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Relationships between Salt Tolerance of Green Soybeans and Calcium Sulfate Applications in Sand Culture

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Summary

Green soybeans (*Glycine max* Merr.) were grown to maturity in sand to determine relationships between salt tolerance of the plants and $CaSO_4$ applications, using diluted sea water. Dry weight of the whole plant, fresh weight of pods and seeds, and number of pods and root nodules were greatest at 0 ppm Cl and decreased with increasing sea water concentrations. Growth at 0 and 250 ppm Cl tended to be greater at 1 mM $CaSO_4$ than at 12 mM $CaSO_4$. There was no effect of $CaSO_4$ treatments on the growth at 500, 1,000 and 2,000 ppm Cl. Na and Cl in leaves and sand solution, and EC values of sand solution increased with increasing sea water concentrations. $CaSO_4$ applications scarcely affected Na and Cl in leaves and sand solution, and EC values of sand solution at each sea water concentration. Ca in leaves, and Ca and SO_4 in sand solution tended to be higher at 4, 8 and 12 mM $CaSO_4$ than 1 mM $CaSO_4$. In the current experiment, salt tolerance of green soybeans in sand culture was not enhanced by the application of $CaSO_4$, indicating that the role of Ca to salt tolerance may differ with crops.

Introduction

Salt tolerance of green soybeans (*Glycine max* Merr.) has been previously reported using sea water diluted with nutrient solution and tap water in sand and soil cultures (11). The sand culture experiment showed that whole plant dry weight decreased as sea water concentrations increased over 250 ppm Cl. This growth reduction seemed to be caused by increasing Na and/or Cl in the sea water diluted with nutrient solution.

There are reports discussing the relationship between Na absorption of plants and Ca concentrations in the medium. LaHaye and Epstein (8) have reported that Ca increases the salt (sodium) tolerance of bean plants. Kawasaki and Moritsugu (6) also found that growth reduction of corn and bean plants by NaCl was smaller at a base Ca concentration (1.0mM) than a low Ca concentration (0.1mM).

Therefore, the purpose of this experiment was to determine relationships between calcium sulfate ($CaSO_4 \cdot 2H_2O$) applications and the salt tolerance of green soybeans grown to maturity in sand receiving sea water diluted with a base nutrient solution. Also the salt tolerance in relation to chemical properties of sand solution was examined.

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Materials and Methods

Twenty-seven seeds of cv. 'Hakucho' were directly sown in a wooden container (40×40×12cm) filled with sand and placed in a plastic house, on April 8, 1977. Seedlings were thinned to 9 uniform plants per container on April 21. Treatments were made in a factorial arrangement involving 5 levels of Cl (0, 250, 500, 1,000 and 2,000 ppm) of diluted sea water and 4 levels of $CaSO_4 \cdot 2H_2O$ (1, 4, 8 and 12mM, but at 2,000ppm Cl only 4 and 12mM $CaSO_4 \cdot 2H_2O$ were combined). Thus there, were 18 treatments each having 5 replications, with a total of 90 container plots. Sea water taken at Miho seaside was diluted with base nutrient solution, as shown in Table 1, to make 5 levels of diluted sea water. The treatment solutions were applied to the sand twice on clear days, once on cloudy days from April 11 to sampling time. There was no application on rainy days. Samplings were made of plants

Table 1. Treatments and composition of nutrient solution.

Treatments			
No.	Sea water[X] concentrations Cl(ppm)	Sea water[X] concentrations %	$CaSO_4$ concentrations (mM)
1	0	0	1
2			4
3			8
4			12
5	250	1.25	1
6			4
7			8
8			12
9	500	2.50	1
10			4
11			8
12			12
13	1,000	5.00	1
14			4
15			8
16			12
17	2,000	10.00	4
18			12

Composition of nutrient solution	
Cl from sea water	designated concentrations
Ca from $CaSO_4 \cdot 2H_2O$	designated concentrations
KH_2PO_4	1 mM
KNO_3	5 mM
$MgSO_4 \cdot 7H_2O$	2 mM
Fe from EDTA-Fe	1 ppm
Zn from $ZnSO_4 \cdot 7H_2O$	0.05 ppm
Cu from $CuSO_4 \cdot 5H_2O$	0.02 ppm
B from H_3BO_3	0.5 ppm
Mo from $Na_2MoO_4 \cdot 2H_2O$	0.05 ppm
Mn from $MnSO_4$	0.5 ppm
pH≒6.0	

X: Sea water contains 20,500 ppm Cl, 2,632 ppm SO_4, 445 ppm K, 10,082 ppm Na, 393 ppm Ca and 1,262 ppm Mg.

Table 2. Effect of sea water concentrations and $CaSO_4$ applications on growth of green soybeans (Average per container).

No.	Cl concns (ppm)	$CaSO_4$ concns (mM)	Whole plant[X] dry wt (g)	Seeds+pods (g) Fresh wt	Seeds+pods (g) Fresh wt per pod	No. of pods	No. of root nodules	No. of podded plants
1	0	1	197^{a}	426^{a}	1.88^{ab}	227^{a}	325^{a}	9
2		4	189^{ab}	392^{b}	1.90^{a}	206^{b}	286^{a}	9
3		8	180^{bc}	373^{b}	1.84^{ab}	204^{b}	226^{ab}	9
4		12	173^{cd}	368^{bc}	1.85^{ab}	199^{bc}	292^{a}	9
5	250	1	173^{cd}	342^{cd}	1.74^{bc}	197^{bc}	292^{a}	9
6		4	159^{e}	314^{d}	1.74^{bc}	181^{cd}	160^{bc}	9
7		8	161^{de}	313^{d}	1.82^{ab}	172^{de}	170^{b}	9
8		12	164^{de}	319^{d}	1.73^{bc}	184^{cd}	125^{bcd}	9
9	500	1	121^{f}	248^{e}	1.55^{d}	161^{ef}	126^{bcd}	9
10		4	125^{f}	251^{e}	1.57^{d}	161^{ef}	117^{bcde}	9
11		8	113^{f}	239^{e}	1.67^{cd}	143^{f}	46^{cde}	9
12		12	117^{f}	245^{e}	1.62^{cd}	151^{f}	52^{cde}	9
13	1,000	1	81^{g}	150^{f}	1.26^{ef}	121^{g}	41^{de}	9
14		4	77^{g}	148^{f}	1.33^{e}	111^{g}	22^{de}	9
15		8	78^{g}	152^{f}	1.32^{e}	115^{g}	14^{de}	8.8
16		12	70^{g}	134^{f}	1.18^{f}	114^{g}	3^{e}	9
17	2,000	4	15^{h}	9^{g}	0.57^{g}	15^{h}	0^{e}	5.2
18		12	18^{h}	18^{g}	0.67^{g}	23^{h}	0^{e}	5.2

X: Mean separation in columns by Duncan's multiple range test, 5% level.

and sand at 1,000 and 2,000 ppm Cl on June 17, and at 0, 250 and 500 ppm Cl on June 20. Measurements were made of plant growth, major elements in leaves, and chemical properties of sand solution. Observations of leaves for salt injury symptom were made several times during the experiment. Sand solution at pF 0 to 3.8 was obtained by the following method. Sand was put into a plastic tube (44 mm in diameter and 55 mm high) and then saturated with distilled water. After 24 hours the sand was centrifuged at 4,570 rpm for 30 min in a Marusan 9B-2 rotor. The analytical methods of plant and sand solution were the same as described previously (11).

Results

Growth and salt injury symptoms (*Table* 2)

Dry weight of the whole plant, fresh weight of pods and seeds, and number of pods, root nodules and podded plants was greatest at 0 ppm Cl and decreased with increasing sea water concentrations. Plants at 2,000 ppm Cl showed chlorosis on whole leaves and some plants began to die approximately 40 days after sowing. At the end of the experiment, the

Table 3. Effect of sea water concentrations and $CaSO_4$ applications on major elements in leaves (% of dry matter).

Treatments			Na[X]	Ca	Cl	SO_4
No.	Cl concns (ppm)	$CaSO_4$ concns (mM)				
1	0	1	0.06^{g}	1.90^{hij}	1.15^{h}	0.78^{bcd}
2		4	0.06^{g}	2.14^{fgh}	1.25^{h}	0.76^{bcd}
3		8	0.07^{g}	2.29^{defg}	0.79^{h}	0.89^{abcd}
4		12	0.07^{g}	2.31^{def}	0.97^{h}	0.61^{cd}
5	250	1	0.07^{g}	2.05^{ghi}	2.98^{g}	0.53^{d}
6		4	0.09^{fg}	2.52^{bcde}	4.14^{f}	0.69^{bcd}
7		8	0.10^{fg}	2.55^{bcd}	4.00^{f}	1.21^{a}
8		12	0.09^{fg}	2.75^{ab}	4.08^{f}	1.02^{abc}
9	500	1	0.23^{de}	2.27^{efg}	6.40^{bcd}	0.91^{abcd}
10		4	0.17^{ef}	2.75^{ab}	5.82^{de}	1.03^{ab}
11		8	0.37^{a}	2.91^{a}	6.48^{bcd}	0.84^{abcd}
12		12	0.24^{cde}	2.71^{abc}	5.93^{cde}	0.84^{abcd}
13	1,000	1	0.26^{bcd}	1.93^{hi}	7.59^{a}	0.64^{bcd}
14		4	0.26^{bcd}	2.48^{cde}	6.73^{bc}	0.66^{bcd}
15		8	0.25^{bcd}	2.72^{abc}	6.86^{ab}	0.64^{bcd}
16		12	0.26^{bcd}	2.35^{def}	6.45^{bcd}	0.50^{d}
17	2,000	4	0.33^{abc}	1.66^{j}	5.26^{e}	0.63^{bcd}
18		12	0.32^{abc}	1.88^{ij}	5.44^{e}	0.56^{d}

X: Refer to Table 2.

Table 4. Chemical properties of sand solution at the end of the experiment.

Treatments			Na[X] (me/l)	Ca (me/l)	Cl (ppm)	SO_4 (ppm)	EC (m℧/cm)	Osmotic[Y] potential (bars)	pH
No.	Cl concns (ppm)	$CaSO_4$ concns (mM)							
1	0	1	7.7^{g}	18.2^{c}	104^{f}	1986de	4.07^{j}	−1.65	7.95^{de}
2		4	8.6^{g}	21.5^{ab}	135^{f}	2261^{abc}	4.90^{ij}	−1.83	7.77^{e}
3		8	10.6^{g}	22.5^{ab}	81^{f}	2459^{a}	5.21^{ij}	−1.71	7.99^{cde}
4		12	6.8^{g}	22.2^{ab}	101^{f}	2428^{a}	4.71^{ij}	−1.71	7.81^{e}
5	250	1	27.8^{f}	11.9^{e}	760^{e}	1772^{ef}	5.87^{hi}	−2.32	8.23^{bcd}
6		4	32.6^{ef}	21.4^{ab}	857de	2365^{ab}	7.15^{fg}	−2.68	8.37^{bc}
7		8	33.0^{ef}	21.6^{ab}	857de	2500^{a}	7.24^{fg}	−2.93	9.00^{a}
8		12	30.0^{f}	21.7^{ab}	763^{e}	2448^{a}	6.85^{gh}	−2.56	8.42^{b}
9	500	1	48.5^{d}	10.8^{ef}	1470^{c}	1663^{f}	7.67^{efg}	−2.99	8.22^{bcd}
10		4	49.9^{d}	20.6^{b}	1477^{c}	2438^{a}	8.77^{cde}	−3.48	7.95^{de}
11		8	47.7^{d}	23.2^{a}	1397^{c}	2479^{a}	8.79^{cde}	−3.66	8.23^{bcd}
12		12	41.8^{de}	22.8^{ab}	1364^{cd}	2386^{ab}	8.15^{def}	−3.05	8.02^{cde}
13	1,000	1	65.4^{bc}	9.5^{f}	2896^{a}	1288^{g}	9.28^{bcd}	−2.87	8.33^{bcd}
14		4	64.6^{bc}	15.3^{d}	1998^{b}	2059^{cd}	9.55^{bc}	−3.84	8.33^{bcd}
15		8	60.9^{c}	22.2^{ab}	2337^{b}	2313^{ab}	9.53^{bc}	−3.84	8.16^{bcde}
16		12	65.7^{bc}	23.3^{a}	2172^{b}	2396^{ab}	10.18^{ab}	−4.39	8.13^{bcde}
17	2,000	4	76.3^{a}	16.3^{cd}	2522^{ab}	1702^{f}	11.17^{a}	−4.94	7.82^{e}
18		12	72.3^{ab}	21.1^{ab}	2168^{b}	2140^{bcd}	10.87^{a}	−4.51	7.82^{e}

X: The same as Table 2. Y: Not subjected to statistical analysis because composite samples were taken.

average number of podded plants was 5.2 at both 4 and 8 mM $CaSO_4$. The fresh weight of pods and seeds decreased markedly at 2,000 ppm Cl as compared to 0 ppm Cl. The growth at 0 and 250 ppm Cl tended to be slightly greater at 1 mM $CaSO_4$ than 4, 8 and 12 mM $CaSO_4$. There was no influence of $CaSO_4$ treatments on the growth at 500, 1,000 and 2,000 ppm Cl. Number of root nodules tended to decrease with increasing $CaSO_4$ concentrations at 250, 500 and 1,000 ppm Cl. The decrement rate of root nodule numbers was greater than that of the growth within the same sea water concentrations.

The visible salt injury was first observed as a marginal chlorosis on lower leaves, which expanded to the leaf and then progressed to a necrosis. The injury was gradually advanced from lower to upper leaves. As the injury advanced, it was observed that plants were wilted and finally died at 1,000 and 2,000 ppm Cl. $CaSO_4$ applications did not reduce the degree of injury. Higher $CaSO_4$ (8 and 12 mM) applications rather induced much more chlorosis at 0 and 250 ppm Cl.

Major elements in leaves (*Table* 3) Na and Cl content increased with increasing sea water concentrations from 0 to 1,000 ppm Cl. $CaSO_4$ applications scarcely affected Na and Cl content at each sea water concentration. Ca content was higher at 4, 8 and 12 mM $CaSO_4$ than 1 mM $CaSO_4$ and was not significantly different at each sea water concentration. There was no distinct difference in SO_4 content.

Chemical properties of sand solution (*Table* 4) Na and Cl content, and EC values increased and osmotic potential decreased as sea water concentrations increased. $CaSO_4$ treatments hardly affected Na and Cl content, EC values, and osmotic potential. Ca and SO_4 content was higher at 4, 8 and 12 mM $CaSO_4$ than 1 mM $CaSO_4$ at all sea water concentrations except for 2,000 ppm Cl. The content ranged from 15.3 to 23.3 me/l for Ca and from 2,059 to 2,500 ppm for SO_4. There was no distinct difference in pH values.

Discussion

Salt tolerance studies of green soybeans showed that with increasing sea water concentrations from 250 to 3,000 ppm Cl, growth decreased and Na and Cl content increased (11). The cause of reduced growth seemed to be related to excess Na, Ca deficiency or low Ca/Na ratio in the plants which was induced by the excess Na, or to the combination of the three. There are many reports stating that Ca plays a crucial role in the response of plants to salinity. LaHaye and Epstein (8, 9) have reported that bean plants exposed to 50 mM NaCl for 1 week suffered no damage if the Ca concentration of the nutrient solution was 1 mM or higher, but at lower Ca concentrations damage was severe and apparently due to a massive breakthrough of sodium into the leaves. Hyder and Greenway (5) showed that adverse effects of high Na on the growth of barley can be due to low Ca/Na ratio in nutrient solutions. Elzam and Epstein (3) stated that the severe effects of NaCl on wheatgrass growth were correlated with extremely low levels of Ca in the roots and that Ca was considered to be the key element in the response of these plants to salinity.

The original purpose of this experiment was to determine the effect of $CaSO_4$ applications on salt injury reduction of green soybeans in sand culture on the basis of the above-mentioned reports (3, 5, 8, 9). In the present experiment, Ca and Na in the sand solution were above 9.5 me/l and below 76.3 me/l. These values seemed to be comparable with nutrient solution concentrations of LaHaye and Epstein's experiment in which Ca was 1 mM or higher and Na is 50 mM. According to their experiment, the salt injury was supposed to be alleviated even if diluted sea water containing 2,000 ppm Cl was applied to the green soybeans. However, in our experiment the growth was suppressed with increasing sea water concentrations from 0 to 2,000 ppm Cl regardless of $CaSO_4$ applications. Moreover, the growth at 0 and 250 ppm Cl was more suppressed at higher $CaSO_4$ concentrations. The result indicated that $CaSO_4$ applications did not ameliorate the salt tolerance of green soybeans. One of the pronounced effects of high Na irrigation water is to lower the availability of Ca in the soil by replacing Ca with Na (7). In this experiment, the Ca concentrations in the sand solution ranged from 15.3 to 23.3me/l and were not significantly different at 4, 8 and 12 mM $CaSO_4$, although Na in the sand solution increased with increasing sea water concentrations from 0 to 2,000 ppm Cl. Therefore, the Ca/Na ratio of the sand solution lowered as diluted sea water concentrations increased. Ca content in leaves at 0 to 1,000 ppm Cl ranging from 1.90 to 2.91% was similar to that of the previous green soybean experiment (11) without an apparent Ca deficiency. The Ca/Na ratio in leaves as well as in sand solution, lowered with increasing sea water concentrations, especially at 500 and 1,000 ppm Cl. With green soybeans the Ca/Na ratio in leaves and in sand solution may be more important than Ca content even if Ca has a crucial role to salt tolerance.

On the other hand, it is considered possible that Ca is not related to a great extent to the salt tolerance of green soybeans, because Ca applications did not alleviate the salt injury at all. Bernstein (2) stated that above-mentioned LaHaye and Epstein's statement is contrary to numerous reports in which appreciable reductions in growth and yield have been observed at such salt concentrations (1, 4). Gauch and Wadleigh (4) reported that whole plant dry weight of red kidney beans grown to the flowering stage in solution culture containing 5.9me/l Ca was 6.49g at control and 4.79g at 48me/l Na. In this case the Ca level in nutrient solution was apparently well above 1mM. Nevertheless the result did not agree with LaHaye and Epstein's results. Similar results indicating that application of Ca did not affect the salinity injury reduction were reported with spinach, Chinese cabbage and tomatoes (13), and with muskmelons (10).

Cl and SO_4 seemed to be other ions which may be effectively deteriorate green soybean growth. Cl content in the sand solution and leaves became higher as sea water concentrations increased, but was scarcely affected by $CaSO_4$ applications. SO_4 content in leaves was not significantly different except for 8 and 12mM $CaSO_4$ at 250 ppm Cl and 4mM $CaSO_4$ at 500 ppm Cl. However, that in the sand solution tended to be higher at 4, 8 and 12 mM $CaSO_4$. In another experiment (12) using nutrient solutions to which various amounts of individual salts were added, it was observed that green soybeans were much more sensitive to chloride than sulfate. Therefore, it is considered that Cl is one of the salt injury causes.

The role of Ca to the salt tolerance of plants may vary with culture regimes, medium conditions and kinds of crops. It is also considered that enhancement of salt tolerance by Ca applications may occur in some Ca sensitive plants. Green soybeans might not exhibit such enhancement. Further studies should be done to clarify this point.

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砂耕におけるエダマメの耐塩性と硫酸カルシウム施用との関係

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摘　要

エダマメの耐塩性と硫酸カルシウム（$CaSO_4$）施用の関係を明らかにするため，希釈した海水を用いて収穫時までエダマメを砂耕栽培した．全植物体乾物重，さやと種子の新鮮重，さや及び根粒数は，0 ppm Cl で最大となり，海水濃度が増すにつれて減少した．0 及び 250 ppm Cl では，1 mM $CaSO_4$ で 12 mM $CaSO_4$ より生育がすぐれる傾向を示した．500，1,000，2,000 ppm Cl では，$CaSO_4$ 処理による生育差はみられなかった．葉中及び砂溶液中の Na と Cl 含量，砂溶液の EC は，海水濃度が増すにつれて増加した．それぞれの海水濃度における葉中，砂溶液中の Na と Cl 含量，砂溶液の EC は，$CaSO_4$ 施用により影響されなかった．葉中 Ca 含量，砂溶液中の Ca と SO_4 含量は，4, 8, 12 mM $CaSO_4$ で 1 mM $CaSO_4$ より高い傾向を示した．本実験では，$CaSO_4$ の施用は砂耕におけるエダマメの耐塩性を増進できなかったが，これは耐塩性に対する Ca の役割が，作物により異なることが一因と考えられる．