Discovery, The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences

Volume 10 Article 12

Fall 2009

Establishing a rapid and effective method for screening salt tolerance in soybean

Mioko Tamura
University of Arkansas, Fayetteville

Pengyin Chen
University of Arkansas, Fayetteville

Establishing a rapid and effective method for screening salt tolerance in soybean

Mioko Tamura and Pengyin Chen†*

ABSTRACT

Chlorine (Cl) toxicity has been recognized as a constraint for soybean production. Although the use of a Cl-tolerant crop easily solves the problem, current screening methodologies for Cl tolerance are often ineffective because of inadequate means of detecting and measuring plant response to salinity. In order to facilitate the evaluation process and selection of Cl-tolerant genotypes, a study was conducted to develop a rapid and effective method for screening Cl tolerance in soybean. Seeds of five soybean cultivars, each representing either the includer or excluder genotype to salt stress, were grown in a greenhouse in two different growing media (potting mix or sandy loam) with four different concentrations of sodium chloride (NaCl) solutions. Visual symptoms of Cl toxicity were rated on a 1 to 6 scale (1 as healthy and 6 as dead), and the score was compared with relative shoot/root dry weight and Cl concentration in shoot/root to corroborate the accuracy of the visual ratings. Reduced dry weight was associated with higher Cl concentrations in both root and shoot tissues. The optimal NaCl concentration for screening was determined as 120 mM NaCl since it effectively differentiated excluders from includers. There were negative, significant correlations between relative shoot dry weight and Cl concentration in shoot tissue ($r = -0.91$ $p = 0.05$), and Cl concentration in shoot was also significantly correlated with visual rating score ($r = 0.79$ $p = 0.05$). The presented methodology is simple, rapid, and effective for screening for salt tolerance in soybean.

*Mioko Tamura is a 2008 honors graduate with a B.S. degree in crop management.
†Pengyin Chen is an associate professor in the Department of Crop, Soil, and Environmental Sciences.

MEET THE STUDENT- AUTHOR

Mioko Tamura

After my graduation from University of Tsukuba Senior High School in Japan, I came to Fayetteville to study English. I did not know anyone here prior to coming here, so I was very fortunate to meet these people who have given me generous support and made it possible for me to achieve my initial goal, enrolling in the university in fall 2004. I was awarded Harvey A & Jo York, Eddie Davis, and Dale & W Hinkle scholarships to pursue my study. While I was an undergraduate student, I participated some student organizations, such as ICT (International Culture Team) and Organic Farming Club. I began working for Dr. Pengyin Chen during my freshman year, involving a hardness testing of food grade soybean. In 2007, the research project was published in Discovery and also in the Journal of Texture Studies in 2008. In summer 2007, I had an opportunity to be a part of a service learning project on a sustainable school farm in Belize. My career goal is to work for an international corporation in agronomy and rural development. In May 2008, I graduated with a B.S.A. degree in crop management and a minor in agribusiness. The following summer, I started as a graduate assistant in the Department of Crop, Soil, and Environmental Sciences in crop physiology.

I would like to thank Dr. Pengyin Chen for his support and guidance, and I also thank members of the soybean research program and the committee; Drs. Richard Norman, Jennie Popp, and Nathan Slaton for my honors thesis research.

INTRODUCTION

Salt toxicity, as evidenced by high concentration of chlorine (Cl) in soil threatens soybean production worldwide (Essa, 2002). Salt accumulation in the soil profile is mainly caused by poor fertilizer practices or excessive use of irrigation water that results in unbalanced in- and out-flow of groundwater (Lee et al., 2004). The toxicity problem is especially severe in arid and semi-arid areas where higher evaporation rates are expected, but the salt accumulation is also found in many irrigated fields in Arkansas (Wilson et al., 2000). Chloride toxicity problems have arisen in soybean production in the Mississippi River Delta in Arkansas since 1990 (Rupe et al., 2000).

Soybean [*Glycince max*] is one of the main crops in the world for producing edible oil and high-protein livestock feed; however, it is categorized as a "salt sensitive" crop in the stress tolerance subdivisions, exhibiting chlorosis or necrosis on leaves in saline growing conditions (Pantalone et al., 1997). Screening based on the leaf chlorosis score and visual foliar symptoms are considered appropriate for salt-sensitive crops. There are two types of salt response in soybean; includer and excluder. The soybean genotype that translocates Cl to the foliage is called includer whereas excluder stores Cl in the roots. High salt tolerance was associated with Cl exclusion from leaves/shoots (Philip and Broadley, 2001). Thus, yield losses are more severe for includers than for excluders, and Cl causes symptoms ranging from faint foliar chlorosis to plant death as leaf and stem Cl concentrations increased in includers.

Genetic variability of Cl tolerance has a potential use for breeding salt-tolerant soybean. About 20% of soybean cultivars released for the southern U.S. are expected to have an economical salt-tolerance level, yet a practical and economically viable method for screening for Cl tolerance has not been established (Lee et al., 2004). Current screening methodologies for salt-tolerant cultivars are often time consuming and labor intensive; the screening is mainly done by hydroponic culture that requires cautious seedling care, gradual exposure to salinity stress levels, and elaborate nutrient maintenance in the solution. Moreover, the plant tissue analysis is the only measurement used to determine the cultivar's tolerance level to the salt stress.

In order to facilitate the evaluation process and selection of Cl-tolerant genotypes, this research was conducted using soil growing media (commercial soil and sandy loam) to develop a simple and reliable methodology based on foliar symptoms for screening Cl-tolerant soybean genotypes. We hypothesized that a high concentration of NaCl hastens stress symptom development, causes more severe foliar symptoms, decreases plant biomass of shoots and roots, and increases Cl concentration in shoot and root tissues. Furthermore, includers should have higher Cl concentration in shoot tissue than excluders, whereas excluders should have higher Cl concentration in roots than includers.

The main objective of this study was to develop a rapid and effective methodology for screening Cl tolerance in soybean. The specific objectives were 1) to identify the optimal Cl concentration for screening Cl tolerance based on visual foliar symptoms, and 2) to determine the effects of Cl uptake on root and shoot growth and Cl concentrations in these plant parts.

MATERIALS AND METHODS

Plant Materials and Growth Conditions. Soybean plants were grown in a greenhouse of the Rosen Center at the University of Arkansas, Fayetteville. They were maintained under 14 h daylength and 25°C day / 20°C night temperature throughout the experiment. Seeds of five soybean cultivars ('Clark', 'Williams', 'Dare', 'Lee 68', and 'S-100'; Table 1) were planted in a 9.8-cm square plastic pot, each containing either commercial potting mix (Redi-earth, Vermiculita and Canadian Sphagnum peat moss, Sun Gro Horticulture Distribution Inc., Bellevue, Wash.) or rocky sandy loam soil collected at Kibler, Ark. 'S-100' and 'Lee 68' represented Cl-tolerant cultivars (excluder) and 'Clark', 'Dare', and 'Williams' represented Cl-sensitive cultivars (includer). After the seedlings emerged, only the healthy, uniform-sized plants were maintained from each cultivar for the experiment. Peters nutrient solution (Peter's Plant Food 20-20-20, Spectrum Group, St. Louis, Mo.) was applied once a week after the second trifoliate growth stage.

Three weeks after planting, at the second to third trifoliolate leaf stage, each pot received one of four concentrations of NaCl solution (0, 80, 120, or 160 mM NaCl) to saturate the growing media (100 ml for potting mix and 75 ml for sandy loam). A total of eight combinations (two growing media and four NaCl levels) of treatments were tested on seedlings in five replications (four plants/pot) for each of the five cultivars in the study.

Measurements and Data Analysis. Symptoms were recorded based on the visual rating scale of 1-6 (Fig. 1). The scale was defined as follows; 1 for healthy plant with no chlorosis, 2 for 25% of leaf chlorosis, 3 for 50% of leaf chlorosis, 4 for 75% of leaf chlorosis, 5 for 100% chlorosis, and 6 for complete leaf necrosis and plant death. The application of NaCl solutions were terminated at 13 d after the stress treatment initiation when the difference between includer and excluder cultivars was apparent. Each plant was then rated as the soil appeared to dry out at 4 d after the treatment termination for the potting mix and 7 d for the sandy loam. Average score of the four plants in each pot was used for data analysis. The NaCl concentration that gave the most contrasting differences between sensitive and tolerant cultivars was defined as the critical-selection NaCl concentration for Cl tolerance. Lastly, the samples were oven-dried at 70°C for 7 d. Total shoot dry weights were recorded for the four plants. However, dried roots of all plants from five replications of each treatment were combined. In order to compare genotypic differences effectively, shoot and root dry weights were also converted into relative shoot dry weight (RSDW) and relative root dry weight (RRDW) based on the dry weight of the control [0 mM NaCl] as 1.0. Two out of five replications of bulked dry shoots were randomly chosen and ground for tissue analysis for Cl concentration (CLSH), and bulked dry roots of all five replications were ground for tissue analysis for Cl concentration (CLRT). Samples were deionized water-extracted and analyzed using a spectrophotometer model CIROS ICP (Specttro Analytical Instruments Inc., Mahwah, N.J.).

Data for plant biomass (RSDW and RRDW), Cl concentrations (CLSH and CLRT), and visual rating score (RATE) were subjected to analysis of variance. Honest significant difference (Tukey) was used to compare means between the includer and excluder cultivars and NaCl concentrations ($P = 0.05$) in each growing media (potting mix or sandy loam), separately. Simple correlation was used to assess relationships among RSDW, RRDW, CLSH, CLRT, and RATE. All statistical analyses were done by the SAS version 9.2 (SAS Institute, Cary, N.C.).

RESULTS AND DISCUSSION

Symptoms of Chloride Toxicity in Shoot and Roots. Visible symptoms, as reflected by visual rating score, were observed on plants in all Cl concentrations in both potting mix and sandy loam soil (Table 2). Symptoms of Cl toxicity ranged from burning of leaves and stunting to interveninal chlorosis and premature chlorosis. Symptom severity increased with NaCl concentration in both growing media. Symptoms were more severe in potting mix than in sandy loam in general. For instance, only a few excluder plants showed chlorosis symptoms with 80 mM NaCl in sandy loam while all plants in potting mix showed 25% or more chlorosis with the same NaCl concentraion. However, visual rating scores of includers were consistently higher than those of excluders at all levels of Cl in potting mix or sandy loam growing media. Statistical analysis showed that the significant difference in visual symptom score between includers and excluders was observed in 120 mM with potting mix (Table 2, illustration and Fig. 2). However, none of the NaCl concentrations with sandy loam showed statistically significant differences although 120 mM NaCl concentraton showed trends toward increased ratings in includers regard to excluders.

Valencia et al. (2008) used a hydroponic culture system to identify a threshold NaCl concentration for Cl toxicity in soybean and found that 120 mM NaCl was the critical NaCl concentration for tolerance selection, which separated includers from excluders at 14 days of Cl stress treatment when interveninal chlorosis on leaves was observed among includers while excluders remained healthy. This agrees with our results using potting mix.

Symptoms of Cl toxicity were also evident in the root systems (Fig. 3). Under the highest concentration of NaCl (160 mM), both includers and excluders developed thin and dark roots with extremely short and sparse secondary roots. Root development was greatly reduced even at the 80 mM NaCl compared to the control. However, root system damage from NaCl cannot be feasibly used as a selection criterion for salt tolerance due to the extensive work involved in root damage evaluation.

Effects of Salt on Plant Biomass. For RSDW and RRDW, relative weights decreased numerically, but not always significantly, as the applied NaCl concentration increased; the higher NaCl concentrations caused more sever toxicity and reduced biomass. A similar inverse relationship between NaCl concentration and plant biomass was also reported by Valencia et al. (2008) using commercial cultivars and by Kao et al. (2006) using wild soybeans.

The RSDW of includers were always, but not significantly, lower than those of excluders. Includers translocate Cl to shoot tissues; therefore, they had more interference with growth due to applied NaCl solution than excluders. In contrast, the RRDW of excluders were generally lower than those of includers though none of the differences were significant. Excluders store Cl in roots, thus the root growth of excluders were hindered by the NaCl solutions by a greater extent than includers.

Overall, RRDW had lower values than RSDW, which indicates that roots were more affected by salinity than shoots. This was also observed by Valencia et al. (2008) and Kao et al. (2006). However, our results conflict with Essa's (2002) finding that root dry weight was less sensitive to salt stress than shoot dry weight (2002).

In comparing the growing media, we found that the sandy loam had higher values for both RSDW and RRDW than the potting mix, suggesting plants grown in sandy loam were less affected by salt stress than those in potting mix. There was twice as much difference between the overall average RRDW of potting mix (0.24) and sandy loam (0.51). In contrast, the average RSDW between potting mix and sandy loam were very similar (0.50 and 0.56, respectively). This result agreed with our observations of visual symptoms that potting mix showed more severe symptoms than sandy loam at the same NaCl concentraions.

Chloride Concentrations in Shoots and Roots. Numerically higher Cl content in shoots (CLSH) was always found in includers than in excluders (Table 5). In contrast, numerically higher Cl content in roots (CLRT) was found in excluders than in includes (Table 6). There were very few significant differences in the means; however, these results demonstrated that the excessive Cl was translocated into leaves in includer cultivars, but it was restricted and accumulated in the root system in excluder genotypes.

Chloride concentrations in both shoots and roots had positive linear relationships with NaCl concentrations (Table 7), which agrees with the report by Essa (2002). This showed that increased, applied NaCl concentration resulted in increased tissue Cl concentrations.

The Cl concentration reached a plateau at 120 mM NaCl concentration for the potting mix. However, CLSH of sandy loam exhibited a linear increase with Cl concentrations up to 160 mM NaCl. The significant difference between includers and excluders was shown at 80 mM NaCl concentration with sandy loam. Overall, shoot from plants grown in potting mix had higher Cl concentration than those in sandy loam.

The significant differences in CLRT were only seen between control and 80 mM NaCl concentration or above in both potting mix and sandy loam, whether for includes and excluder plants. This implied that roots reached their nearly maximum Cl intake capacity with 80 mM NaCl solution. Unlike CLSH, roots of plants grown in sandy loam contained more Cl than those grown on in potting mix, probably because plants grown in sandy loam had higher RRDW than those in potting mix.

Correlations Among Measurements. There were positive, significant correlations between RSDW and RRDW (Table 7). Positive and significant correlations were also found between CLSH and both CLRT and RATE. The negative, significant correlations between CLSH/CLRT and biomass (RSDW and RRDW) imply that higher Cl concentration in shoots/roots limited plant growth to a greater extent. Finally, the validity of visual rating scale (RATE) was supported by the significant correlation between RATE and both RSDW (negative) and CLSH (positive). In other words, a higher degree of visual symptoms was a sign of reduced shoot growth and increased Cl concentration in shoot. This result agrees with reports by Pantalone et al. (1997) and Lee et al. (2004).

The trends of negative or positive correlation among measurements were found to be exactly the same between potting mix and sandy loam; however, the stronger correlations were found in sandy loam than in the potting mix between CLRT and both RSDW and RRDW. Although the correlation between RATE and RSDW was not significant for potting mix, this correlation was significant for the sandy loam.

Our research showed that the visual, foliar evaluation of soybean for chloride tolerance screening with 120 mM NaCl is a rapid and effective screening method with both potting mix and sandy-loam media. Additional experiments are ongoing to confirm these results.

ACKNOWLEDGMENTS

Financial support for this project was provided by an Honors College Undergraduate Research Grant, the Arkansas Soybean Promotion Board, and the University of Arkansas Agricultural Experiment Station. The author wishes to thank Dr. Pengyin Chen, honors thesis mentor; Drs. Richard Norman and Jennie Popp, thesis committee members; Dr. Nathan Slaton for plant tissue analysis; and Dr. Tetsuaki Ishibashi for statistical analyses. All of their help was necessary for the success of the project and is greatly appreciated.

LITERATURE CITED

Essa, T.A. 2002. Effect of salinity stress on growth and nutrient composition of three soybean (*Glycine max* L. Merrill) cultivars. J. Agron.Crop Sci.188:86-93.

Kao, W., T.T. Tyng, C.T. Hung, and N.S. Chen. 2006. Response of three Glycine species to salt stress. Environmental and Experimental Botany. 56:120-125.

Lee, G.J., H.R. Boerma, M.R. Villagarcia, X. Zhou, T.E. Carter Jr., Z. Li, and M.O. Gibbs. 2004. A major QTL conditioning salt tolerance in S-100 soybean. Theor. Appl. Genet. 109 (8):1610-1619.

Pantalone, V.R., W.J. Kenworthy, L.H. Slaughter, and B.R. James. 1997. Chloride tolerance in soybean and perennial Glycine accessions. Euphytica 97(2):235-239.

Philip, J. and R. Broadley. 2001. Chloride in soils and its uptake and movement with the plant: A review. Annals of Botany. 88(6):967-988.

Rupe, J.C., J.D. Widick, W.E. Sabe, R.T. Robbins, and C.B. Becton. 2000. Effect of chloride and soybean cultivar on yield and the development of sudden death syndrome, soybean cyst nematode, and southern blight. Plant Dis. 84:669-674.

Wilson, C.E. Jr., T.C. Keisling, D.M. Miller, C.R. Dillon, A.D. Pearce, D.L. Frizzell, and P.A. Counce. 2000. Tillage influence on soluble salt movement in silt loam soils cropped to paddy rice. Soil Sci. Soc. Am. J. 64:1771-1776.

Valencia, R., P. Chen, T. Ishibashi, and M. Conatser. 2008. A rapid and effective method for screening salt tolerance in soybean. Crop Sci. 48:1173-1779.

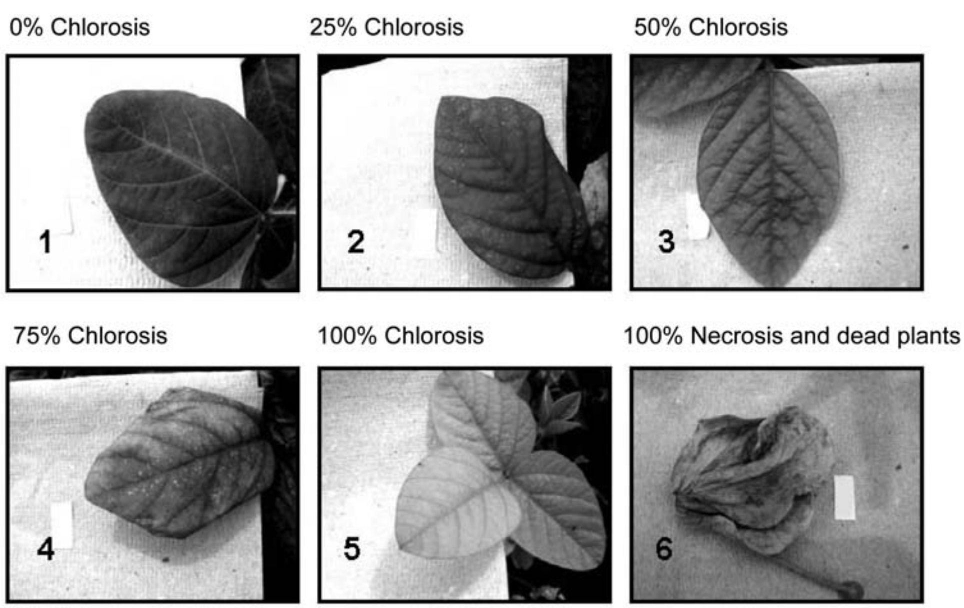

Fig. 1. Visual rating scale (1~6) used for evaluating foliar chloride symptoms in soybean.

Fig. 2. Comparison of the symptoms of at 13 d on 'Clark' (left, includer) and 'Dare' (right, excluder) soybean in potting mix with 120 mM NaCl.

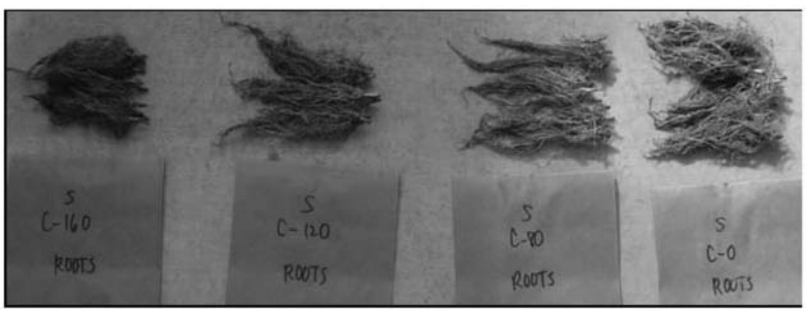

Fig. 3. Root development of Clark soybean (includer) in sandy loam soil with 160, 120, 80, and 0 mM NaCl, respectively.

Table 1. Soybean cultivar with known reactions to salt used in this greenhouse study to evaluate the response to salt stress.

Genotypes	Reaction	Classification
Clark	Sensitive	Cl-includer
Dare	Sensitive	Cl-includer
Williams	Sensitive	Cl-includer
Lee 68	Tolerant	Cl-excluder
S-100	Tolerant	Cl-excluder

Table 2. Visual rating scores of foliar chlorosis symptoms averaged for five soybean cultivars grown in potting mix and sandy loam with different concentrations of NaCl.

NaCl concentration (mM)	RATE†							
	Potting Mix				Sandy loam			
	Includer		Excluder		Includer		Excluder	
80	3.56	Aa‡	2.33	Ba	2.86	Ba	1.87	Aa
120	4.57	Aa	3.29	ABb	4.55	Aa	3.28	Aa
160	5.25	Aa	3.88	Aa	4.92	Aa	3.74	Aa
Mean	4.46		3.17		4.11		2.96	

† RATE = Foliar symptoms rating scale; 1 for healthy plant with no chlorosis, 2 for 25% of leaf chlorosis, 3 for 50% of leaf chlorosis, 4 for 75% of leaf chlorosis /scorching, 5 for 100 % chlorosis, and 6 for completely dead plant.

‡ In a column, scores followed by the same upper case letter are not significantly different by Tukey (p=0.05). Scores of includer and excluder in a row followed by the same lower case letter are not significantly different by Tukey (p=0.05).

Table 3. Relative shoot dry weights (RSDW) of five soybean cultivars grown in potting mix and sandy loam with different concentraions of NaCl.

NaCl concentration (mM)	RSDW (g)†							
	Potting mix				Sandy loam			
	Includer		Excluder		Includer		Excluder	
0	1.00	A‡	1.00	A	1.00	A	1.0	A
80	0.59	Ba	0.68	Aa	0.60	Ba	0.63	Ba
120	0.43	BCa	0.44	Aa	0.50	Ba	0.66	Ba
160	0.37	Ca	0.49	Aa	0.44	Ba	0.54	Ba
Mean	0.46		0.54		0.51		0.61	

† Relative Shoot Dry Weight = treated / control; averaged over genotypes.

‡ In a column, relative dry weight followed by the same upper-case letter are not significantly different by Tukey (p=0.05).

The relative dry weights of includer and excluder in a row followed by the same lower-case letters are not significantly different by Tukey (p=0.05).

Table 4. Relative root dry weights (RRDW) averaged over five soybean cultivars grown in potting mix and sandy loam with different concentrations of NaCl.

NaCl concentration (mM)	RRDW (g)†							
	Potting mix				Sandy loam			
	Includer		Excluder		Includer		Excluder	
0	1.00	A‡	1.00	A	1.00	A	1.0	A
80	0.43	Ba	0.38	Aa	0.66	ABa	0.58	ABa
120	0.12	Ba	0.17	Aa	0.51	Ba	0.47	Ba
160	0.21	Ba	0.14	Aa	0.49	Ba	0.37	Ba

† Relative root dry weight = treated / control; averaged over genotypes.

‡ In a column, relative dry weights followed by the same upper case letter are not significantly different by Tukey (p=0.05).

The relative dry weights of includer and excluder in a row followed by the same lower-case letter are not significantly different by Tukey (p=0.05).

Table 5. Chloride concentrations in shoot (CLSH) averaged for five soybean cultivars grown in potting mix and sandy loam with different concentrations of NaCl.

NaCl concentration (mM)	CLSH† (mg/Kg)							
	Potting Mix				Sandy Loam			
	Includer		Excluder		Includer		Excluder	
0	5229	Ca‡	1552	Cb	3404	Ca	2512	Ca
80	46583	Ba	40467	Ba	41463	Ba	24743	Bb
120	61478	Aa	50689	Aa	46788	Ba	34017	Ba
160	60768	Aa	49632	Ab	64640	Aa	54769	Aa
Mean	43515		35585		39074		29010	

† CLSH= Chloride concentration in shoots (mg/Kg).
Plants were grown in either potting mix or sandy loam with 0, 80, 120, or 160 mM NaCl. Cl concentrations were determined by tissue analysis.

‡ In a column, chloride concentrations followed by the same upper case letter are not significantly different by Tukey (p=0.05).

The chloride concentrations of includer and excluder in a row followed by the same lower-case letter are not significantly different by Tukey (p=0.05).

Table 6. Chloride concentrations in root (CLRT) averaged for five soybean cultivars grown in potting mix and sandloam with different concentrations of NaCl.

NaCl concentration (mM)	CLRT† (mg/Kg)							
	Potting mix				Sandy loam			
	Includer		Excluder		Includer		Excluder	
0	7523	Ba‡	1984	Ba	18920	Ba	21765	Ba
80	19717	Aa	26398	Aa	36933	Ab	42225	Aa
120	18487	Ab	32775	Aa	42133	Aa	48900	Aa
160	18420	Aa	40294	Aa	36350	Ab	47625	Aa
Mean	16037		25363		33584		40129	

† CLRT= Chloride concentration in roots (mg/Kg).
Plants were grown in either potting mix or sandy loam with 0, 80, 120, or 160 mM NaCl.
Cl concentrations were determined by tissue analysis.
The chloride concentrations of includer and excluder in a row followed by the same lower-case letter are not significantly different by Tukey (p=0.05).

‡ In a column, chloride concentrations followed by the same upper-case letter are not significantly different by Tukey (p=0.05).

Table 7. Correlations among relative shoot, root dry weights, shoot and root chloride concentrations, and rating scores of Cl toxicity of NaCl stressed soybean.

Overall	RSDW	RRDW	CLSH	CLRT	RATE
RSDW		0.81***	−0.91***	−0.48**	−0.52**
RRDW			−0.83***	−0.40*	−0.30NS
CLSH				0.44**	0.79***
CLRT					−0.27NS
RATE					

Potting Mix	RSDW	RRDW	CLSH	CLRT	RATE
RSDW		0.91***	−0.93***	−0.61**	−0.48NS
RRDW			−0.91***	−0.72***	−0.22NS
CLSH				0.59**	0.84***
CLRT					−0.34NS
RATE					

Sandy Loam	RSDW	RRDW	CLSH	CLRT	RATE
RSDW		0.69***	−0.88***	−0.74***	−0.56*
RRDW			−0.77***	−0.83***	−0.34NS
CLSH				0.69***	0.80***
CLRT					−0.15NS
RATE					

*Significant at P < 0.05.
**Significant at P < 0.01.
***Significant at P <0.001.
RSDW= Relative shoot dry weight.
RRDW= Relative root dry weight.
CLSH= Chloride concentration in shoot.
CLRT= Chloride concentration in root.
RATE= Rating score of visual Cl toxicity symptoms.
NS= Not significant.