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Effect of salinity on phosphate accumulation and injury in soybean

I. Influence of $CaCl_2$/NaCl ratios

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Abstract

Many soybean [*Glycine max* (L.) Merr.] genotypes that are grown in solution cultures are highly sensitive to the combination of both salinity and inorganic phosphate (Pi) in the substrate. This effect has been observed on numerous occasions on plants grown in a saline medium that contained a substantial amount of Ca (*i.e.*, $CaCl_2$/NaCl = 0.5 on a molar basis). Because Ca is important in regulating ion transport and membrane permeability, solution culture experiments were designed to examine the effects of various concentrations of Pi and ratios of $CaCl_2$/NaCl (0 to 0.5 on a molar basis) at a constant osmotic potential (−0.34 MPa) on this adverse interaction. Four soybean cultivars ('Lee', 'Lee 74' 'Clark' and 'Clark 63') were tested.

No adverse salinity × Pi interaction was found on Lee at any ratio and leaf P and Cl were maintained below 300 and 200 mmol kg^{-1} dry wt, respectively. Clark, Clark 63 and Lee 74 soybean plants, on the other hand, were severely injured by solution salinity (−0.34 MPa osmotic potential) when substrate Pi was $\geqslant$ 0.12 m*M*. Reduced substrate Ca did not intensify the salinity × Pi interaction. On the contrary, the onset of injury was hastened and more severe with increased $CaCl_2$/NaCl ratios in isotonic solutions. Shoot and root growth rates decreased as injury increased. Leaf P concentrations from these cultivars grown in saline solutions with 0.12 m*M* Pi were excessive (> 600 mmol kg^{-1} dry wt) compared with concentrations commonly found in soybean leaf tissue yet they were independent of the severity of injury. Since leaf Cl increased with increased $CaCl_2$/NaCl ratio, we suspect that the severity of foliar injury was related to the combined effects of excessive P and Cl within the tissue. Lee 74, the only injured cultivar examined that excluded Cl from its leaves, was less sensitive than either Clark cultivar and its injury was characteristically different. Other ion interactions were reported that may have played a role in injury susceptibility.

Introduction

Differential sensitivity among soybean [*Glycine max* (L.) Merr.] cultivars to inorganic phosphate (Pi) has been known for nearly 70 years (Shive, 1918). 'P-Sensitive' and 'P-Tolerant' were relative terms used by Howell and Bernard (1961) to describe the response of a soybean cultivar to 1.6 m*M* Pi when grown in non-saline nutrient solutions. Sensitive cultivars developed a reddish-brown discoloration on their leaves and growth was reduced. In recent studies, certain soybean cultivars were killed at phosphate concentrations an order of magnitude lower than these used by

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Howell and Bernard (1961) when grown in saline solutions (Grattan and Maas, 1984; 1985). This effect was not observed when they were grown in saline solutions at Pi concentrations two orders of magnitude lower than that used by Howell and Bernard. Injured plants contained excessive quantities of P (800 mmol kg^{-1} dry wt) in their leaves. It was concluded that salinity enhanced P sensitivity in soybean.

The adverse salinity × Pi effects described above were observed on plants grown in nutrient solution salinized with NaCl and $CaCl_2$ (2:1 on a molar basis). Salination with this salt mixture has often been used at this laboratory to avoid the specific effects of high Na concentrations. In view of the effect of Ca in stimulating Pi uptake on the one hand (Leggett and Egli, 1977) and its essential role in maintaining membrane integrity on the other (Greenway and Munns, 1980), it seemed prudent to evaluate the effects of the Ca/Na ratio on the adverse interaction of salinity and Pi on soybeans. Numerous studies of the effects of NaCl on soybeans have been reported (Shere *et al.*, 1974; Läuchli and Wieneke, 1979; Wieneke and Läuchli, 1979, 1980; Nukaya *et al.*, 1982; Roeb *et al.*, 1982) but the low Ca/Na ratios resulting from increasing NaCl concentrations may have increased membrane permeability (Greenway and Munns, 1980).

Although Läuchli and Wieneke (1979) found that substrate Ca concentrations of 1 m*M* were optimal for soybean growth in both saline (NaCl only) and non-saline media, later experiments (Wieneke and Läuchli, 1980) showed that increased NaCl concentrations substantially decreased Ca uptake and translocation. Conversely, increasing Ca concentrations in the media markedly decreased Na uptake and translocation. They suggested that the enhanced foliar injury of the salt sensitive cultivar 'Jackson' at high NaCl levels was caused by the increased accumulation of Na and the concommitment reduction in Ca uptake. No data are available on the effects of varying the Ca/Na ratio on Pi uptake and accumulation in soybean.

The objective of this study was to evaluate leaf element accumulation, shoot and root growth, and foliar injury in soybeans as affected by variable Pi concentrations and changing $CaCl_2$/NaCl ratios at a constant osmotic potential (−0.34 MPa). Four soybean cultivars (Clark, Clark 63, Lee and Lee 74) were used in this study. Clark and Lee have been classified as P-sensitive and P-tolerant, respectively (Howell and Bernard, 1961). Clark 63 and Lee 74, to our knowledge, have not been classified with respect to tolerance to Pi.

Materials and methods

Soybean seeds were coated with Thiram[1] seed protectant (50% tetramethylthiuram) and germinated in paper towels saturated with 0.5 m*M* $CaSO_4$ solution in the laboratory. Seedlings between four to seven days old were transplanted into 190 L plastic drums filled with a nutrient solution consisting of 2.5 m*M* $Ca(NO_3)_2$, 3 m*M* KNO_3, 1.5 m*M* $MgSO_4$, 50 μM Fe (as sodium ferric diethylenetriamine pentaacetate), 23 μM H_3BO_3, 5 μM $MnSO_4$, 0.4 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.1 μM H_2MoO_4 and either 0.02, 0.12, 0.15, or 0.30 m*M* KH_2PO_4. Inorganic phosphate concentrations of the nutrient solutions were determined weekly by the Bartlett (1959) modification of the Fiske and Subbarow (1925) assay and KH_2PO_4 was added to maintain the desired Pi concentrations. The solutions were aerated continuously and the pH was maintained between 5.5 and 6.5 with either H_2SO_4 or KOH. Salination began when seedlings were 7 to 10 days old. Over a three-day period, NaCl and $CaCl_2$ were added to the nutrient solutions in five molar ratios of Ca/Na (0.5, 0.25, 0.125, 0.0625, and 0) at a rate that reduced the osmotic potential of the solution 0.1 MPa day^{-1}. The final m*M* concentrations of NaCl and $CaCl_2$ for the respective ratios were: 38 and 19, 49 and 12, 56 and 7, 60 and 4, and 64 and 0. Therefore, the final calculated osmotic potential of all saline cultures, regardless of the Ca/Na ratio, was −0.34 MPa.

Three solution culture experiments were conducted in a greenhouse. Thirty-six or 48 drums were divided into 3 or 4 replicated and completely randomized blocks. Each block contained an unsalinized control (−0.04 MPa OP) and several salinity treatments where the nutrient solutions were salinized to −0.34 MPa with NaCl and $CaCl_2$ at various molar ratios of Ca/Na. Each salinity treatment maintained Pi at a prescribed level. Specific information with regards to cultivars tested, Pi and Ca/Na treatments, replications, number of plants per drum and sampling dates for each experiment

Table 1. Cultivars and treatments for the three experiments

	Experiment I	*Experiment II*	*Experiment III*
Cultivars	Clark, Lee	Clark 63, Lee 74	Clark, Lee, Clark 63, Lee 74
Treatments			
Pi (m*M*)	0.02, 0.12	0.02, 0.12 0.3	0.02, 0.15
$CaCl_2$/NaCl ratio at − 0.34 MPa	0, 0.063 0.125, 0.25, 0.5	0, 0.125, 0.5	0, 0.125, 0.5
Replications	4	4	2
Number of plants of each cultivar/drum	3	8	6[a]
Days sampled[b]	17	7, 11, 14, 21	6, 12, 18

[a] only 4 of Lee.
[b] days after salination began.

is reported in Table 1. Two-way analyses of variance were conducted on all dry-weight and tissue-element-concentration data.

The relative humidity in the greenhouse was uncontrolled but extreme temperature fluctuations were reduced by evaporative coolers and heaters. The average daily maximum and minimum temperatures (°C) in the greenhouse for experiments I, II, and III were 30 and 19, 31 and 21, and 32 and 21, respectively. Air pollutants were removed by passing incoming air through activated charcoal filters. All drums were wrapped with aluminum foil-faced fiberglass insulation to minimize temperature fluctuations in the culture media. Lighting was natural sunlight through glass.

At the time of harvest, roots were separated from shoots and were washed three times, 30 s each, in separate 15 l quantities of deionized water. The shoots and roots were placed in separate paper bags and dried in an oven at 65°C. After dry weights were obtained, leaves were separated from stems and petioles. Replicate samples were composited for both leaves and roots in experiments II and III. The samples were then ground in a blender and stored in glass vials for elemental analysis. Sodium, K, Ca, Mg, Fe, Mn, and Zn were determined in nitric-perchloric acid digests of the ground tissue by atomic absorption spectrophotometry. Phosphorus was determined on the tissue digests by the molybdate-vanadate colorimetric method (Kitson and Mellon, 1944). Chloride was determined on dilute nitric-acetic acid extracts of the dry, ground plant material by the Cotlove (1963) coulometric-amperometric titration procedure.

Results

Visual foliar injury

Extreme differences in sensitivity to the combination of salinity and Pi ($\geqslant 0.12$ m*M*) were observed among cultivars. Clark and Clark 63 were the most sensitive of those tested followed closely by Lee 74. Lee, on the other hand, was insensitive to the adverse interaction and plants remained healthy during the course of the experiment regardless of the treatment.

Plants grown in non-saline solution cultures at all Pi levels and in saline cultures at 0.02 m*M* Pi, never developed visual foliar injury. Under saline treatments at Pi $\geqslant 0.12$ m*M*, Clark, Clark 63, and Lee 74 developed injury. Furthermore, as the Ca/Na ratio increased, the foliar injury was not reduced but rather intensified. This effect was most obvious on both Clark cultivars and less on Lee 74.

The foliar injury symptoms on Clark and Clark 63 were identical, but were characteristically different from the injury observed on Lee 74. Clark and Clark 63 were first to show injury. Injury developed as a general chlorosis on the primary leaves, usually within a week after salination began. Injury then progressed towards first and second trifoliolates. As injury became more severe, the leaves became hyponastic and appeared greenish-grey in color. Eventually, necrosis developed and leaves abscised. The necrotic tissue was beige in color. Lee 74 was slower than the Clark cultivars to develop injury and its symptoms were unique. Lee 74 developed a reddish-brown coloration on the adaxial leaf surface of the primary leaves which

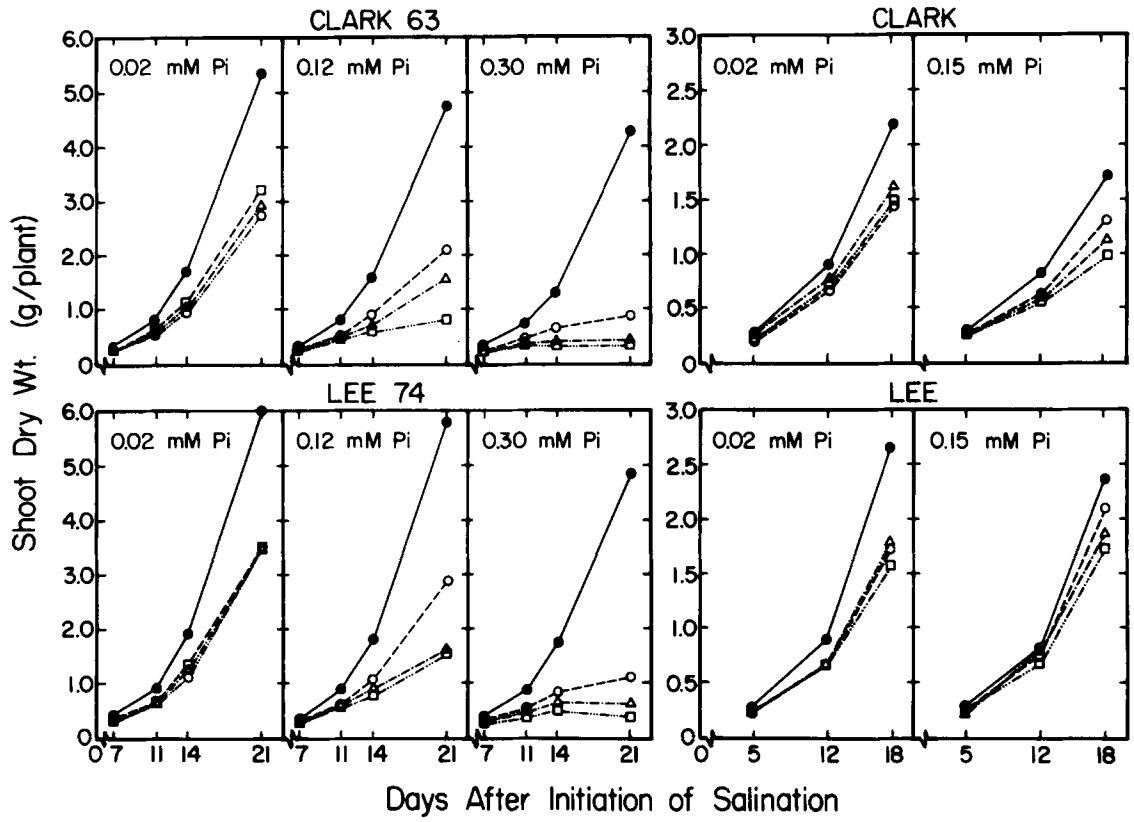

Fig. 1. Effect of different $CaCl_2$/NaCl ratios and substrate Pi on the shoot dry weights of Clark 63, Clark, Lee 74, and Lee at various times after salination began. ● denotes the unsalinized control. ○, △, and □ denote salinity treatments with the Ca/Na at 0, 0.125, and 0.5, respectively.

later migrated to the oldest trifoliolates. This coloration became more intense with time under treatment until leaves abscised. Most often, leaves would abscise before they dried, unlike Clark and Clark 63.

Plant growth

The effects of the different Ca/Na ratios and substrate Pi on the shoot growth of Clark, Clark 63, Lee, and Lee 74 soybeans are shown in Figure 1. The data for Clark 63 and Lee 74 are from experiment II, while the data for Clark and Lee are from experiment III. No data are given for experiment I because it was not a time-course study. Increased Pi significantly ($\alpha < 0.05$) decreased the shoot growth of Clark 63 and Lee 74 at all Ca/Na ratios and Clark at Ca/Na = 0.5. The effect on unsalinized controls was significant but much smaller than on salt-treated plants. Under saline conditions, this effect was greatest at high Ca/Na and least where NaCl was the only salt. Growth rates of Clark were not significantly different among Ca/Na treatments at low Pi (0.02 m*M*), but were significantly ($\alpha < 0.01$) decreased by increased Ca/Na at high Pi (0.15 m*M*). Increased Ca/Na significantly ($\alpha < 0.01$) decreased the growth rates of Lee 74 and Clark 63 at the two highest Pi levels, but slightly increased the growth rates at the lowest Pi level for Clark 63. No significant differences in the growth rates of Lee were found among Pi levels at a given salinity (except for Ca/Na = 0, $\alpha < 0.05$) or between Ca/Na ratios at a given Pi level. The effects of variable Ca/Na ratios and Pi on shoot growth of Clark and Lee from experiment I, and Clark 63 and Lee 74 from experiment III (data not presented), were identical to the response of the respective cultivars in the experiments described above.

The effects of Ca/Na ratios and Pi on the dry

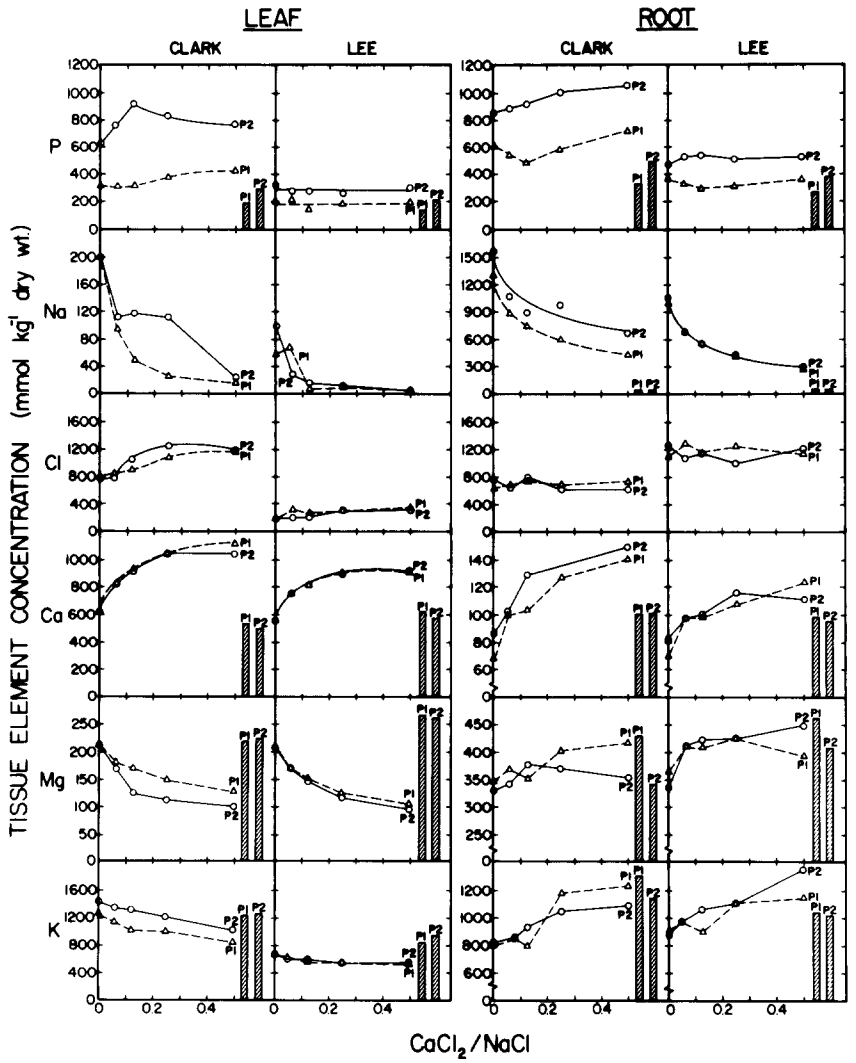

Fig. 2. Interactive effects of different $CaCl_2/NaCl$ ratios and substrate Pi on tissue element concentrations of Clark and Lee. P_1 and P_2 = 0.02 and 0.12 m*M* Pi; OP of saline treatments = −0.34 MPa. Nonsalinized control values indicated by *enclosed bar graph*. Data from experiment I.

root weights (data not presented) were analogous to the effects on the shoot weights. Although dry weights of roots were only 15 to 35% of those of shoots, relative differences among treatments were the same.

Leaf element concentration

The effects of isotonic salinity treatments with variable Ca/Na and Pi on leaf element concentration of Clark and Lee are shown in Figure 2. Levels of significance are reported in Table 2. Concentrations for nonsaline control treatments are represented by the bar graphs. Both salinity and increased substrate Pi increased leaf P in both cultivars but the increase was only slight for Lee. Clark accumulated significantly more P than Lee at all Ca/Na ratios. This contrast was more pronounced at the highest Pi level. At high Pi, leaf P increased in Clark as the Ca/Na ratio increased from 0 to 0.125 then decreased. Regardless of the differences between Ca/Na treatments, injured Clark leaves contained abnormally high amounts of P (> 600 mmol kg^{-1} dry wt). No significant differences in leaf P were

Table 2. Levels of significance from two-way analyses of variance of the leaf and root element concentration data from experiment I

Cultivar	Source of variation	P	Na	Cl	Ca	Mg	K
Leaf							
Clark	Ca/Na Ratio	**[a]	**	**	**	**	**
	Pi	**	*	ns	ns	**	**
	Ca/Na × Pi	**	ns	ns	ns	**	ns
Lee	Ca/Na Ratio	ns	**	**	**	**	**
	Pi	**	ns	ns	ns	ns	ns
	Ca/Na × Pi	ns	**	ns	ns	ns	ns
Root							
Clark	Ca/Na Ratio	*	**	ns	**	ns	**
	Pi	**	**	ns	ns	ns	ns
	Ca/Na × Pi	ns	ns	ns	ns	ns	ns
Lee	Ca/Na Ratio	ns	**	ns	**	*	**
	Pi	**	ns	ns	ns	ns	ns
	Ca/Na × Pi	ns	ns	ns	ns	ns	ns

[a] * and ** denote statistical significance at the 5 and 1% confidence level, respectively.
n.s. indicates data are not statistically significant.

found among the Ca/Na ratios at a given Pi level for Lee.

Increased Ca/Na ratios significantly increased leaf Cl in both Clark and Lee cultivars (Fig. 2). The increase, however, was only slight for Lee, a cultivar known to exclude Cl from its leaves (Abel and MacKenzie, 1964; Abel, 1969). The increase in leaf Cl was probably related to the increased Cl concentration in the substrate at higher Ca/Na ratios. No differences in leaf Cl were found between Pi levels. Lee 74 retained its parent's (Lee) ability to exclude Cl from its leaves (data not presented), as leaf Cl concentrations remained under 320 mmol kg^{-1} dry wt. Both Clark 63 and the parent, Clark, were unable to exclude Cl from leaf tissue.

Sodium analyses indicated that the ability of soybean to exclude Na from leaves was directly related to the substrate Ca concentration (Fig. 2). Leaf Na was diminished by relatively small increases in $CaCl_2$ concentrations in the saline media. Lee excluded Na more effectively than Clark. Clark 63 and Lee 74 were affected similarly to Clark and Lee, respectively (data not shown).

Leaf Ca increased asymtotically to a maximum of 1100 and 900 mmol kg^{-1} dry wt for Clark and Lee, respectively, as Ca/Na increased. No significant differences were caused by different Pi levels. Leaf Ca concentrations in plants that were salinized with only NaCl were comparable to those in nonsalinized controls. In later experiments where Lee 74 was treated with NaCl alone, leaf Ca was slightly suppressed but there was no evidence of Ca deficiency in any plant.

Increased Ca/Na ratios significantly decreased leaf Mg concentration in both Clark and Lee presumably because of competition from increased substrate Ca. Whereas this effect was significantly greater at the higher Pi level for Clark, there was little difference in Mg concentration at the two Pi levels in Lee. Although leaf Mg concentrations in both cultivars at the highest Ca/Na ratios were about half the control values, no Mg deficiency symptoms were evident during the course of the experiment. Leaf Mg in Lee 74 and Clark 63 in expriment II was affected similarly to that in Clark.

Leaf K in Clark decreased with increased Ca/Na ratios and K levels were less at the low Pi level. In Lee, K was decreased by salinity at all Ca/Na ratios. Furthermore, leaf K significantly decreased as the Ca/Na ratio increased. Leaf K in Lee 74 and Clark 63 in experiment II was affected similarly to that in Clark.

Accumulation of leaf Fe was influenced by both salinity and substrate Pi. Nonsalinized control plants consistently contained less Fe (100 to 140 mg kg^{-1} dry wt) than salinized plants (120 to 350 mg kg^{-1} dry wt). However, no differences in leaf Fe were found among the Ca/Na treatments. Increased Pi, on the other hand, decreased leaf Fe. This interactive effect occurred with all cultivars.

Accumulation of leaf Mn was influenced by Pi and the Ca/Na ratio. Regardless of the Ca/Na ratio, increased Pi slightly decreased leaf Mn. Increased Ca/Na generally decreased leaf Mn. Maximum and minimum concentrations were 170 and 60 mg kg^{-1} dry wt, respectively. No differences were found among cultivars.

No consistent differences in leaf Zn were found among salinity treatments, Pi treatments, or cultivars. Concentrations were between 50 and 100 mg kg^{-1} dry wt.

Root element concentration

The effects of different Ca/Na ratios on root element concentration (Fig. 2) were analogous, for

the most part, to those on leaf elemental concentration.

Phosphorus concentrations were slightly higher in roots than in leaves. Increased Ca/Na from 0 to 0.5 and from 0.125 to 0.5 increased root P in ark grown at 0.12 and 0.02 m*M* Pi, respectively. The ratio had no effect on root P in Lee.

Root Cl concentration in Lee was significantly higher than in Clark. This effect was opposite that in the leaves. No significant differences in Cl concentration were found among Ca/Na or Pi treatments.

Sodium concentrations in general were 10-fold higher in roots than in leaves. In both leaf and root tissue, increased concentrations of substrate Ca enhanced Na exclusion. Increased Pi, on the other hand, weakened this exclusion ability in Clark, Clark 63, and to a lesser extent in Lee 74.

NaCl alone decreased root Ca, Mg, and K concentration with respect to the non-salinized controls in all four cultivars examined. The decrease in K was greatest in those cultivars that exhibited an adverse salinity-Pi interaction (Clark, Clark 63, and Lee 74). The concentration of all three elements generally increased with increased Ca/Na.

Except for Mn, neither Pi concentrations nor Ca/Na ratios had any obvious effects on micronutrient concentrations in roots. Root Mn concentrations were 2 to 10 times higher than those in leaves. In salinity treatments with $CaCl_2$ present, increased substrate Pi decreased root Mn. Root Fe concentrations were 10 to 30 times higher than those in the leaves. Zn concentrations (50 to 100 mg kg^{-1} dry wt) were comparable to those in the leaves.

Discussion

Salination of nutrient solutions with NaCl, rather than a mixture of NaCl and $CaCl_2$, did not aggravate the adverse salinity × Pi interaction. On the contrary growth rates decreased with increased Ca/Na ratios at high Pi ($\geqslant 0.12$ m*M*) concentrations. The lower growth rates were related to increased foliar injury, at least for Clark and Clark 63. Growth rates in Lee 74 decreased with increased Ca/Na at high Pi yet the injury differences were more subtle. $CaCl_2$ salinity combined with high substrate Pi may have produced some nutritional imbalance that, in addition to toxic effects (*i.e.*, Cl and P), retarded plant growth. Although differences in leaf P concentration were found among Ca/Na treatments, there was no indication that these abnormal P concentrations (> 600 mmol kg^{-1} dry wt) were the sole cause of foliar injury, especially in the Cl-accumulators (Clark and Clark 63). Both leaf Cl and Ca were found to increase with increased Ca/Na. Therefore, increased severity of injury with increased Ca/Na may be related to one or both of these elements. Shive (1918) found that $Ca(H_2PO_4)_2$ was considerably more toxic to soybeans than KH_2PO_4. It is likely therefore that Ca plays an important role in the enhancement of P-sensitivity. This role of Ca merits further research.

Clark and Clark 63 were not only more susceptible to foliar injury from combined salinity and high Pi ($\geqslant 0.12$ m*M*) than Lee 74 but injury symptoms were characteristically different. The reddish-brown injury in Lee 74 may be characteristic of P-toxicity whereas the beige-necrotic injury in Clark and Clark 63 might be related to combined P and Cl toxicity. Since leaf Cl concentrations in Clark and Clark 63 increased with increased Ca/Na, it is not surprising that injury became more pronounced. This effect was small in Lee 74, a leaf Cl excluder.

The finding of inherited P-sensitivity in Lee 74 was unexpected. Evidently, Lee 74 acquired P-sensitivity from either Lee 68 or R66–1517 (See Caviness *et al.*, 1975 for pedigree) yet at the same time maintained Lee's ability to exclude Cl from its leaves. This suggests that the mechanisms of Cl and Pi uptake and translocation may be independent of one another.

The literature contains numerous articles on the interactions of P and Ca on the Fe status within the plant (*e.g.* Clark, 1983). Although Fe concentrations in our soybean leaf samples were well within the range of adequacy, high concentrations of P and Ca can interact with Fe and thereby inhibit its reduction from Fe^{3+} to Fe^{2+} (Clark, 1983). Therefore, these leaf Fe concentrations may not represent active Fe in the plant and its role in this adverse synergism between salinity and phosphate becomes difficult to assess.

The possibility of P × Zn interactions has also been explored by several investigators. Although leaf Zn concentrations reported herein might be adequate under most conditions, Millikan *et al.*

(1968) suggested that excessive P accumulation in plants might increase the physiological requirement for Zn. Paulsen and Rotimi (1968) found, however, that increasing the Zn supply to soybean was only effective in eliminating the deleterious effect of P in the P-tolerant Chief cultivar and not in the P-sensitive Lincoln cultivar. More recently, Loneragan *et al.*, (1982) questioned the concept of 'P-induced Zn deficiency' and concluded that the necrotic symptoms of high P are not the result of impaired Zn metabolism but rather are caused by toxic levels of P in the leaves.

The role of Na in the adverse synergism between salinity and Pi remains unresolved. Although increased substrate Ca decreased leaf and root Na concentrations in all cultivars, a response observed earlier on soybeans (Wieneke and Läuchli, 1980), root Na increased with increased substrate Pi only in cultivars susceptible to the harmful salinity × Pi interaction. Since a significant interaction occurred between Na and P, it may be possible that Na is involved in Pi uptake and/or transport to the shoots. The role of Na and Cl on the salinity × Pi interaction will be evaluated in a subsequent study.

References

Abel G H 1969 Inheritance of the capacity for chloride inclusion and chloride exclusion by soybeans. Crop Sci. 9, 697–698.

Abel G H and MacKenzie A J 1964 Salt tolerance of soybean varieties (*Glycine max* L. Merrill) during germination and later growth. Crop Sci. 4, 157–161.

Bartlett G H 1959 Phosphorus assay in column chromatography. J. Biol. Chem. 234, 466–468

Caviness C E, Riggs R D and Walters H J 1975 Registration of Lee 74 soybean (Reg. No. 106). Crop Sci. 15, 100.

Clark R B 1983 Plant genotype differences in the uptake, translocation, accumulation, and use of mineral elements required for plant growth. Plant and Soil 72, 175–196.

Cotlove E 1963 Determination of true chloride content of biological fluids and tissues. II. Analysis by simple nonisotopic methods. Anal. Chem. 35, 101–105.

Fiske C H and Subbarow Y 1925 The colorimetric determination of phosphorus. J. Biol. Chem. 66, 375–400.

Grattan S R and Maas E V 1984 Interactive effects of salinity and substrate phosphate on soybean. Agron. J. 76, 668–676.

Grattan S R and Maas E V 1985 Root control of leaf phosphorus and chlorine accumulation in soybean under salinity stress. Agron. J. 77, 890–895.

Greenway H and Munns R 1980 Mechanisms of salt tolerance in nonhalophytes. Annu. Rev. Plant Physiol. 31, 149–190.

Howell R W and Bernard R L 1961 Phosphorus response of soybean varieties. Crop Sci. 1, 311–313.

Kitson R E and Mellon M G 1944 Colorimetric determination of phosphorus as molybdovanado-phosphoric acid. Ind. Eng. Chem. Anal. Ed. 16, 379–383.

Läuchli A and Wieneke J 1979 Studies on growth and distribution of Na, K, and Cl in soybean varieties differing in salt tolerance. Z. Pflanzenernaehr. Bodenkd. 142, 3–13.

Leggett J E and Egli D B 1977 Ion accumulation by crop plants. 2–33, In Physiological Aspects of Crop Nutrition and Resistance, Ed. U S Gupta, Atma Ram, Delhi, India.

Loneragan J F, Grunes D L, Welch R M, Aduayi E A, Tengab A, Lazar V A and Cary E E 1982 Phosphorus accumulation and toxicity in leaves in relation to zinc supply. Soil Sci. Soc. Am. J. 46, 345–352.

Millikan C R, Hanger B C and Bjarnason E N 1968 Effect of phosphorus and zinc levels in the substrate on ^{65}Zn distribution in subterranean clover and flax. Aust. J. Biol. Sci. 21, 619–640.

Nukaya A, Masui M and Ishida A 1982 Salt tolerance of green soybeans as affected by various salinites in sand culture. J. Japan. Soc. Hort. Sci. 50, 487–496.

Paulsen G M and Rotimi O A 1968 Phosphorus-zinc interaction in two soybean varieties differing in sensitivity to phosporus nutrition. Soil Sci. Soc. Am. Proc. 32, 73–76.

Roeb G W, Wieneke J and Führ F 1982. Effect of high NaCl concentration in the nutrient medium on transpiration, abscisic acid, cytokinin and proline content of two soybean varieties. Z. Pflanzenernaehr. Bodenkd. 145, 103–116.

Shere S M, Memon K S and Khanzada A N 1974 Effect of salinity on the growth and mineral uptake in soybeans (Glycine), Pakistan J. Sci., Ind. Res. 17, 148–151.

Shive J M 1918 Toxicity of monobasic phosphate towards soybeans grown in soil and solution cultures. Soil Sci. 5, 87–122.

Wieneke J and Läuchli A 1979 Short-term studies on the uptake and transport of Cl by soybean cultivars differing in salt tolerance. Z. Pflanzenernaehr. Bodenkd. 142, 799–814.

Wieneke J and Läuchli A. 1980 Effect of salt stress on distribution of Na and some other cations in two soybean varieties differing in salt tolerance. Z. Pflanzenernaehr. Bodenkd. 143, 55–67.