

SALINITY ADAPTABILITY RESPONSES OF WILD SOYBEAN (*GLYCINE SOJA* SIEB. & ZUCC.) UNDER HIGH SALINE SOIL STRESS OVER WHOLE GROWTH PERIOD

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Abstract

Wild soybean is expected to improve stress tolerance of soybeans. However, few salt-tolerance identifications are done through whole growth period in this plant. We identified 895 wild soybean samples in a high-salinity soil during the whole growth period. The results showed that there were five types in salinity adaptability response (A, non-germinable; B, seedling death; C, before-flowering death; D, before-maturity death and E, lived to seed maturity). Under high-salinity conditions, the most severe loss was above-ground dry weight (lost 87.25%) followed by yield per plant (82.58%), the number of seeds per plant (73.71%). However, 100-seed weight had a relatively low reduction (40%). The number of seeds per plant, 100-seed weight, above-ground dry weight, harvesting index, growth period, and plant height were significantly positively correlated with yield mainly indirectly via the number of seeds, with higher indirect path coefficients. The highly salinity-tolerant lines (E type) possessed more rapid growth and lesser growth inhibition, however, they were evolved into different levels of adaptability to salinity according the comprehensive assessment *D* values. Our present study suggested that early or short-term or staged-identification would have the hazard of misjudgement of salinity tolerance and whole growth period identification should be adopted for soybean breeding program.

Key words: Wild soybean, Germplasm, Screening, Salt tolerance.

Introduction

Soil salinization is a global problem for crop production. Most crops, particularly leguminous crops are sensitive to saline-alkali soil. In China, there are salinized lands of approximately 3.3×10^7 ha, of which 1×10^7 ha are moderate or low saline-alkali lands. Salinity can harm the development of plants and cause yield reduction of crops (Abel & Mackenzie, 1964; Weil & Khalil, 1986; Chang *et al.*, 1994; Maas, 1996). Although several salt-tolerant soybean (*Glycine max*) varieties were developed by traditional cross breeding methods using the existing salt-tolerant germplasm accessions (Shao *et al.*, 2009; Wang & Li, 2001) yet current soybean production is becoming low on salt affected lands due to loss of salt tolerance in salt-tolerant cultivars in China. Nevertheless, there has not been any breakthrough in soybean salt-tolerance breeding in China over the past few decades. The reason, from the breeding practices, seems to be the absence of high-salinity-tolerant germplasm, which is likely due to fallacious technique and/or use of improper selection criteria for salt tolerance. Using nutrient solutions containing salinity to identify salt tolerance has prevailed at present in screening salt-tolerant plants, by evaluating root growth parameters and morphological performances (Ahmad & Wainwright, 1976; Humphreys, 1982; Shannon, 1984; Ab-Shukor *et al.*, 1988; Kik, 1989; Wang *et al.*, 1997; Zhu *et al.*, 1998; Bayuelo-Jiménez *et al.*, 2002; Mguis *et al.*, 2008; Lee *et al.*, 2009), and analyzing physiological traits and chemical composition (Yu *et al.*, 2001; Sairam & Tyagi, 2004) at germination and seedling stages. These short-term identification methods can quickly collect data under controllable conditions, however, some researchers have noted that efforts to evaluate salt tolerance for crop species at germination and

emergence stage are generally not successful. Salt tolerance is a developmentally regulated, stage-specific phenomenon, such that one growth stage usually is not related to another (Shannon, 1997; Bayuelo-Jiménez *et al.*, 2002). Short-term identification might only demonstrate phasic tolerance to the designated level of salinity at the identification period, and it cannot reflect stable tolerance throughout the entire life cycle, because of the differences in salt tolerance between growth stages. In soybeans, no correlation of salt tolerance between germination and subsequent growth stages was observed (Abel & Mackenzie, 1964; Shao *et al.*, 1986). Epstein *et al.*, (1980) and Jones & Qualset (1984) suggested that plant growth attributes must be measured throughout the growth period so that salt-sensitive growth stages could be identified, and tolerance selection of salt stress over the entire growth cycle could be used. Field identification of salt tolerance has also been attempted for Chinese soybean (Shao *et al.*, 1986) and wild soybean (Wang *et al.*, 2005). Moderately saline fields were treated by seawater irrigation prior to sowing, and the development of plants was investigated over the entire growth period. This method could not severely control uniform field conditions and environmental alterations, particularly in the rainy season. The leaching of precipitation can briefly reduce the salinity damage, which allows some traits to escape the salt stress.

The productivity of crops is inherently limited by the germplasm employed in commercial varieties. In order to exploit biotic and abiotic stress-tolerant germplasms or genes of crops, the wild relatives have been identified as good genetic resources (Harlan, 1976). Some soybean perennial wild relatives (*Glycine* Willd.) have been identified for chloride tolerance (Pantalone *et al.*, 1997). The genus *Glycine* subgenus *Soja* has only two species:

cultivated soybean (*Glycine max*) and its annual wild progenitor wild soybean (*Glycine soja*). Both have the same genome ($2n=40$, GG) and can cross each other without any reproductive isolation. This annual herbaceous species *G. soja* is distributed across most parts of China, and grows in a broad range of environments such as riverbanks, roadsides, ponds, wastelands, sidehills, woods, grass, droughty lands, and saline-alkali lands. In China, wild soybean grows well in some coastal areas with moderate inorganic salts. Several seedling-staged salt-tolerant germplasm accessions of *G. soja* have been screened from saline-alkali lands by evaluating germination and seedlings (Yang *et al.*, 2003; Yu *et al.*, 2001) in China. However, so far, few salt-tolerance identifications through whole growth period are done in this plant, and also little is known about the adaptive differentiation of wild soybean natural populations that grow in coastal saline zones.

The objectives of the present study were to focus on the population dynamics response of wild soybean exposed to high-salinity stress over the whole growth period (i) to screen wild germplasm lines that have whole growth period salt tolerance for soybean breeding utilization, and (ii) to understand the ecological adaptation of this coastal wild soybean population under high-salinity stress.

Materials and Methods

Collection area and study materials: Wild soybean single-plant lines were sampled from a large coastal region growing in the middle Bohai Bay, North China (Fig. 1). This is a saline zone of about 1000 km² with non-uniform total salinity of 0.3% to >5% that mainly consisted of chlorides and other inorganic compounds from seawater. In the soil-improved, low-salinity patch lands, some crops such as cotton and maize and rice are cultivated. This regional wild soybean population was distributed in fragmented patterns from a few square meters to a few thousand square meters, even infrequently to a few kilometres far. Generally, the soil where wild soybean grew contained <1.5% total salinity and the wild soybean samples collected should had low salt tolerance. We collected wild soybean samples from the following habitats: roadsides, crop field verges, gutters, ponds and tree nurseries. A large number of 895 lines were sowed with five seeds for each line in the pots without setting repeats.

Entire growth period identification and experimental soil conditions: If salinity tolerance identification for these coastal wild soybean samples is conducted under conditions of low or moderate salinity, it will not be meaningful because they grow well in such saline soil conditions. For insight into the tolerance response of the study population to high salinity, soil near to the sea was used in the pot identification. The soil contained about 3.33% total salinity (0.94% Na⁺, 1.86% Cl⁻, 0.06% Ca²⁺, 0.05% K⁺, 0.11% Mg²⁺, 0.27% SO₄²⁻, 0.04% HCO₃²⁻). The identification of these lines was conducted in a plastic pot (35 cm diameter × 30 cm high) with 13 kg coastal salinity soil (Fig. 2). A large number of 895 lines

were sowed with five seeds for each line in the pots without setting repeats, and synchronously, a non-saline soil contrast was set for every line. The non-saline pot soil was usual experimental field soil from the experimental station of the Institute of Crop Science (Beijing). Prior to sowing, wild soybean seeds were gently abraded on the cotyledons of the hilum back with a blade. Watering used trickle irrigation once or twice weekly depending on the pot water status and was controlled to the extent of no water effusing from the bottoms of the pots. All experiments were carried out under an around-ventilated, rainproof plastic film shed. These identification pots including control pots were not supplied with any nutrient during the whole growth period for simulating field environment and maintaining a strict identification.

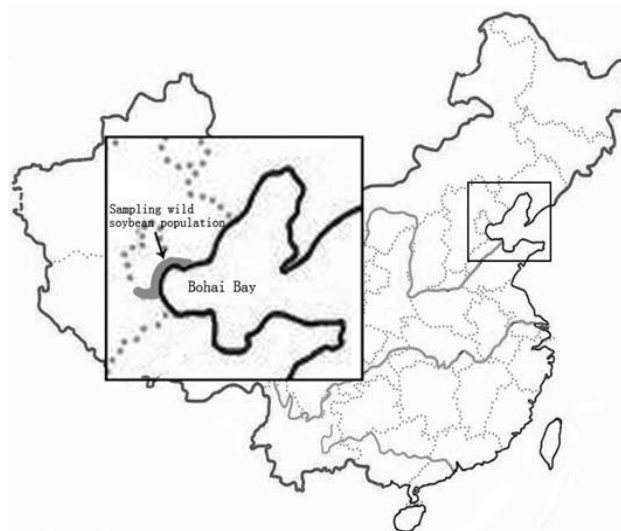


Fig. 1. Sampling wild soybean population at the middle Bohai Bay coastal saline region. The wild soybeans were distributed in fragmented patterns from a few square meters to a few thousand square meters in this saline zone. A total of 895 single plants were separately collected from in the following habitats: roadsides, crop field verges, gutters, ponds and tree nurseries.

Standard salt-tolerant control soybean varieties employed were Wenfeng No. 7 (WF7) and Tiefeng No. 8 (TF8). The two varieties were among the several most salt-tolerant soybean germplasm accessions that were screened out from Chinese soybean collection by Shao *et al.*, (1986). WF7 and TF8 were gene-mapped for their salt-tolerance genes (Guo *et al.*, 2000). WF7 has been confirmed to have stronger salt-tolerant gene expression of *GmCNGC* (cyclic nucleotide gated cation channel), *GmGLR3* (glutamate receptor) and *GmNKCC* (Na⁺/K⁺/Cl⁻ co-transporter) (Phang 2008; Shao *et al.*, 2009).

Data collection and statistics: The investigation characters included the dates of seedling emergence and death (date of the last death), vegetative-stage growth rate, defoliation rate per plant, plant height, yield/plant, yield components/plant (pod number, seed number, 100-seed weight), above-ground dry weight, and harvesting index [yield/(above-ground dry weight plus yield) × 100%]. In the maturation period, the character mean values for every line were measured from the surviving plants in a pot.

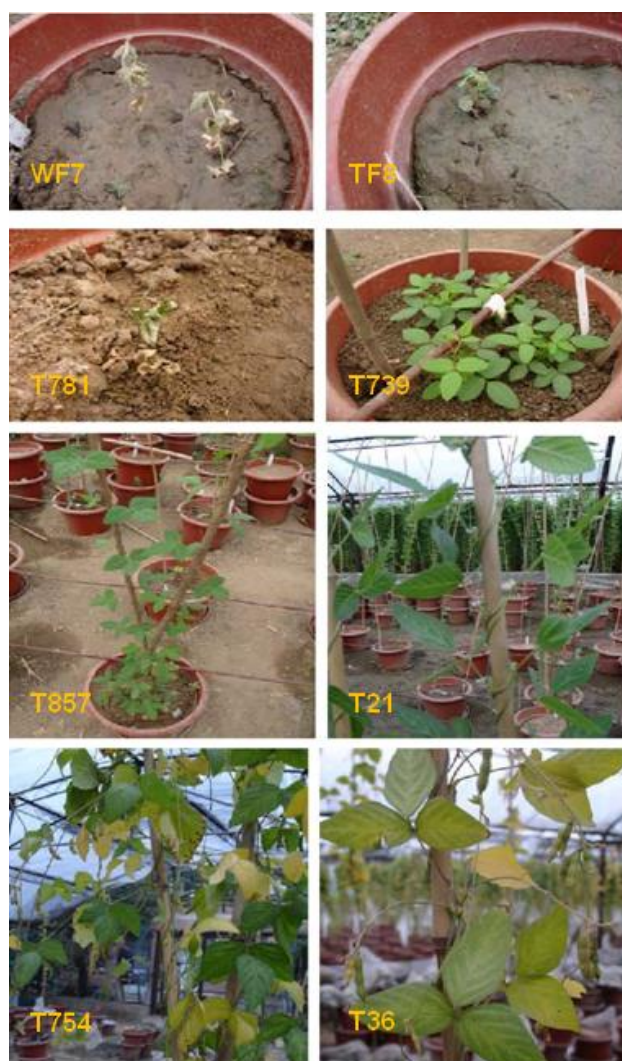


Fig. 2. Growth response of wild soybean lines collected in the middle Bohai coastal area in North China. Two standard highly salt-tolerant soybean varieties, WF7 and TF8, could not survive after 40 days from sowing (photos taken on day 39 after sowing). Sensitive T781 line died and highly salinity-tolerant T739 line survived at the seedling stage (17 days from sowing). Other lines were highly salinity-tolerant lines (photographs taken for T557 and T21 lines at 70 days and for T754 and T36 lines bearing pods at 127 days).

Correlation analyses between characters were made using simple correlation analysis, path analysis, and partial correlation analysis with SPSS version 16.0 software. Nine characters used for correlation analyses were: number of seeds per plant (X_1); 100-seed weight (X_2); above-ground dry weight per plant (X_3); harvesting index per plant (X_4); vegetative growth stage (days from sowing to the first flowering) (X_5); growth period (days from sowing to maturity); harvest index (X_6); plant height (cm) (X_7); defoliation rate per plant on 60 days after sowing (X_8); and yield per plant (X_9).

To evaluate the degree of salinity tolerance for the survival lines at maturation, we used the subordinate function method (Niu *et al.*, 1996) to calculate the comprehensive evaluation value (D) of salinity tolerance of each line. Prior to the evaluation of D , all observed data were standardized using the formula

$X_s = (X - \bar{X}) / SD$: where X is the observed value, \bar{X} is the mean value of samples, and SD is the standard deviation. The D value was estimated using the formula:

$$D = \sum_{j=1}^n [u(x_j) \cdot \left(r_j / \sum_{j=1}^n |r_j| \right)]$$
 referring to Niu *et al.*,

(1996). $u(x_j) = \frac{x_j - x_{\min}}{x_{\max} - x_{\min}}$ is the subordinate function

value of character j ; x_j is the relative value of character j [relative value: (observed value on saline soil/value on control soil) $\times 100\%$]; and x_{\max} and x_{\min} are the minimum and maximum relative values of the character j among samples, respectively. $r_j / \sum_{j=1}^n |r_j|$ is the weight of the

character j among all characters, which denotes the degree of importance, and r_j is the eigen value of the eigen vector of the character j in the primary principal component. Principal components analysis (PCA) was conducted using NTSYScp version 2.1 (Rohlf, 2000) to select the primary principal components of tolerance. The character factors of the eigen vector of the first principal component with the greatest contribution rate were used to estimate the D values of salinity tolerance for each mature line (E type). The maximal D value denoted the highest salinity tolerance.

Results

Population dynamics response to high-salinity stress across the developmental period: In high-salinity soil stress, two standard salt-tolerant soybean varieties, WF7 and TF8, could not survive long and died within 40 days from sowing (Fig. 2). We investigated the death and survival lines from sowing to maturity. The wild soybean samples had different duration types of salinity tolerance among samples. A series of individual lines were able to survive to different days from seedling emergence; even until seed maturity (Table 1). At the germination stage, 270 wild soybean lines (30.17% of the total samples) could not germinate, i.e. 625 lines (about 70% of the total samples) germinated and seedlings emerged. During the growth period, individual lines ceaselessly died at various days of development after seedling emergence (Table 1). Over half (335 lines, 53.6%) of the germinated 625 lines died within 30 days, of which, 60% (201 lines) died within 14 days. Subsequently, the number of death and survival lines was gradually reduced with growing time (Table 1). However, the survival lines far exceeded the death lines in number at every development stage (Table 1). A total of 118 lines (18.88% of the 625 germinated lines) could tolerate the high-salinity stress to flowering, and 88 lines (14.08% of the 625 lines) could survive to maturity to bear pods (Fig. 2, Tables 2 and 8). Seventy-six lines matured during 121–151 days after seedling emergence (Table 1).

Differences in growth of different duration types of salinity tolerance: According to the growth stages of death lines at different development times, the samples

were divided into five duration types of salinity tolerance: no germination (A type, 30.17% lines); death at seedling stage (B type, within 30 days after seedling emergence, 37.43%); death before flowering (C type, shorter-duration type, 22.23%); death from flowering and before seed maturity (D type, medium- duration type, 3.35%); and survival to seed maturity (E type, perpetual-duration type, 9.83%) (Table 2).

The results showed that differences in plant height appeared between three duration types (C, D and E) (Table 2). At the seedling stage, C, D and E types averaged 11.8, 16.6 and 15.8 cm in plant height (at 20 days from seedling emergence) under this salinity stress. The plant growth in C type was more significantly inhibited than the D and E types that could survive longer ($P<0.05$), and there were no significant differences in rate of plant growth between D and E types. The mean growth reduction (control value minus stress value for a line) was in the order in intensity: C (70.57%), D (61.75%) and E (61.43%).

When entering into the medium-term stage of vegetative growth, the three duration types C, D and E had mean plant heights of 91.0, 109.3 and 130.0 cm (at 51 days from seedling emergence). There were significant differences between these types ($P<0.05$), and the degree of growth reduction was also in the order: C (61.41%), D (55.62%) and E (44.66%), where only the E type that survived upto seed maturity was more mitigated for growth inhibition. However, C, D and E types in non-saline control soil showed no difference in plant height at any growth stage (Table 2).

The experimental data in Table 2 also showed that D and E types could tolerate this salinity stress longer and had higher growth rates, as shown in Table 3. At the seedling stage, the growth rates of D and E types (0.79 and 0.75 cm/day) were significantly higher than that of C type (0.56 cm/day) ($P<0.01$). At the medium-term stage of vegetative growth, the growth rate was 3.57 cm/day for E type, >3.09 cm/day for D type, and >2.61 cm/day for C type, with significant differences between them ($p<0.05$) (Table 3).

These results confirmed that longer-duration salinity-tolerant types also possessed more rapid growth. At the

seedling stage, growth rate reduced by 70.68% in the C type and by 61.84–61.93% in the D and E types. During growth, at the medium-term vegetative stage, growth rate inhibition abated to 60.27% in C type, 54.29 % in D type, and 45.83% in E type ($p<0.05$), which showed that the E type was obviously less suppressed in growth rate (Table 3).

Variation of main phenotypic characters in high-salinity stress:

Under high-salinity stress, most of the lines died at the two growth stages before flowering and maturity, and only E-type lines (88 lines, 9.8% of the total samples) completed their life cycles (of which, three lines bore sterile seeds). The surviving 88 lines suffered major damage, and their loss of characters relative to the controls are listed in Table 5. The most severe loss was above-ground dry weight and yield per plant, which reduced on average by 87.25% and 82.58%, respectively, followed by the number of seeds per plant (73.71%), but 100-seed weight had a relatively low reduction (40%). However, the minimal and maximal losses had rather larger gaps (Table 4), which showed great variation and heterogeneity in the adaptive response of these salinity-tolerant lines to high-salinity stress in this coastal population. Such great heterogeneity among the tolerant lines was also reflected from the coefficients of variation in the main characters (Table 5); plant height and seed weight were two relatively stable characters at the maturity stage.

All the character parameter values under high-salinity stress had higher coefficients of variation than those in the non-saline soil control (Table 5). The yield per plant had the highest coefficient of variation (105.38%) at maturation, and the other coefficients of variation were: number of seeds (93.09%); number of pods (89.97%); harvesting index (73.91%); above-ground dry weight (51.58%); 100-seed weight (39.42%); and plant height (19.00%) (Table 5). However, plant height of all types had a higher coefficients of variation at the early vegetative growth stage (seedling stage, 20-day old) than at the medium-term vegetative growth stage (51 day-old) (Tables 2 and 5).

Table 1. Developing dynamics, death, survival, and maturity for the middle Bohai Bay coastal wild soybean population at different development times after emergence under high-salinity-soil stress.

Days from emergence	No. of deaths (%)	No. surviving			Description of death	
		Total (%)	Flowering			Maturity (%)
			Yes	No		
0 (20 May)	270(30.17) ^a	625(69.83) ^b				
1–14	201(32.16)	424(67.84)				
15–30	134(21.44)	290(46.40)			335 lines (53.60%) died within 30 d	
31–60*	89(30.69)	201(32.16)			Died before flowering	
61–90	69(34.33)	132(21.12)	70	62	Died before flowering	
91–120	36(27.27)	96(15.36)	87		9 (1.44) 14 lines died before and 22 during flowering	
121–150	8(9.20)	79(12.64)	3		76(12.16) 8 lines died between flowering and maturity	
≥151		3(0.48)	3		3(0.48) Normally matured	

^aUngerminated lines; ^bgerminated lines; numbers in parentheses are the percentage of the total 895 lines; ^cmaturity; percentage relative to the 625 germinated lines. Other percentages were all relative to the total number of surviving lines at the anterior stage 118 lines (18.88% of the 625 germinated lines) survived to flowering, 88 lines (14.08% of the 625 lines) survived to maturity, out of the 118 flowering lines

Table 2. Duration of salinity tolerance and comparison of plant height at three developing stages of the middle Bohai Bay coastal wild soybean population.

Type (duration of salinity tolerance)	Test time of plant height	Variable	Plant height (cm)		Growth reduction in saline soil (%)
			Saline soil	Control	
A (non-germinable)	No	Number of lines	270 (died)		
B (died within 20 d after seedling emergence)	No	Number of lines	335 (died)		
C (died before flowering)	20 d after seedling emergence (Seedling stage of vegetative growth)	Number of plants	199	199	
		Maximum	44.0	71.0	
		Minimum	1.5	7.0	
		Mean	11.8 ± 10.0	40.1 ± 14.1	70.57
		CV (%)	84.81	35.06	
D (died before maturity)		No. of plants	30	30	
		Maximum	43.3	69.0	
		Minimum	3.0	19.0	
		Mean	16.6 ± 12.0	43.4 ± 12.0	61.75
		CV (%)	72.48	27.68	
E (survived to maturity)		No. of plants	88	88	
		Maximum	46.0	63.0	
		Minimum	2.0	12.0	
		Mean	15.8 ± 11.7	41.3 ± 12.0	61.43
		CV (%)	74.05	29.06	
Total		No. of plants	317	317	
		Maximum	46.0	71.0	
		Minimum	1.5	7.0	
		Mean	13.4 ± 10.9	40.7 ± 13.3	67.08
		CV (%)	81.34	32.68	
C	51 d after seedling emergence (Medium-term stage of vegetative growth)	No. of plants	108	108	
		Maximum	173.0	276.0	
		Minimum	10.0	130.0	
		Mean	91.0 ± 30.9	235.8 ± 21.5	61.41
		CV (%)	34.01	9.14	
D		No. of plants	30	30	
		Maximum	186.0	275.0	
		Minimum	48.0	216.0	
		Mean	109.3 ± 32.8	246.3 ± 13.1	55.62
		CV (%)	30.04	5.31	
E		No. of plants	88	88	
		Maximum	253.0	268.0	
		Minimum	48.0	153.0	
		Mean	130.0 ± 32.7	234.9 ± 13.31	44.66
		CV (%)	25.15	7.92	
Total		No. of plants	226	226	
		Maximum	253.0	275.0	
		Minimum	10	130.0	
		Mean	107.1 ± 35.8	236.8 ± 19.8	54.77
		CV (%)	33.43	8.36	

Correlations between phenotypes in high-salinity stress environments: The correlations among nine investigated characters were estimated in the 88 high-salinity-tolerant E-type lines. Correlations between the characters in the controls and under high-salinity-stress conditions were not always consistent (Table 6), and exhibited differences in the response of characters to both soil environments. The correlation between characters had three tendencies between the salinity stress and controls: both positive correlations; one positive and one negative correlation; or almost no correlation in both soils.

Five groups of character correlations under both salinity stress and control soil showed largely different correlation between X_1 and X_2 , X_1 and X_6 , X_2 and X_4 , X_3 and X_4 , X_4 and X_5 , X_4 and X_6 (Table 6, indicated by

boxes). There was a marked positive correlation ($r=0.625^*$) between X_1 and X_2 under high-salinity stress and a markedly negative correlation ($r=-0.263^*$) under control soil conditions (Table 6). The negative correlation between the number and weight of seeds is most common in normal environments in the subgenus *Soja* (Xu and Wang 2009; Liu and Zhou 1995). Nevertheless, under high-salinity conditions, the high-salinity-tolerant plants bore relatively more and enlarging seeds (Table 6). Baker (1972) has found that plant in California had a tendency of an increase in seed weight, with an increased likelihood of the seedlings being exposed to drought after germination. Our data also showed that injured leaves (defoliation rate) (X_8), although not significantly, influenced the above-ground dry mass weight (X_3) ($r=-0.204$) (Table 6).

Table 3. Differences in growth rate for the different duration-types of salinity tolerance in the middle Bohai Bay coastal wild soybean population in high-salinity and control soil.

Type (duration of salinity tolerance)	No. of surviving plants	Growth rate							
		20 d after emergence				51 d after emergence			
		Sample	Max.	Min.	Mean (%)*	Sample	Max.	Min.	Mean (%)*
C (salinity stress)	199	199	2.10	0.01	0.56 (70.68)	108**	5.27	0.18	2.61 (60.27)
C (control)	199	199	3.38	0.33	1.91	108	8.23	2.37	6.57
D (salinity stress)	30	30	2.05	0.14	0.79 (61.84)	30	5.83	1.07	3.09 (54.29)
D (control)	30	30	3.29	0.90	2.07	30	8.37	5.38	6.76
E (salinity stress)	88	88	2.19	0.10	0.75 (61.93)	88	7.60	1.50	3.57 (45.83)
E (control)	88	88	3.00	0.57	1.97	88	6.45	3.13	6.59
Total salinity stress	317	317	2.19	0.07	0.64 (67.01)	226	7.60	0.18	3.16 (51.68)
Total control	317	317	3.38	0.33	1.94	226	8.37	2.37	6.54

*Number in parentheses is the rate of growth inhibition. **number of survival lines on 51 d after seedling emergence, out of the 199 lines at 20 d after seedling emergence. Other types (D and E) each had the same number of survival lines at the two stages

Table 4. Percentage loss of character values (to control) for high salinity-tolerant E-type lines harvested at maturity in the middle Bohai Bay coastal wild soybean population under high-salinity-soil stress.

Character	Loss amount (%)			
	Sample size	Line with minimal loss	Line with maximal loss	Mean
Above-ground dry weight per plant (g)	88	83.42	91.68	87.25 ± 9.29
Yield per plant (g)	85	51.57	99.75	82.58 ± 21.42
Number of seeds per plant	85	30.82	99.41	73.71 ± 27.24
100-seed weight (g)	85	19.93	85.71	40.00 ± 24.89

Table 5. Overall single plant agronomic characters for 88 high salinity-tolerant E-type lines in the middle Bohai Bay coastal wild soybean population under high-salinity-soil stress.

Characters	Sample size	Saline soil				Nonsaline soil control			
		Max.	Min.	Mean	CV %	Max.	Min.	Mean	CV %
Plant yield (g)	85	7.85	0.01	1.47 ± 1.57	105.38	16.21	4.01	8.44 ± 2.58	30.56
No. of seeds	85	541	1	112.29 ± 104.53	93.09	782	168	427.16 ± 127.43	29.83
No. of pods	85	245	1	49.00 ± 44.22	89.97	—	—	—	—
Harvesting index	85	0.55	0.003	0.22 ± 1.16	73.91	0.32	0.08	0.22 ± 0.04	19.74
Dry wt. (g) *	88	10.22	1.29	3.90 ± 2.01	51.58	61.66	15.50	30.59 ± 9.18	30.00
Seed wt. (g)**	85	2.33	0.20	1.20 ± 0.47	39.42	2.91	1.40	2.00 ± 0.28	14.09
Plant ht. (cm)	88	361.30	130.90	260.70 ± 49.60	19.04	391.70	270.00	321.60 ± 21.20	6.58

*Above-ground dry weight; number of measured samples was 88 lines. The other yield character data were calculated based on 85 lines because three mature lines did not bear any seeds and three did not bear effective pods. **100-seed weight: some values were estimated because some plants produced fewer than 100 seeds

Path analysis showed that number of seeds per plant, 100-seed weight, above-ground dry weight, harvesting index, growth period, and plant height were significantly positively correlated with yield by simple correlation, however, they were related with the yield mainly indirectly via the number of seeds, with higher indirect path coefficients (Table 7).

Comprehensive valuation for salinity tolerance: Through PCA analysis, six principal eigen vectors were validated to contribute to the first principal component composed of seed number per plant (X₁), 100-seed weight (X₂), above-ground dry weight per plant (X₃), harvesting index (X₄), plant height (X₇), and single-plant yield (X₉). These eigen vector factors gave an 87.61% accumulative contribution rate of variation to the first principal component, and these factors were used to estimate the comprehensive D values for salinity-tolerant lines (Table 8).

According to the D values, the 10 most tolerant lines (T754, T51, H384, T49, H859, T28, H812, T56 and T36) were identified, as shown in Fig. 3. On average, these highly tolerant lines had relatively more seeds, larger seed weights, higher yields, higher harvesting indexes, higher plant heights and heavier above-ground dry weights. Higher plant height could increase seed numbers. However, the most tolerant group with the highest D values did not have always the most outstanding single traits. If salt tolerance was evaluated on the basis of one single prominent character, the hazard of mistaken identification increased for a line. In two cases, the eighth high D value line (H812) had a lower above-ground dry weight (2.79 g) but it had a higher harvesting index (54.7%), more seeds (260 seeds) and a higher yield (3.37g); whereas the 51st D value line (T688) had a relatively higher above-ground dry weight (7.11 g) but its yield and yield components were not the best among all the samples (Table 7).

Table 6. Coefficients of correlation between traits in 88 high salinity-tolerant E-type lines from the middle Bohai Bay coastal wild soybean population under salinity stress and control conditions.

	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈
X ₂ salinity	0.625**							
X ₂ control	-0.263*							
X ₃ salinity	0.563**	0.334**						
X ₃ control	0.393**	0.284**						
X ₄ salinity	0.769**	0.800**	0.091					
X ₄ control	0.490**	-0.149	-0.490**					
X ₅ salinity	0.068	0.146	0.130	0.063				
X ₅ control	-0.129	0.191	0.346**	-0.413**				
X ₆ salinity	0.508**	0.640**	0.285**	0.576**	0.340**			
X ₆ control	-0.097	0.471**	0.243*	-0.169	0.757**			
X ₇ salinity	0.495**	0.277**	0.291**	0.368**	0.313**	0.275*		
X ₇ control	-	-	-	-	-	-		
X ₈ salinity	0.018	-0.080	-0.204	0.039	0.069	-0.048	0.165	
X ₈ control	-	-	-	-	-	-	-	
X ₉ salinity	0.982**	0.687**	0.584**	0.771**	0.050	0.524**	0.468**	-0.006
X ₉ control	0.894**	0.175	0.526**	0.441**	-0.016	0.142	-	-

X₁, number of seeds per plant; X₂, 100-seed weight (g); X₃, above-ground dry weight per plant (g); X₄, harvesting index per plant; X₅, No. of days from sowing to first flowering; X₆, growth period; X₇, plant height (cm); X₈, defoliation rate per plant (%); X₉, yield per plant (g)

*Significant at 1 % level; **significant at 5 % level. X_{7 control}: not measured for plant height at maturity period. X₈ was investigated at 51 d after seeding emergence (medium-term stage of vegetative growth). X_{8 control}: no leaf injury

Discussion

High levels of heterogeneity in salinity tolerance for the coastal wild soybeans: This study clearly showed high levels of heterogeneity in the coastal wild soybean samples for salinity adaptation when exposed to high-salinity stress over the entire growth period. About 70% of the samples had germination ability and 30% did not, and death occurred at any developmental stage (Table 1). The first 30 days after seedling emergence was the most sensitive stage with a higher death rate (53.60%), followed by 31–60 days (30.69%) and 61–90 days (34.23%) (Table 1). The germinated lines could be divided into three duration types of salinity tolerance according to survival time: C type (died before flowering), D type (died before maturity), and E type (lived to maturity) (Table 2). Death lines at different stages implied differences among individual lines for degree of salinity tolerance; 88 lines (~10%) could tolerate this high-salinity stress to complete the life cycle (Table 4, Fig. 2).

Types C, D, and E also showed differences in growth rate, with the more tolerant types (such as E) showing more rapid growth (Table 3). Usually, tolerant species or individual genotypes are related with low-degree damages in early plant growth and vegetative organs (Ahmad & Wainwright, 1976; Humphreys, 1982; Shannon, 1984; Ab-Shukor *et al.*, 1988; Wang *et al.*, 1997; Kik, 1989; Bayuelo-Jiménez *et al.*, 2002; Mguis *et al.*, 2008; Lee *et al.*, 2009). In this study, all 88 E-type lines that survived to maturity could be regarded as having high salinity tolerance. However, they had great variation in each single character and comprehensive evaluation *D* values (Tables 5 and 8). Coefficients of variation for the measured characters varied greatly among the 88

individual lines; the plant height was the most stable character at maturation (Table 5) but the growth of E-type lines was rather heterogeneous (Table 3). The seed weight was the second trait with smaller variation.

Ab-Shukor *et al.*, (1988) reported that populations of *Trifolium repens* L. growing in salt-marsh sites showed highly salt-tolerant root growth, and the non-saline inland population had no such tolerance. Kik (1989) observed that genotypes that do not have maximal salt resistance remained within a salt marsh population of *Agrostis stolonifera* L. The coastal population of wild soybean in the present study clearly exhibited high levels of heterogeneity in salinity tolerance, which was reflected by different survival times of individual lines (Tables 1 and 2), and greater variation in characters and different *D* values among these E-type lines (Table 8).

The heterogeneity of salinity tolerance may be explained to have been caused by the long-term natural selection on heterogeneity of saline soil. This region was originally salt marsh lands, but latter-day social development and human agricultural exploitation and crop cultivation have made this region fragmented in terms of land and salinity concentration. It could be hypothesized that this regional population of wild soybean originally had high genetic variability in salinity tolerance, including a series of genotypes that did not have maximal salt resistance and possess salinity tolerance to some extent, such that the population could grow normally in this coastal region and colonize wide ecological habitats because of genetic variability (Ashraf *et al.*, 1986). When we subjected these coastal wild soybean samples to high-salinity-stress conditions, the highly tolerant genotypes were identified, and it seemed that they were not evolved by rapid evolution, as reported for copper tolerance in *A. stolonifera* L. by Wu *et al.*, (1975).

Table 7. Correlations of agronomic characters (X₁₋₈) to single-plant yield (X₉) in 85 high salinity-tolerant E-type lines that bore seeds within the middle Bohai Bay coastal wild soybean population under salinity stress and control conditions.

Character	Coefficients of correlations					
	Type of correlation	Salinity stress			Non-salinity control	
			X ₁	X ₂		X ₁
No. of seeds per plant (X ₁)	Simple correlation	0.982**			0.894**	
	Partial correlation	0.989**			0.890**	
	Direct path	0.945**			0.846**	
	Indirect path			0.088		-0.102
100-seed weight (g) (X ₂)	Simple correlation	0.687**			0.175	
	Partial correlation	0.455**			0.825**	
	Direct path	0.176**			0.357**	
	Indirect path		0.589			-0.246
Above-ground dry weight (g) (X ₃)	Simple correlation	0.584**			0.526**	
	Partial correlation	0.024			0.345**	
	Direct path	0.008			0.162*	
	Indirect path		0.543	0.047		0.342
Harvesting index (X ₄)	Simple correlation	0.771**			0.441**	
	Partial correlation	-0.162			0.382**	
	Direct path	-0.089			0.172**	
	Indirect path		0.702	0.111		0.448
Vegetative growth period (days from sowing to flowering) (X ₅)	Simple correlation	0.050			-0.016	
	Partial correlation	-0.179			0.049	
	Direct path	-0.032			0.012	
	Indirect path		0.079	0.014		-0.154
Growth period (X ₆)	Simple correlation	0.524**			0.142	
	Partial correlation	-0.037			0.160	
	Direct path	0.008			0.041	
	Indirect path		0.436	0.081		-0.134
Plant height (cm) (X ₇)	Simple correlation	0.468**			-	
	Partial correlation	-0.029			-	
	Direct path	-0.005			-	
	Indirect path		0.447	0.044		-
Defoliation rate (%) (X ₈)	Simple correlation	-0.006			-	
	Partial correlation	-0.037			-	
	Direct path	-0.006			-	
	Indirect path		-0.044	-0.001		-

*Significant at 1 % level; **Significant at 5 % level

Shorter-term or staged-identification or early identification would increase the hazard of misjudgement of salinity tolerance: Salinity tolerance identification is generally involved in growth phases, growth parameters and identification criteria. Many researchers have evaluated salt tolerance at germination or the vegetative seedling stage by measuring the morphological performances (Humphreys, 1982; Ahmad & Wainwright, 1976; Bayuelo-Jiménez *et al.*, 2002; Mguis *et al.*, 2008; Lee *et al.*, 2009) and root growth parameters (Shannon, 1984; Wang *et al.*, 1997; Ab-Shukor *et al.*, 1988; Kik, 1989). Jones & Qualset (1984) have asserted that plant growth attributes must be measured throughout the growth period in order to identify particularly salt-sensitive growth stages, because tolerance at one growth stage usually is not related to another. Our present identification study was a whole-growth-period identification with high salinity pressure, which made the tolerant lines adequately expressive.

Usually, the various growth trait parameters that are used for salt-tolerance evaluation are based upon close correlation between the parameters and salinity concentration. Yield components and growth trait parameters always show differential response to salinity stress. (Tables 2–5). Our results demonstrated that number of seeds was most directly close to the goal character—yield because all coefficients of three correlations (bivariate simple, partial and direct pass) between the number of seeds and the yield were very high, followed by seed size (100-seed weight) (Table 7). The vegetative growth stage (days from sowing to flowering) and defoliation rate (leaf injury) among the growth trait parameters did not show any correlation with yield under high-salinity stress (Table 7). This suggests that, for wild soybean, defoliation rate might be a poor indicator of salinity tolerance among all the characters in early-stage identification of salt tolerance. However, leaf

injury index at seedling stage has been used for analysing the inheritance of salt tolerance for a wild soybean germplasm (Lee *et al.*, 2009) and for soybeans (Shao *et al.*, 1994). Other traits (above-ground dry weight, harvesting index, and plant height) were related indirectly to yield through the factor of number of seeds (Table 7). This suggests that, for salinity tolerance evaluated throughout the growth cycle, number of seeds and seed weight are superior to other single traits, or that salinity tolerance is better evaluated using a comprehensive evaluation method that includes yield components (Table 8, Fig. 3). Single traits with good tolerance performance do not always relate to other growth trait parameters, particularly yield.

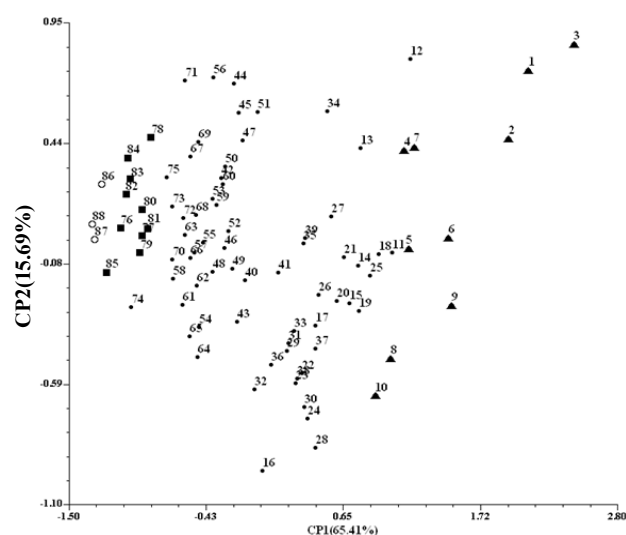


Fig. 3. Evaluation of salinity-tolerant wild soybean germplasm using PCA based on six characters (above-ground dry weight, plant height, number of seeds, yield, single-seed weight, and harvesting index) for 88 salinity-tolerant lines (E-type). Strongly and poorly tolerant lines were distinctly separated. The first two axes explained 81.10% of tolerance. ▲: 10 lines with highest D values; ■: 10 lines with lowest D values; ●: lines with D values between the highest and lowest groups; ○: three lines (nos. 86, 87 and 88) only bore a few sterile seeds that lacked tolerant genes supporting the seed-reproductive system.

High-salinity stress intensity is necessary for salinity tolerance identification in crops. The death time is also one of the evaluation criteria in salinity tolerance. The present identification throughout the growth period revealed a series of different salinity-tolerant types and various phenomena and responses that could not be obtained in short-term identification at germination or seedling stages. Our high salinity tolerance criterion was survival until seed maturation, because the lines that were not viable until maturation under high-salinity stress and had no practical value in breeding application. Regardless of how well some lines performed in terms of salinity tolerance at different days (Table 1) or stages (Table 2) of development, even if they lived to 60–90 days and more (Table 1) or to the later phases before maturity, but they did not belong to salinity-tolerant germplasm. Indeed, many lines that died at near the flowering time or later stages showed exceptional salt tolerance with less damaged or fewer damaged leaves. Our results revealed

that a early or shorter-term or staged-identification would have the hazard of misjudgement of salinity tolerance. Our data showed a high risk at any developing stage: 30.69% (89 lines) died at 31–60 days among the 290 living lines at 15–30 days; 34% died at 61–90 days among the 210 survival lines at 31–60 days. Even at the flowering stage at 91–120 days, 27.27% lines died, of which 22 lines (16.7%) died during flowering (Table 1).

Life-maintaining and reproductive system genes in high salinity tolerance: Salinity tolerance is a complex, quantitative, genetic character that is controlled by many genes. Perhaps the single character of leaf injury at seedling stage could be controlled by a pair of single alleles in salt tolerance of soybean (Shao *et al.*, 1994) and wild soybean (Lee *et al.*, 2009). Seawater irrigation treatment at the seedling stage reduces yield components in soybean to allow salinity identification (Chang *et al.*, 1994). In the present evaluation, 88 lines survived the high-salinity stress and showed large differences in number of seeds per plant (Table 8); the highest D value line, T51, bore 541 seeds per plant, whereas nine lines with low D values produced <10 seeds. Four of these lines (H470, T763, T22 and H494) had abortive seeds and three (T586, T598 and T694) did not bear seeds, notwithstanding all 88 lines being highly salinity-tolerant through their life cycles.

Flower shedding was common for wild soybean under salt stress, nevertheless, some individual lines only flowered but large number of flowers were shed off, which resulted in only a small number of pods and seeds. A reasonable explanation for the genetic differences between the seed-prolific and seed-unproductive lines among the 88 salinity-tolerant lines could be that the high and low seed-bearing ability can be attributed to two salinity-tolerant gene systems, i.e., life-maintaining and seed-reproductive system genes. The highly salinity-tolerant lines possessed both systems. Early-stage death lines or salinity-sensitive lines lacked strongly salinity-tolerant life-maintaining genes. However, these wild soybean lines were evolved into different levels of adaptability to salinity, as shown by the lines with the lowest D values ($D < 0.1$, Table 8). Most lines with higher D values bore more seeds and lines with lower D values produced, a small amount of seeds. These lines with high D values (> 0.5) and prolificness identified here might possess both highly salinity-tolerant life-maintaining and seed-reproductive system genes, and they could be utilized in breeding programs.

Our results revealed that if a salinity-tolerant germplasm that only had the life-maintaining system genes but lacked highly salinity-tolerant seed-reproductive system genes, the breeding work would become difficult to achieve a desired threshold yield. A highly salt-tolerant germplasm should be provided with strongly tolerant genes supporting life-maintaining and seed-reproductive systems. Therefore, seed-reproductive system genes deserve our full attention at the present time when there are few studies on the salinity tolerance of seed-reproductive system in soybean. Salt tolerance identification can not merely focus on vegetative growth stage.

Table 8. Agronomic characters in 88 high salinity-tolerant E-type wild soybean lines of the middle Bohai coastal population under high-salinity-soil stress.

Line no.	Line name	D value	Precedence of tolerance	Agronomic characters					
				No. of seeds	Single-seed weight (mg)	Yield (g)	Harvesting index (%)	Plant height (cm)	Above-ground dry weight (g)
1	T754	0.80	1	313	23.29	5.78	36.64	361.3	9.99
2	T37	0.76	2	438	17.28	6.13	43.95	287.2	8.14
3	T51	0.69	3	541	13.39	7.85	45.31	332.8	9.48
4	H384	0.63	4	243	16.94	3.67	32.88	291.9	7.49
5	T49	0.60	5	234	17.21	3.63	40.86	320.6	5.26
6	H859	0.59	6	341	15.43	4.66	46.27	321.1	5.41
7	T28	0.56	7	308	14.87	3.62	33.77	304.7	7.11
8	H812	0.55	8	260	13.34	3.37	54.74	319.8	2.79
9	T56	0.52	9	335	15.19	4.92	54.16	321.7	4.17
10	T36	0.50	10	167	19.75	3.09	50.74	282.5	3.00
11	H464	0.46	11	295	12.92	3.70	43.19	286.3	4.87
12	T742	0.45	12	227	19.74	3.58	25.93	257.5	10.22
13	T739	0.43	13	159	20.53	2.62	24.40	247.1	8.12
13	T109	0.43	13	187	16.50	2.70	36.45	298.1	4.70
15	H887	0.40	14	212	13.25	2.58	42.15	305.2	3.54
16	T766	0.40	14	65	18.87	1.02	42.77	199.7	1.36
17	H360	0.40	14	161	14.62	1.98	37.92	274.3	3.24
18	T750	0.39	15	224	15.62	3.03	38.79	317.8	4.79
19	T263	0.38	16	194	15.32	2.66	42.40	313.5	3.61
20	T152	0.38	16	176	16.48	2.68	37.80	241.7	4.41
21	H867	0.38	16	191	15.59	2.19	32.95	307.3	4.46
22	T18	0.38	16	138	17.59	1.99	38.73	210.1	3.15
23	T124	0.38	16	112	14.36	1.59	42.74	277.4	2.13
24	H816	0.37	17	107	15.96	1.43	47.20	300.0	1.60
25	H487	0.37	17	228	15.20	2.65	39.16	325.7	4.11
26	H869	0.36	18	151	17.15	2.24	33.30	234.7	4.48
27	T719	0.36	18	136	17.20	2.12	27.26	286.9	5.65
28	T557	0.35	19	96	21.93	1.80	44.87	231.0	2.21
29	T106	0.33	20	121	14.96	1.60	36.15	248.5	2.82
30	H870	0.33	20	123	14.45	1.64	47.49	282.2	1.81
31	T26	0.33	20	106	13.22	1.24	36.44	319.0	2.16
32	T179	0.33	20	99	13.78	1.36	37.69	198.6	2.26
33	H882	0.32	21	134	14.61	1.59	34.70	266.6	3.00
34	T729	0.32	21	130	16.19	1.82	19.14	293.7	7.69
35	H363	0.31	22	129	13.53	1.59	27.63	315.1	4.16
36	H857	0.31	22	101	16.30	1.15	32.05	244.0	2.45
37	T605	0.31	22	134	17.01	2.16	38.99	247.3	3.38
38	T19	0.31	22	138	15.05	1.96	41.98	226.5	2.71
39	H878	0.30	23	153	11.75	1.58	28.54	326.7	3.96
40	T138	0.27	24	99	08.38	0.83	25.96	311.3	2.37
41	T95	0.27	24	132	13.99	1.60	27.35	227.8	4.26
42	H397	0.26	25	61	7.30	0.45	10.89	332.4	3.64
43	H809	0.26	25	81	12.23	0.99	26.76	231.4	2.71
44	T505	0.26	25	47	9.49	0.45	6.41	303.1	6.51

Table 8. (Cont'd.).

Line no.	Line name	<i>D</i> value	Precedence of tolerance	Agronomic characters					
				No. of seeds	Single-seed weight (mg)	Yield (g)	Harvesting index (%)	Plant height (cm)	Above-ground dry weight (g)
45	H387	0.25	26	98	8.70	0.85	11.75	249.7	6.41
46	T508	0.24	27	42	11.21	0.47	14.08	309.4	2.87
47	T660	0.23	28	91	9.92	0.90	13.18	253.5	5.95
48	T21	0.23	28	63	12.24	0.69	16.47	208.2	3.52
49	H874	0.22	29	74	12.03	0.76	18.88	256.3	3.26
50	T280	0.22	29	83	7.48	0.62	12.31	292.4	4.43
51	T688	0.22	29	65	13.43	0.87	10.93	256.7	7.11
52	T147	0.21	30	94	9.04	0.85	18.41	251.6	3.77
53	T258	0.21	30	76	7.71	0.59	13.02	265.3	3.91
54	H808	0.21	30	41	10.34	0.42	20.07	246.8	1.69
55	T604	0.20	31	20	13.45	0.27	7.36	256.7	3.39
56	T517	0.20	31	30	9.23	0.28	3.89	267.6	6.83
57	H371	0.19	32	8	11.50	0.09	4.34	316.3	2.03
58	T256	0.18	33	29	6.48	0.19	11.61	278.9	1.43
59	T590	0.18	33	70	9.00	0.63	13.37	257.3	4.08
60	T722	0.18	33	67	11.67	0.78	12.51	208.4	5.47
61	T232	0.18	33	34	10.24	0.35	14.39	221.7	2.07
62	H883	0.17	34	60	8.23	0.49	17.89	245.8	2.27
63	T764	0.17	34	45	8.04	0.36	10.46	239.9	3.10
64	T169	0.17	34	25	16.32	0.41	16.07	184.3	2.13
65	T225	0.17	34	45	10.98	0.49	19.94	197.8	1.98
66	T720	0.17	34	25	9.36	0.23	9.55	285.4	2.22
67	H298	0.17	34	16	7.44	0.12	2.95	311.6	3.91
68	H802	0.16	35	61	8.26	0.50	11.64	231.1	3.83
69	T620	0.16	35	15	9.60	0.14	2.84	282.7	4.93
70	T108	0.16	35	17	6.59	0.11	7.48	306.8	1.39
71	H380	0.16	35	28	5.02	0.14	2.27	269.4	5.93
72	H886	0.15	36	28	7.11	0.20	6.90	294.0	2.64
73	T253	0.13	37	14	6.07	0.09	3.45	311.1	2.38
74	T41	0.11	38	40	6.28	0.25	11.24	130.9	1.98
75	H470	0.11	38	14	4.64*	0.07	2.20	305.8	2.89
76	T6	0.10	39	3	5.67	0.02	0.58	164.7	2.92
77	T763	0.10	39	27	3.96*	0.11	4.88	233.4	2.08
78	T717	0.10	39	19	5.58	0.11	1.96	182.8	5.30
79	T515	0.09	40	17	7.65	0.13	4.47	172.5	2.78
80	H799	0.09	40	3	6.00	0.02	0.64	236.1	2.81
81	T690	0.09	40	12	7.08	0.09	2.85	213.8	2.90
82	T22	0.09	40	7	2.71*	0.02	0.65	216.7	2.89
83	T726	0.06	41	9	5.78	0.05	1.17	154.1	4.39
84	H494	0.06	41	1	2.00*	0.001	0.27	219.9	3.72
85	T241	0.05	42	5	5.00	0.03	1.35	149.6	1.82
86	T586**							184.6	2.99
87	T598**							203.2	1.29
88	T694**							181.7	1.93

*Lines that did not bear germinative seeds; **lines that did not bear seeds and had no *D* value

Conclusion

The identification of 895 wild soybean samples under a high-salinity soil during the whole growth period revealed five salinity response types (A, non-germinable; B, seedling death; C, before-flowering death; D, before-maturity death and E, maturity). The stronger salinity-tolerant E type possessed more rapid growth and more mitigated growth inhibition. Under high-salinity conditions, the most severe inhibition by high saline stress was above-ground dry weight and yield per plant, followed by the number of seeds per plant, and 100-seed weight was relatively lowly reduced. The number of seeds per plant, 100-seed weight, above-ground dry weight, harvesting index, growth period, and plant height were significantly positively correlated with yield mainly indirectly via the number of seeds. Our results revealed important implications for soybean salt-tolerance breeding: high salinity-tolerant wild soybean germplasm should hold two salinity-tolerant system genes, i.e. life-maintaining and seed-reproductive system genes.

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