural greenhouse conditions in Kyushu University during the summer months. In both cases, the nitrogen source was 4 mM $NaNO_3$ prior to the initiation of salt treatment.

Twenty–day–old soybean plants were subjected to salt treatment for 11 days and 23–day–old cucumber plants for 13 days. For both nitrate (4 mM $NaNO_3$) and enhanced ammonium (1.75 mM $(NH_4)_2SO_4$ + 0.5 mM $NaNO_3$) nutrition, the salinity was imposed by adding the appropriate salts to the growth medium in order to give the selected concentrations; 25 and 50 mM for NaCl and 12.5 and 25 mM for $CaCl_2$. Controls and treatment pots were set in triplicate. The nutrient solution was renewed every 3 days. Prior to changing, the pH was measured using a pH meter (HM–1K, TOA Electronics Ltd., Tokyo).

Harvested plants were washed with water and separated into root, stem (plus petiole) and leaf parts. All the plant parts were dried at 70 °C for 3 days. After the dry weight was recorded, these materials were milled. Chloride from powdered samples was extracted in water at 45 °C and the levels determined by ion chromatography (ICA–5000 system, TOA Electronics Ltd., Tokyo). Powdered samples were digested by heating with salicylic acid

– H_2SO_4 – H_2O_2 (Cataldo *et al.*, 1974) for the determination of K, Na, Ca, Mg and N. Potassium, Na, Ca and Mg in the digest solution were determined by atomic absorption (Z–5300, Hitachi, Tokyo). Nitrogen in the digest solution was determined by the indophenol method (Cataldo *et al.*, 1974).

RESULTS

Dry matter production of soybean and cucumber plants is shown in Fig. 1. In the control soybean plants, dry mass of all the organs was greater in nitrate nutrition than in enhanced ammonium nutrition. The supply of 25 mM NaCl and 12.5 mM $CaCl_2$ did not affect dry mass, but the supply of 50 mM NaCl and 25 mM $CaCl_2$ clearly decreased dry

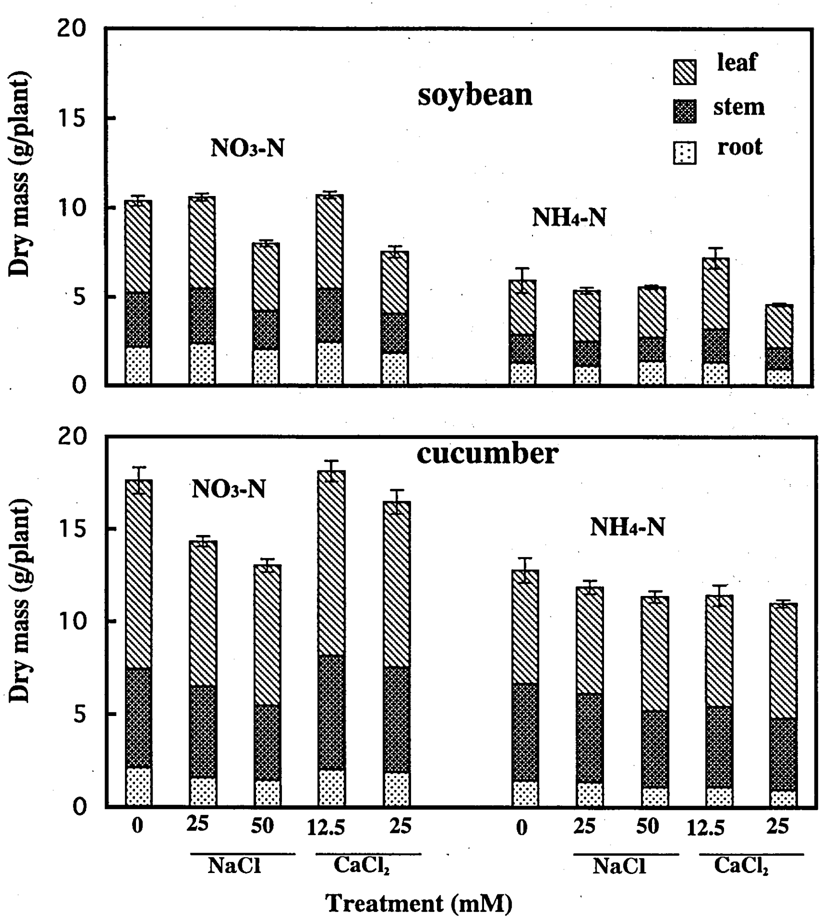

Fig. 1. Effect of salinity and nitrogen sources on dry mass of soybean and cucumber plants. Error bars on columns indicate S. D. of means (n=3).

mass in nitrate nutrition, particularly in stem+petiole and leaf parts. In enhanced ammonium nutrition, dry mass was slightly decreased by the supply of higher concentrations of both salts, although the supply of 12.5 mM $CaCl_2$ resulted in an increase in dry mass.

The dry mass of cucumber plants was lower in enhanced ammonium nutrition than in nitrate nutrition, especially for leaf and root part (Fig. 1). In nitrate nutrition, the supply of NaCl clearly decreased dry mass, whereas the supply of $CaCl_2$ had no effect. In the case of enhanced ammonium nutrition, the extent of growth suppression was lower with NaCl supply than with $CaCl_2$ supply although growth suppression was not marked.

The pH of the nutrient solution increased from pH 6 to pH 8 in nitrate nutrition for both plants. In the case of enhanced ammonium nutrition, it decreased from pH 6 to pH 5 for soybean and to pH 4 for cucumber during the first period of treatment and to pH 4 for soybean and to pH 3 for cucumber during the final period of treatment. The pH decreases in enhanced ammonium nutrition were similar for both plants with or without added salts, suggesting that the uptake of ammonium was little affected by added salts. There is a possibility that the lowering of pH might affect the growth of both plants in the case of enhanced ammonium nutrition regardless of added salts.

When soybean plants were supplied with NaCl, the Na concentration remained very low in the leaf parts irrespective of nitrogen nutrition conditions, whereas it increased in the root and stem. This increase was found to be related to the concentration of NaCl supplied (Table 1). In the case of cucumber, Na concentrations in all the plant parts including the leaf were greatly increased by the supply of NaCl.

The supply of NaCl and $CaCl_2$ greatly increased Cl concentrations in all the parts of both plants (Table 1). The nature of the added salts and nitrogen sources did not clearly cause consistent differences in the increase of Cl concentrations. Even in nitrate–fed cucumber plants treated with 25 mM $CaCl_2$, despite the extremely high observed Cl levels, the growth was similar to that of the control plants (see Fig. 1).

Mineral concentrations in leaf parts affected by the supply of NaCl and $CaCl_2$ are shown in Table 2. Potassium concentrations were decreased by the supply of NaCl in

Table 1. Effect of NaCl and $CaCl_2$ on the leaf Na and Cl concentrations in soybean and cucumber plants grown in different nitrogen nutrition.

Treatment		Soybean		Cucumber	
	(mM)	Na	Cl	Na	Cl
		(g/kg dry matter)			
N control	–	0.15±0.05	14.5±0.8	2.7±0.4	6.3±0.4
N NaCl	25	0.33±0.23	20.1±1.1	11.4±3.5	22.3±1.2
N NaCl	50	0.22±0.12	21.2±1.2	14.2±0.7	29.2±1.6
N $CaCl_2$	12.5	0.25±0.16	22.8±1.3	2.1±0.5	22.9±2.3
N $CaCl_2$	25	0.22±0.19	18.2±1.0	1.9±0.5	32.2±1.8
A control	–	0.10±0.01	10.4±0.6	2.0±0.2	14.3±0.8
A NaCl	25	0.23±0.09	18.6±1.0	10.7±0.4	36.7±3.0
A NaCl	50	0.31±0.01	29.1±1.6	25.7±0.7	40.2±2.2
A $CaCl_2$	12.5	0.20±0.15	22.6±1.3	2.6±0.3	31.8±1.8
A $CaCl_2$	25	0.20±0.15	31.9±1.8	1.8±0.2	36.4±2.0

N: nitrate nutrition, A: enhanced ammonium nutrition; Data are shown as means ±S. D. (n=3).

cucumber, but not in soybean regardless of the nitrogen source. The supply of $CaCl_2$ slightly decreased K concentrations in soybean, but not in cucumber. Ca concentrations in both plants were increased by the supply of $CaCl_2$ in both types of nitrogen nutrition, but the supply of NaCl did not show a consistent trend with regards to Ca concentrations. Magnesium concentrations were decreased by the supply of $CaCl_2$ while added NaCl afforded little effect on Mg concentrations in both plants.

In both plants, leaf nitrogen concentrations appeared higher in enhanced ammonium nutrition than in nitrate nutrition. Salinity treatment with NaCl and $CaCl_2$ did not affect leaf nitrogen concentrations (Table 2).

Mineral accumulation in soybean and cucumber plants grown in nitrate and enhanced ammonium nutrition under salt-stress conditions is shown in Table 3. K accumulation in soybean was greatly lowered by the addition of the higher concentration of either NaCl or $CaCl_2$ in both the nitrogen nutrition conditions while it was lowered in cucumber only by the addition of NaCl irrespective of different nitrogen nutrition. In both plants, Ca accumulation was, as a whole, increased by the addition of $CaCl_2$. In the case of enhanced ammonium nutrition, this increase did not always show a dependence on the treatment concentration of $CaCl_2$. Only NaCl treatment in nitrate-fed soybean plants reduced Ca accumulation. Supply of NaCl and $CaCl_2$ reduced Mg accumulation except for soybean plants grown in enhanced ammonium nutrition. The extent of increases of Na accumulation was large compared to that of Cl accumulation in both plants. There was

Table 2. Effect of NaCl and $CaCl_2$ on K, Ca, Mg, and N concentrations in the leaf part of soybean and cucumber plants grown in different nitrogen nutrition.

Treatment		K	Ca	Mg	N
	(mM)	(g/kg dry matter)			
			Soybean		
N control	–	20.4±1.3	23.2±3.0	5.2±0.3	47.6±1.7
N NaCl	25	22.2±3.4	13.8±0.7	4.6±0.2	47.6±0.9
N NaCl	50	21.3±0.8	15.0±4.7	4.8±0.1	45.7±1.9
N $CaCl_2$	12.5	17.2±1.3	24.6±4.4	3.9±0.1	44.4±1.3
N $CaCl_2$	25	16.2±1.0	28.5±1.4	3.6±0.2	41.4±1.9
A control	–	24.6±4.9	8.8±3.5	3.9±0.2	48.1±1.0
A NaCl	25	26.7±1.0	10.0±3.2	4.2±0.1	55.9±1.5
A NaCl	50	28.8±1.2	15.1±4.2	5.2±0.2	57.4±0.8
A $CaCl_2$	12.5	20.5±0.9	20.8±5.3	3.8±0.1	52.2±1.5
A $CaCl_2$	25	20.3±0.7	26.2±6.3	4.0±0.4	47.8±3.9
			Cucumber		
N control	–	31.1±1.2	33.5±2.5	4.7±1.0	38.9±5.1
N NaCl	25	25.3±3.5	42.1±2.2	4.7±0.5	44.5±4.6
N NaCl	50	21.6±2.4	41.4±6.7	5.3±0.2	40.3±3.6
N $CaCl_2$	12.5	31.1±3.0	53.2±4.4	3.4±0.2	42.1±0.7
N $CaCl_2$	25	32.3±4.2	70.5±1.7	3.5±0.3	47.4±1.3
A control	–	37.0±3.2	35.1±1.5	3.6±0.4	56.6±2.0
A NaCl	25	27.7±1.2	33.1±2.3	3.3±0.2	54.8±1.8
A NaCl	50	23.7±3.0	36.2±1.0	2.8±0.1	53.3±3.7
A $CaCl_2$	12.5	32.8±0.8	45.5±0.7	2.9±0.3	56.5±1.1
A $CaCl_2$	25	36.0±7.4	53.1±2.1	2.9±0.1	53.0±1.7

N: nitrate nutrition, A: enhanced ammonium nutrition; Data are shown as means ±S. D. (n=3).

Table 3. Effect of salinity on mineral content of soybean and cucumber plants grown in different nitrogen nutrition.

Treatment	(mM)	K	Ca	Mg	Na	Cl
				(mg per plant)		
				Soybean		
N control	–	304	176	57	8	128
N NaCl	25	298	120	54	35	206
N NaCl	50	210	92	41	70	212
N $CaCl_2$	12.5	269	213	50	6	243
N $CaCl_2$	25	187	165	34	4	175
A control	–	190	53	21	5	66
A NaCl	25	171	50	21	26	114
A NaCl	50	142	61	24	80	184
A $CaCl_2$	12.5	205	130	29	5	145
A $CaCl_2$	25	127	108	19	3	137
				Cucumber		
N control	–	818	475	86	83	181
N NaCl	25	554	453	64	250	383
N NaCl	50	421	423	66	302	496
N $CaCl_2$	12.5	848	728	76	53	446
N $CaCl_2$	25	841	824	68	42	550
A control	–	567	278	41	37	282
A NaCl	25	417	245	34	202	380
A NaCl	50	350	273	33	327	514
A $CaCl_2$	12.5	479	404	31	30	446
A $CaCl_2$	25	508	452	30	23	473

N: nitrate nutrition, A: enhanced ammonium nutrition.

little difference in Cl accumulation between NaCl and $CaCl_2$ supply and between nitrate and enhanced ammonium nutrition.

DISCUSSION

The effects of NaCl and $CaCl_2$ salinity with the same concentration of Cl were compared in this study, because the effect of Cl rather than Ca was expected to appear in the $CaCl_2$ treatment. Enhanced ammonium nitrogen was adopted as the nitrogen source in place of ammonium–only solution since nitrate was reported to be necessary for the synthesis and transport of the plant hormone (Walch–Liu *et al.*, 2000). Nevertheless, even without salt treatments, the growth of enhanced ammonium–fed plants was inferior to that of nitrate–fed plants.

The observed responses of soybean and cucumber plants to NaCl and $CaCl_2$ in nitrate media were similar to those reported in the previous paper (Dabuxilatu and Ikeda, 2003). In enhanced ammonium nutrition, however, the supply of both salts resulted in slightly reduced growth of plants except for soybean treated with 12.5 mM $CaCl_2$, although the growth of control plants fed with enhanced ammonium nitrogen was already suppressed compared to that of nitrate–fed plants. Hence the extent of growth inhibition due to salinity was less in enhanced ammonium nutrition than in nitrate nutrition. Other studies also showed similar results concerning salinity effects on different plants whose growth was inhibited by ammonium nutrition regardless of salinity. These studies, however, did not make reference to pH changes in ammonium and nitrate media. (Hawkins and Lewis, 1993; Ashraf and Sultana, 2000; Ashraf, 1999). It should be noted that ammonium nutrition induced the lowering of solution pH unless it was adjusted automatically or frequently. If the solution acidity in enhanced ammonium nutrition increases to a level where it inhibits root growth during salinity treatment, the low pH might affect the growth of the plant to a greater extent than the tested level of salinity. The pH of the enhanced ammonium nutrient solution decreased to pH 3 in some cases. Thus, the masking of salinity effects by the effects of acidity could be a reason for the apparent lack of sensitivity to salinity in enhanced ammonium nutrition. On the contrary, Speer and Kaiser (1994a) reported that ammonium nutrition which had no negative effect on plant biomass production drastically increased the sensitivity of pea plants to 50 mM NaCl (moderate salinity). It was suggested that the lower intracellular compartmentation capacity of ammonium-fed pea plants compared to that of nitrate-fed plants was responsible for the higher sensitivity (Speer and Kaiser, 1994b).

The extent of pH decrease observed in enhanced ammonium nitrogen solutions was similar in any treatment, although the decrease was somewhat larger in cucumber than soybean. Because increases in proton activity (pH decreases) are almost parallel to the amount of ammonium ion absorbed by plants from nutrient solution containing ammonium nitrogen (Breteler, 1973), it is assumed that the absorption of ammonium nitrogen may not be restricted by the supply of salts.

Al-Mutawa and El-Kantony (2001) reported that two wheat cultivars exhibited greater tolerance to NaCl in nitrate nutrition than in ammonium nutrition. They also suggested that in the case of NaCl salinity, the lower K/Na ratio observed in ammonium-fed plants compared to nitrate-fed plants might be responsible for the low salinity tolerance in ammonium nutrition. When NaCl was added in our experiment, Na concentration did not increase in soybean leaves but increased in cucumber leaves irrespective of the nitrogen source. Consequently, K/Na ratios did not decrease in soybean leaves, but did decrease markedly in cucumber leaves. In addition, cucumber leaves had much larger K/Na ratios than soybean leaves. These results suggest that growth suppression caused by NaCl salinity might not be due to low K/Na ratios in nutrient balance especially in leaves. Although soybean growth was suppressed more in 50 mM NaCl than in 25 mM NaCl, Cl concentrations for both salinity treatments were similar in the case of nitrate nutrition. Hence, the high observed concentrations of Cl in soybean leaves might be excluded as being a reason for growth suppression in 50 mM NaCl.

Changes in the accumulation of Na, Ca and Cl in leaf parts affected by respective salt treatments were similar between nitrate nutrition and enhanced ammonium nutrition in soybean and cucumber plants, although plant growth was inhibited by enhanced ammonium nutrition with or without added salts. These results suggest that the presence of ammonium at the present concentration in the nutrient solution would not suppress uptake of Na and Ca that were added at high concentrations in the salinity treatments. Low accumulation of K, Ca and Mg in whole plant in enhanced ammonium nutrition compared to nitrate nutrition can be accounted for by lower dry matter production in enhanced ammonium nutrition.

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