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Synergistic interaction of NaCl and Cd on growth and photosynthetic parameters in soybean genotypes differing in salinity tolerance*

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Abstract: The effects of salinity (50 mmol/L NaCl) and Cd (1 μmol/L $CdCl_2$) as sole and combined on growth and photosynthetic parameters were studied using two soybean genotypes, Huachun 18 and NGB. The concentrations of Cd^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, K^+ and Na^+ were also determined in seeds and pods. Huachun 18 suffered a more serious decrease than NGB in net photosynthetic rate (P_n) in the treatments of salinity stress alone and combined stress (NaCl+Cd), showing that it is relatively sensitive to salinity. The decrease in P_n caused by salt stress in Huachun 18 was mainly due to the reduced total chlorophyll content and photosynthetic efficiency (the ratio of variable fluorescence to maximal fluorescence, F_v/F_m), whereas the decease in NGB was mainly related to reduced stomatal conductance (G_s). The combined stress of both Na and Cd did not induce further decrease in photosynthesis and fluorescence in the two genotypes relative to salt or Cd stress alone. Greater change in the pod concentrations of Zn^{2+}, Ca^{2+}, Mg^{2+}, K^+ and Na^+ was detected under salt stress for Huachun 18 than for NGB. The results suggested that the interactive effect of NaCl-Cd on growth and nutrient uptake differs between the two soybean genotypes.

Key words: Soybean (*Glycine max* (L.) Merr.), Salinity, Cadmium, Photosynthesis, Fluorescence
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INTRODUCTION

Soil salinity is one of the major abiotic stresses in crop production. Much has been written about the extent of salt-affected land and its impact on agriculture (Szabolcs, 1989; 1994; Tanji, 1990; Rhoades and Loveday, 1990), with this problem being especially serious in arid and semi-arid regions. On the other hand, rapid urbanization, industrialization, and increased utilization of pesticides and fertilizers have aggravated heavy metal pollution. It was reported that soil salinity could significantly enhance Cd bioavailability in soil and promote Cd uptake and translocation in plant (McLaughlin *et al*., 1994; Smolders *et al*., 1997; Weggler-Beaton *et al*., 2000). Although the mechanism of salinity-Cd interaction is not fully understood, it has been postulated that the formation of Cd-Cl complex is the main factor of Cd uptake enhancement (Weggler-Beaton *et al*., 2000).

Both Cd and salt stresses can pose several problems for plant growth and development by inducing physiological dysfunctions. Excessive Cd could disturb mineral nutrient uptake, carbohydrate metabolism (Moya *et al*., 1993), therefore, strongly inhibit chlorophyll biosynthesis (Padmaja *et al*., 1990) with this being the same in case of salinity. Sudhir and Murthy (2004) reported that salt stress causes various inhibitory effects on bio-energetic processes of photosynthesis. It is known that the deleterious effect of salinity occurs due to (1) osmotic stress, (2) interruption of metabolic activities by ionic excess and imbalance, and (3) interference of salt ions on the uptake of essential macro-nutrients and micro-nutrients (Pasternak, 1987). However, few researches were focused on the combined effect of Cd and NaCl on plant growth and development, although it has been believed that it is not simply an additive influence

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(Mühling and Läuchli, 2003).

Long-term consumption of Cd-contaminated food may threaten human health by inducing Cd disease, such as proximal tubular renal dysfunction. Cd is chronically toxic to human even at lower concentration. Therefore, it is important to minimize Cd accumulation in plants, particularly in edible parts. Whereas, current reports concerning the response of the plants to Cd exposure are mainly aimed at evaluating its effect on the development and growth of plants and Cd accumulation in roots and shoots (Leita and Nobili, 1991; Costa and Morel, 1993; Jalil *et al*., 1994). Little work has been carried out on Cd accumulation and the relative effect on cations such as Ca, Zn, Mg in soybean seed.

The aim of this work was to determine effects of single and combined stress of Cd and NaCl on photosynthesis and fluorescence in leaves and cation relations in soybean seeds. Two soybean genotypes, a salt-sensitive (Huachun 18) and a salt-tolerant (NGB) were treated by low Cd concentration and moderate saline stress. The present study was also carried out to test the hypothesis that plants exposed to salinity stress would absorb more Cd.

MATERIALS AND METHODS

Experimental treatments and design

The experiment was carried out during 2004~2005 at Huajiachi campus, Zhejiang University, Hangzhou, China. Two genotypes of soybean (*Glycine max* (L.) Merr.) were used, i.e. Huachun 18, a salt-sensitive genotype and NGB, a salt-tolerant genotype. The seeds of the two genotypes were surface-sterilized in 2% H_2O_2 for 10 min, rinsed with distilled water 5 times and germinated in moist quartz sand in a greenhouse. At the second leaf stage (14 d old), seedlings were selected for uniformity and transplanted onto 30-L containers, covered with a foamed plastic plate with evenly spaced holes and placed in a greenhouse. Sixteen seedlings of each genotype were planted in a container. The composition (μmol/L) of the basic nutrient solution was: NH_4NO_3 362.7, NaH_2PO_4 182.2, K_2SO_4 91.2, $MgSO_4$ 508.8, $CaCl_2$ 590.3, Fe-citrate 4.47, $MnCl_2$ 0.45, $ZnSO_4$ 0.4, $CuSO_4$ 0.22, H_3BO_3 2.9, Na_2MoO_4 0.01, $CoCl_2\cdot 6H_2O$ 0.025, and the pH was 5.0. The NaCl and Cd treatments were started two weeks after transplanting. Cd and NaCl were added to corresponding containers to form the following 4 treatments: (1) control; (2) 1 μmol/L Cd; (3) 50 mmol/L NaCl; (4) 1 μmol/L Cd+50 mmol/L NaCl. The experiment was laid out as completely random black design with four replicates. The solution pH in each container was adjusted every other day with HCl or NaOH as required. The nutrient solution was renewed every 5 d. On the day (73 d after transplanting) when the treatments were ended, soybean pods were harvested, dried and then seeds in them gathered.

Measurements

The measurements were carried out on the topmost secondary fully expanded leaves. At 10 and 20 d after treatment, chlorophyll (Chl) concentration, expressed as SPAD value, was measured with a chlorophyll meter (Minolta Co. Ltd., Japan). The ratio of variable fluorescence to maximal fluorescence (F_v/F_m), which is an indicator of the efficiency of the photosynthetic apparatus, was measured with a portable fluorometer (model FMS-2 Hansatech Instruments Ltd., England). The leaves of measured plants were first adapted to total darkness with a Hansatech clip for 15 min. The unquenchable portion of fluorescence (F_0) was determined by measuring beam [<0.05 μmol/(m^2·s)]. The maximal fluorescence (F_m) was determined using a saturating pulse [1200 μmol/(m^2·s)]. Actinic light was obtained from a light emitting diode [180 μmol/(m^2·s)]. The variable fluorescence (F_v) was determined by the formula, $F_v=F_m-F_0$. In parallel to the fluorescence measurement, photosynthetic parameters, including net photosynthetic rate (P_n), stomatal conductance (G_s), intracellular CO_2 concentration (C_i) and transpiration (T_r), were determined using a photosynthesis system (ADC Bio-scientific Ltd., UK).

The seeds were ground, weighed and concentrations of Cd^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, K^+ and Na^+ were determined by an atomic absorption spectroscope (Shimadzu, Japan) after the samples were ashed in a muffle furnace and prepared with HNO_3:H_2O (1:1).

Statistical analysis

All data were subjected to statistical analysis using software of SPSS 11.0 and means were compared by Student's *t*-test. Comparisons with *P* values <0.05 were considered significantly different.

RESULTS

Plant growth

Effect of Cd and NaCl on soybean growth was assessed by shoot height. Both Cd and NaCl treatments inhibited shoot elongation, although the difference between the control and Cd alone treatment was not significant (Table 1). At 10 d after treatment, no significant difference was found among the treatments, except for the difference of control and the treatment Cd+NaCl for Huachun 18. At 20 d, NaCl stress led to significant decrease in shoot height in both genotypes, with the reduction being 19.2% for Huachun 18 and 13.9% for NGB, respectively. However, the combined two stresses (Cd+NaCl) did not cause further decrease in shoot height (Table 1). The two genotypes showed obvious difference in the response of shoot length to the two stresses, with NGB being more tolerant than Huachun 18.

Table 1 Effect of different stress treatments on shoot length (cm) of two soybean genotypes

Genotype	Treatment	Shoot length (cm)	
		10 d	20 d
Huachun 18	Control	37.8 a*	41.7 a
	1 μmol/L Cd	33.9 ab	39.8 ab
	50 mmol/L NaCl	33.4 ab	33.7 c
	1 μmol/L Cd+50 mmol/L NaCl	30.0 b	37.2 bc
NGB	Control	46.1 a	51.9 a
	1 μmol/L Cd	44.8 a	47.0 ab
	50 mmol/L NaCl	42.3 a	44.7 b
	1 μmol/L Cd+50 mmol/L NaCl	47.7 a	49.1 ab
Interaction between variety and treatment		s#	ns

* The same letter after the data within a column for the same cultivar means no significant difference at 95% probability level; # s and ns mean significant and not significant at 95% probability level

Chlorophyll concentration, fluorescence and photosynthesis

The dose- and time-responses of chlorophyll concentration, which is expressed as SPAD value, in soybean leaves is shown in Table 2. There was a significant difference between genotypes in SPAD value. Treatment with 1 μmol/L Cd did not reduce SPAD value significantly relative to the control. However, the plants exposed to 50 mmol/L NaCl reduced SPAD value by 32.8% and 35.2% at 10 and 20 d after treatment for Huachun 18, respectively, but no significant difference was found for NGB. The combined stress did not induce further decrease of SPAD value in the plants only exposed to NaCl. The difference between Huachun 18 and NGB was observed in response of SPAD value to the stresses.

The effect of both NaCl and Cd treatments on photosynthetic parameters is presented in Tables 2 and 3. In photosynthetic efficiency (F_v/F_m), no significant inhibition was found in the plants exposed to both Cd and NaCl treatments, except for Huachun 18 under NaCl stress (Table 2). Moreover, the plants exposed to 1 μmol/L Cd showed no significant difference from the control in all the five photosynthetic parameters, irrespective of genotypes (Tables 2 and 3), indicating that 1 μmol/L Cd had little influence on the photosynthetic function of soybean plants. On the other hand, there was an obvious difference in P_n between the two genotypes. Huachun 18 showed dramatic reduction in P_n 20 d after NaCl or NaCl+Cd treatment, while NGB had relatively small change. It was also noted that P_n under combined stress was slightly higher than NaCl alone, except for NGB at 20 d. NaCl treatment caused marked decrease in G_s

Table 2 Effects of the different stress treatments on chlorophyll concentration (SPAD value) and photosynthetic efficiency (F_v/F_m) of the topmost secondary fully expanded leaves for the two soybean genotypes

Genotype	Treatment	SPAD value		F_v/F_m	
		10 d	20 d	10 d	20 d
Huachun 18	Control	30.5 a*	39.8 a	0.746 a	0.776 a
	1 μmol/L Cd	28.5 ab	38.9 a	0.637 a	0.755 a
	50 mmol/L NaCl	20.5 c	25.8 b	0.777 a	0.327 b
	1 μmol/L Cd+50 mmol/L NaCl	23.2 bc	25.4 b	0.763 a	0.702 a
NGB	Control	36.0 ab	42.2 a	0.726 a	0.767 a
	1 μmol/L Cd	38.3 a	45.9 a	0.761 a	0.774 a
	50 mmol/L NaCl	34.6 ab	40.1 a	0.762 a	0.751 a
	1 μmol/L Cd+50 mmol/L NaCl	30.8 b	39.7 a	0.773 a	0.721 a
Interaction between variety and treatment		s#	s	s	s

* The same letter after the data within a column for the same cultivar means no significant difference at 95% probability level; # s means significant at 95% probability level

Table 3 Effect of the different stress treatments on net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i) and transpiration (T_r) of the topmost secondary fully expanded leaves for the two soybean genotypes

Genotype	Treatment	P_n [μmol CO_2/(m^2·s)]		G_s [mol/(m^2·s)]		C_i (μmol/mol)		T_r [mmol/(m^2·s)]	
		10 d	20 d	10 d	20 d	10 d	20 d	10 d	20 d
Huachun 18	Control	17.3 a*	17.5 a	0.577 a	0.585 a	317.7 a	323.7 a	5.72 a	2.86 a
	1 μmol/L Cd	18.7 a	16.4 a	0.435 a	0.728 a	286.3 ab	335.0 a	5.03 a	3.12 a
	50 mmol/L NaCl	8.4 b	1.2 b	0.152 b	0.070 b	282.3 ab	367.7 a	2.70 b	0.65 b
	1 μmol/L Cd+50 mmol/L NaCl	12.5 b	3.6 b	0.182 b	0.084 b	252.0 b	321.7 a	3.42 b	0.87 b
NGB	Control	19.0 a	18.6 a	0.548 a	0.892 a	298.0 a	335.0 a	6.45 ab	3.96 ab
	1 μmol/L Cd	19.7 a	18.9 a	0.509 a	0.879 a	295.7 a	331.3 ab	6.71 a	4.20 a
	50 mmol/L NaCl	16.5 a	15.6 ab	0.193 b	0.398 b	215.7 b	307.7 c	4.82 b	3.05 b
	1 μmol/L Cd+50 mmol/L NaCl	16.9 a	14.5 b	0.233 b	0.434 b	237.7 b	315.3 bc	5.70 ab	3.30 ab
Interaction between variety and treatment		s#	s	ns	ns	s	s	ns	s

* The same letter after the data within a column for the same cultivar means no significant difference at 95% probability level; # s and ns mean significant and not significant at 95% probability level

(Table 3). Although the two genotypes showed significant decrease in G_s for both NaCl alone and combined Cd treatment, the extent of decrease was more severe in Huachun 18 than in NGB.

The difference between the two genotypes in response of T_r to the stresses was also quite clear. The plants of Huachun 18 exposed to NaCl alone and combined Cd treatments reduced T_r by 77.3% and 69.6% 20 d after the treatments, respectively in comparison with the control, but no significant differences were found between the treatments and the control for NGB.

Cd, Na and some cation concentrations in seeds and pods

The changes of Cd^{2+}, Na^+ and some nutrient element concentrations in seeds and pods are shown in Tables 4 and 5, respectively. The data on Huachun 18 seeds under NaCl and combined treatments were not presented because no seed was obtained due to its high sensitivity to salt stress.

It was obvious that Cd concentration of seeds increased under Cd stress for the two genotypes although the increase in NGB was not significant (Tables 4 and 5). Similarly Cd stress increased Cd concentration in pods for both genotypes although the difference with the control was not significant. In contrast, Cd concentration of seeds and pods in both genotypes was little affected by salt stress. The combined treatment further increased Cd concentration in both seeds and pods in comparison with Cd treatment alone.

In terms of Na concentration, salt stress led to significant increase in Na^+ concentration in both seeds and pods (Tables 4 and 5). However, significantly lower Na^+ concentration was observed in pods of Huachun 18 under combined stress than NaCl stress, but the differences were not found in both seeds and pods of NGB.

There was a genotypic difference in influence of Cd and NaCl stresses on some nutrient element concentration. The seeds of the plants exposed to Cd stress contained higher Zn^{2+} concentration (Table 4) and for the seeds of NGB, NaCl stress increased the concentrations of Zn^{2+}, Mg^{2+} and K^+. The combined stress showed significant positive influence on Zn^{2+}, Ca^{2+} and Mg^{2+} accumulation.

The genotypic difference in salt tolerance can also be retested in Table 5. Under NaCl stress, the K^+/Na^+ of Huachun 18 (1.26) was much lower than that of NGB (1.69). Significant differences of other cations, such as Zn^{2+}, Ca^{2+}, Mg^{2+}, were also found in the two genotypes. The ions balances were seriously influenced under stress for Huachun 18, but for NGB, little changes were found due to Cd and NaCl treatments in this study.

Table 4 Effect of NaCl and Cd stress on cation concentration of soybean seeds at maturity

Genotype	Treatment	Zn (μg/g)	Cd (μg/g)	Ca (μg/g)	Mg (mg/g)	K (mg/g)	Na (mg/g)
Huachun 18#	Control	23.5 b*	0.82 b	85.2 b	0.44 b	9.9 a	0.10 a
	1 μmol/L Cd	24.5 a	2.04 a	95.4 a	0.48 a	10.7 a	0.05 b
NGB	Control	24.4 c	0.66 b	118.5 b	0.48 c	9.9 b	0.02 b
	1 μmol/L Cd	25.5 c	1.39 ab	116.7 b	0.47 c	10.3 ab	0.03 b
	50 mmol/L NaCl	29.7 b	1.05 ab	110.5 b	0.79 b	13.2 ab	5.54 a
	1 μmol/L Cd+50 mmol/L NaCl	31.6 a	1.96 a	163.8 a	1.01 a	13.5 a	5.87 a

* The same letter after the data within a column for the same cultivar means no significant difference at 95% probability level; # The plants exposed to NaCl and CaCl+Cd stress had no seed production

Table 5 Effect of NaCl and Cd stress on cation concentration of soybean pods at maturity

Genotype	Treatment	Zn (μg/g)	Cd (μg/g)	Ca (μg/g)	Mg (mg/g)	K (mg/g)	Na (mg/g)
Huachun 18	Control	23.71 c*	0.81 b	347.67 c	0.47 b	12.57 b	0.26 c
	1 μmol/L Cd	24.11 c	1.79 ab	317.26 c	0.48 b	11.94 b	0.18 c
	50 mmol/L NaCl	67.23 a	1.40 ab	733.01 a	1.54 a	27.08 a	21.55 a
	1 μmol/L Cd+50 mmol/L NaCl	34.69 b	2.48 a	417.89 b	0.66 b	14.71 b	9.01 b
NGB	Control	23.09 b	0.63 b	207.82 c	0.47 a	18.79 a	0.18 b
	1 μmol/L Cd	25.69 a	1.46 ab	259.40 a	0.46 a	12.19 b	0.13 b
	50 mmol/L NaCl	24.86 a	0.74 b	234.97 b	0.48 a	11.29 b	6.68 a
	1 μmol/L Cd+50 mmol/L NaCl	25.83 a	2.44 a	244.33 ab	0.52 a	12.11 b	7.43 a
Interaction between variety and treatment		s#	ns	s	s	s	s

* The same letter after the data within a column for the same cultivar means no significant difference at 95% probability level; # s and ns mean significant and not significant at 95% probability level

DISCUSSION

It has been reported that the decrease in shoot growth rate for Cd-stressed plants was correlated with external Cd concentration, exposure duration, plant species or genotype (Dražić *et al*., 2004). In this study, it is obvious from Table 1 that there was no significant difference in growth between plants of the control and of 1 μmol/L Cd treatment, which is consistent with the results reported by Wu *et al*.(2003). The combined stress (Cd+NaCl) did not show further influence upon plant growth compared with salinity, which might also be attributed to the too low Cd concentration. However, both genotypes exposed to Cd showed obvious increase in Cd accumulation in both seeds and pods.

The decrease of plant growth was mainly associated with the decrease of photosynthesis, which decreased biomass assimilation in plants. The inhibition of P_n by NaCl may be a mixed consequence of salinity induction of stomatal closure caused by the decrease in osmotic potential, and non-stomatal inhibition of photosynthesis, caused by direct effects of NaCl on other photosynthetic parameters independent of stomatal closure. This study found that the decrease of P_n was significantly correlated with G_s, T_r, SPAD value and F_v/F_m (data not shown). However, further analysis revealed that the inhibition mechanism of photosynthesis was different between genotypes. For Huachun 18, the relatively seriously inhibited genotype, the decrease of G_s was accompanied by significant increase of intercellular CO_2 concentration (C_i), suggesting that not stomatal closure but decrease of chlorophyll concentration and F_v/F_m contributed to the decrease of P_n (Tables 2 and 3). The ratio of F_v/F_m is always used as a stress indicator, describing the potential yield of the photochemical reaction. The decrease of F_v/F_m and chlorophyll concentration suggests that salinity induced irreversible physiological destruction to photosynthetic function in Huachun 18, whereas the reduction of P_n in NGB was probably due to the salinity modification of G_s (a less reduction in P_n than in G_s and concomitantly a lower C_i under NaCl stress), which is reversible. This is consistent with the finding in wild soybean species (Kao *et al*., 2003) and mangrove (Parida *et al*., 2004).

It is pertinent to mention that the photosynthesis related parameters are always slightly less suppressed under combined stress (NaCl+Cd) than NaCl in both genotypes, especially for F_v/F_m in Huachun 18 (Tables 2 and 3). However, the mechanism is still unclear.

No seeds were harvested in Huachun 18 under NaCl and combined treatments, which could be most likely attributed to the sharp decline of P_n. To date, little work has been done on cation accumulation in seeds under Cd and NaCl stress. Therefore, in addition to Cd^{2+} and Na^{+}, some essential elements, such as Zn^{2+}, Ca^{2+}, Mg^{2+} and K^{+} were also determined. In general, cation concentrations in seeds are positively associated with those in pods. The role of salinity in increasing Cd^{2+} uptake was also found in earlier investigations (Bingham *et al*., 1984; Helal *et al*., 1999; McLaughlin *et al*., 1994; Weggler-Beaton *et al*., 2000; Mühling and Läuchli, 2003). In the present study, the increased Cd^{2+} concentration in soybean seeds and pods provided another proof for the hypothesis that more Cd would be assimilated by plant under salt stress. Thus it is very important to find the exact range of NaCl and Cd concentrations in the external environment for safe soybean production.

There was a significant genotypic difference in Zn^{2+}, Ca^{2+}, Mg^{2+} and K^{+} concentrations of soybean pods (Table 5). Under salt stress, Huachun 18 showed extremely high concentration of all these elements in pods, revealing that ion toxicity which led to ion uptake disorder and serious ions imbalance occurred in Huachun 18. However, for the relatively tolerant genotype NGB, no significant change was found in concentrations of these elements under all treatments.

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