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Identification, inheritance and QTL mapping of root traits related to tolerance to rhizo-spheric stresses in soybean (*G. max* (L.) Merr.)

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Abstract A sample of soybean accessions (*Glycine max* (L.) Merr.) from Huanghe–Huaihe–Haihe and Middle–Lower Changjiang Valleys in China was used to identify their tolerance to rhizo-spheric stresses, including drought, aluminum toxin and low phosphorus. A total of 15 accessions highly tolerant to at least one of the abiotic stresses were screened out. The correlation between drought tolerance and the relative values of total root length, root volume and dry root weight (relative to dry plant weight) were all significant at 0.01 level, respectively. So did for the correlation between aluminum toxin tolerance and the stress to non-stress ratios of the number of lateral roots, tap root length, total root length, root volume and dry root weight. The inheritance study on the above three root traits related to drought tolerance under segregation analysis indicated that between the two parents of the recombinant inbred line (RIL) population of (Kefeng 1 × Nannong 1138-2), the relative values of dry root weight, total root length and root volume were controlled by two major genes plus polygenes with their major gene heritability values 62.26%–91.81% and polygene heritability values 2.99%–24.75%, respectively, and for the latter two traits, the two major genes linked together with recombination value 4.30% and 1.93%, respectively. The inheritance study on the five root traits related to aluminum toxin tolerance revealed that between the two parents of the recombinant inbred line

(RIL) population of (Bogao × NG94-156), the stress to non-stress ratios of lateral root number, tap root length, total root length and dry root weight were controlled by three major genes plus polygenes with their major gene heritability values 80.22%–91.81% and polygene heritability values 3.52%–11.39%, while the stress to non-stress ratio of root volume was controlled by three major genes with their major gene heritability value 93.44%. The (Kefeng 1 × Nannong 1138-2) RIL population was also used for mapping QTLs of relative dry root weight, total root length and root volume related to drought tolerance. Five, three and five QTLs located on Linkage group N6-C2, N8-D1b+W, N11-E and N18-K for each of the three traits, respectively, were identified. Each of the traits appeared to have one locus (*Dw1*, *R11*, *Rv1*) with relatively large effect in comparison with their other loci, and the three loci in above parentheses were located in the same region STAS8_3T-STAS8_6T on N6-C2 with a same distance to the flanking markers. Thus, *Dw1*, *R11*, and *Rv1* even might be a same locus and performed as pleiotropic of a same gene. The results between segregation analysis and QTL mapping appeared relatively consistent, therefore could be used for verification each other.

Keywords drought tolerance, aluminum toxin tolerance, low phosphorus tolerance, abiotic stress tolerance related root trait, genetic resource, segregation analysis, quantitative trait locus (QTL)

Received March 8, 2007; accepted March 20, 2007

Part of the results was published in Chinese in *Acta Genetica Sinica*, 2005, 32(8): 855–863 [部分译自: 遗传学报]

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1 Introduction

Abiotic stresses are a group of limiting factors for the implement of the yield potential of a soybean cultivar in addition to biotic stresses (Sloane et al., 1990; Von Uexkull, 1995; Carter et al., 1992; Hudak and Patterson, 1995). For soybeans, there exists the full seasonal drought stress problem in arid and semi-arid areas (northern part of China) and the seasonal drought stress problem also occurs in non-arid areas (southern part of China) where during the hot summer, temporary

drought also damages pod-setting and seed-filling and causes yield losses. There also exists the aluminum toxin problem and the related low phosphorus problem in acid red soil areas in southern China. In addition, salinity in coastal areas is also a serious problem for soybean production.

The firstly stressed organ of a plant under rhizo-spheric stresses is the underground part or root, and it conveys the stresses to the above ground parts (Hoogenboom et al., 1987; Garay and Welhelm, 1983; Liu et al., 2004; Hudak and Patterson, 1996). So root traits should be studied with priority in addition to the above ground parts as far as tolerance to stresses in rhizo-sphere is concerned. Unfortunately, very few studies on soybean root traits, especially related with rhizo-spheric stresses were reported.

A great number of genetic studies of quantitative traits, especially the studies of quantitative trait locus (QTL) analysis, indicated that there existed both major genes and minor genes controlling a quantitative trait, not necessary all being minor genes even with equal effects. Gai et al. (2003) indicated that for a quantitative trait, the major gene plus minor gene model was the general model, while the pure major gene or pure minor gene model was only the specific cases of the general model. Accordingly, Gai and Wang (1998), Wang and Gai (1998), Gai et al. (2000), Zhang and Gai (2000a, b), Zhang et al. (2003) and Gai (2006) established the procedures of segregation analysis to detect the genetic structure for a quantitative trait.

Quantitative trait locus mapping is a procedure utilizing segregation data in linkageship analysis to locate QTLs to the nearest markers on a molecular genetic map. In comparing the segregation analysis with QTL mapping, both procedures are based on a similar set of genetic assumptions and use the same set of segregation data. But the former can detect both major gene and polygene effects, and it does not need a genetic linkage map and therefore does not provide the information about the location of QTLs; while the latter can detect and locate the possible QTLs if a linkage map is available. It is obvious that the precision of the results from the latter procedure depends on the precision of the linkage map in addition to that of the phenotypic data which are of basic importance to the precision of both the procedures. Gai et al. (2007) compared the results in a study on segregation analysis and QTL mapping of some agronomic traits and indicated both the procedures could detect the main major genes or QTLs, and therefore, could be used as a mutual check and supplement.

From a plant breeder's point of view, to reveal the inheritance of a quantitative trait is for recognizing some major genes or major QTLs with large genetic effects at first so that the plant breeder can use simple cross or backcross procedures to transfer the major gene(s) into a material to be improved; otherwise, an intermating procedure followed with recurrent selection may be used if there are only polygenes involved. The objective of the present paper is to identify elite germplasm accessions highly tolerant to rhizo-spheric stresses, including drought, aluminum toxin and low phosphorus, to explore the inheritance and to map the QTLs of

root traits related to tolerance to the rhizo-spheric stresses in soybean, and at the same time, to make a further comparative study on segregation analysis and QTL mapping to demonstrate if both of them can provide meaningful results in recognizing major QTLs with the same set of data.

2 Materials and methods

A total of 301 accessions were sampled from various eco-regions in Huanghe-Huaihe-Haihe and Middle-Lower Changjiang Valleys in China and surveyed for their root morphological performances around the full podding stage. Among them, 62 entries with different root types were selected from ten provinces for further stress tolerance study (Table 1).

2.1 Experiment 1: Identification and evaluation of drought tolerance

Evaluation of drought tolerance at the seedling stage of 59 accessions (Table 1) was carried out in the pot experiment in the greenhouse in 2001 and 2002, with pots in size of $\phi 25$ cm \times h28 cm, filled with soil medium (sand : clay = 85 : 15), two plants per pot, three replications, split plot design with major-plot treatment watering (optimum watering versus non-watering) and sub-plot treatment accession (59 varieties). Watering treatment started at the first pair of leaves unrolled.

The plant height, number of leaves, dry weight above ground (above cotyledon node) and dry weight under ground (cotyledon node) were measured and evaluated on the 18th day after water treatment.

For each trait of each accession, the membership index F_{ij} value was calculated from the ratio of drought stress value to non-stress value (Liu et al., 1989), and then the mean membership index averaged over the four traits F_i was used to indicate drought tolerance of an accession

$$F_{ij} = \frac{X_{ij} - X_{\min}}{X_{\max} - X_{\min}}, \quad F_i = \Sigma F_{ij} / n.$$

In the above equations, X_{ij} is the ratio of the i th accession, j th trait, X_{\max} and X_{\min} are the maximum and minimum ratios of the trait, F_{ij} is the membership index value of the i th accession, j th trait, and F_i is the mean membership index averaged over n traits of the i th accession. The rank of an accession's drought tolerance is classified according to the following criteria: Rank 1: $F_i > 0.8$, highly tolerant; Rank 2: $0.6 \leq F_i < 0.8$; tolerant; Rank 3: $0.4 \leq F_i < 0.6$, medium; Rank 4: $0.2 \leq F_i < 0.4$, sensitive; Rank 5: $F_i < 0.2$, highly sensitive.

2.2 Experiment 2: Identification and evaluation of aluminum toxin tolerance

The experiment design and evaluation procedure were similar to those of drought tolerance at the seedling stage except that

Table 1 Materials used in tests for stress tolerances

Code	Name	Source	D	A	P	Code	Name	Source	D	A	P
1	HZ Bayuehuang	Shaanxi	D	A		32	GC Yishuhou	Hubei	D	A	P
2	LY Yaoheidou	Shaanxi	D	A		33	You 91-11	Hubei	D	A	P
3	NQ Laoshupi	Shaanxi	D	A		34	Zhongdou 19	Hubei	D		
4	ZB Xiaobaihuangdou	Shaanxi	D			35	Zhongdou 8	Hubei	D		
5	Bianheidou	Shanxi	D	A		36	1138-2×86-53	Jiangsu	D	A	
6	Jinda 53	Shanxi	D	A		37	NG94-156	Jiangsu		A	
7	Jindou 14	Shanxi	D	A		38	Bogao	Jiangsu		A	
8	Jindou 16	Shanxi	D			39	FX Sunlouzihuangdou	Jiangsu	D	A	P
9	Jindou 19	Shanxi	D			40	JR Dabianqingdou	Jiangsu	D	A	P
10	Yuanheidou	Shanxi	D			41	LH Dafengqing	Jiangsu	D	A	
11	Kefeng 1	Beijing	D	A	P	42	Lvbaozhu	Jiangsu	D	A	
12	3-29	Hebei	D	A		43	Nannong 1138-2	Jiangsu	D	A	P
13	5-5	Hebei	D	A	P	44	Nannong 18-6	Jiangsu	D	A	
14	6-13	Hebei	D			45	Nannong 86-4	Jiangsu	D	A	P
15	Dawudou	Hebei	D	A	P	46	Nannong 88-29	Jiangsu	D	A	
16	Naiyinheidou	Hebei	D	A		47	Nannong 88-31	Jiangsu	D	A	P
17	Yixianheidou	Hebei	D	A	P	48	Nannong 96B-2	Jiangsu	D	A	P
18	CS Xianheidou	Shandong	D	A	P	49	QD Heidou	Jiangsu	D	A	
19	WM Tiezhugan	Shandong	D	A	P	50	SH Dalihuangdou	Jiangsu	D	A	
20	Qihuang 10	Shandong	D	A	P	51	Suxie 1	Jiangsu	D	A	
21	Qihuang 1	Shandong	D	A	P	52	HF Guizilan	Jiangxi	D	A	
22	WS Gunlongzhu	Shandong	D	A	P	53	NC Zaibuyao	Jiangxi	D	A	P
23	CF Xiaotianedan	Henan	D	A	P	54	TG Xiazhidou	Jiangxi	D		
24	RN Pingdingdou	Henan	D	A	P	55	YG Zaowudou	Jiangxi	D	A	
25	XX Xiaoli Huang	Henan	D	A	P	56	CM Tiegendou	Shanghai	D	A	
26	XX Taipingzihuadou	Henan	D	A	P	57	SH Daqingdou (xuan)	Shanghai	D	A	P
27	XS Dalihuang	Henan	D	A		58	DQ Xianzhudou	Zhejiang	D	A	
28	CX Nongjiawuming	Anhui	D	A		59	NH Wanhuangdou	Zhejiang	D	A	
29	liufeng	Anhui	D	A	P	60	XJ Xiaomaodou	Zhejiang	D	A	P
30	SC Qujiahuangdou	Anhui	D			61	XC Liuyuedou	Zhejiang	D	A	P
31	GY Heidou	Anhui	D	A	P	62	PI416937	USA		A	

Note: D, A and P represent the materials used for identification of tolerance to drought, aluminum toxin and low phosphorus, respectively.

only one year's (2002) test with major plot treatment is 28 ppm aluminum concentration versus 0 ppm aluminum (CK) and subplot treatment is 51 accessions (Table 1). The plant height, number of leaves, dry weight above ground (above cotyledon node) and dry weight under ground (cotyledon node) were measured and evaluated after two weeks. The calculation of the mean membership index and the classification of tolerance rank were similar to those of drought tolerance.

2.3 Experiment 3: Identification and evaluation of low phosphorus tolerance

The experiment was similar to the above two except that major plot treatment is high phosphorus (1 000 mmol/L) versus low phosphorus (0.2 $\mu\text{mol/L}$) and subplot treatment is 30 accessions (Table 1). The prescription was K_2SO_4 0.75 mmol/L, KCl 0.1 mmol/L, $\text{Ca}(\text{NO}_3)_2$ 2 mmol/L, H_3BO_3 1 $\mu\text{mol/L}$, MnSO_4 1 $\mu\text{mol/L}$, CuSO_4 0.1 $\mu\text{mol/L}$, $(\text{NH}_4)_5\text{Mo}_7\text{O}_{24}$ 0.005 $\mu\text{mol/L}$, ZnSO_4 10 $\mu\text{mol/L}$, and FeEDTA 10 mmol/L (Yan and Zhang, 1997). After eight days of treatment, the plants were analyzed for their phosphorus content by using the method of H_2SO_4 - H_2O_2 Digestion—Colorimetric Vanadium-Molybdate. Five ranks of tolerance to low

phosphorus were classified based on the ratio of P content of low phosphorus treatment to that of high phosphorus treatment according the following criteria: Rank 1: ratio value >0.85 ; Rank 2: $0.70 \leq$ ratio value <0.85 ; Rank 3: $0.55 \leq$ ratio value <0.70 ; Rank 4: $0.40 \leq$ ratio value <0.55 ; Rank 5: ratio value <0.40 .

2.4 Correlation analysis between stress tolerance and root traits

In Experiments 1 and 2 on tolerance to drought and tolerance to aluminum toxin, the following root traits were measured and evaluated on per plant base: number of lateral roots (>1 cm), tap root length, dry root weight, total root length and root volume. The latter two were obtained from scanning data and processed with WinRhizo software. Based on that, relative dry root weight, relative root volume and relative total root length were calculated from the counterparts under drought treatment divided by the dry weight of the whole plant; and root weight ratio, root volume ratio, total root length ratio, tap root length ratio and lateral root number ratio were calculated from the related measurements under aluminum toxin stress to their respective counterparts under non-stress condition.

2.5 Experiment 4: Inheritance of root traits related to drought tolerance

The 184 RILs derived from Kefeng 1 × Nannong 1138-2 along with the two parents were tested in 2002 in the same way as in Experiment 1 except that only drought treatment was used in a randomized block design for analyzing the inheritance of the relative values (relative to dry plant weight) of the total root length, root volume and dry root weight per plant related to drought tolerance. The segregation analysis under the major gene plus polygene model mixed with the inheritance model was used (Zhang and Gai, 2000a; Gai et al., 2003) to reveal the inheritance of the traits.

2.6 Experiment 5: Inheritance of root traits related to tolerance to aluminum toxin

The 164 RILs derived from Bogao × NG94-156 (Rank 4 × Rank 2) along with the two parents were tested in 2002 in the same way as in Experiment 3 for analyzing the inheritance of the stress to non-stress ratio of root weight, root volume, total root length, tap root length and lateral root number related to aluminum toxin tolerance by using segregation analysis.

2.7 QTL mapping of root traits related to drought tolerance

The data of total root length, root volume and dry root weight relative to whole plant dry weight from the 184 RILs derived from Kefeng 1 × Nannong 1138-2 in Experiment 5 were analyzed for mapping QTLs by using the QTL Cartographer V2.0 software (Wang et al., 2001–2004) based on the genetic linkage map constructed by Wang et al. (2004). LOD value 3.0 was used as the threshold value for the existence of a QTL.

3 Results

3.1 Identification and evaluation of tolerance to rhizo-spheric stresses

3.1.1 Drought tolerance

The membership index values for each trait (data omitted) were pretty close to each other in both years, which indicated that the mean membership index value averaged over the four traits (plant height, number of leaves, above ground dry weight and under ground dry weight) could be used as an appropriate indicator for all the traits in tolerance to drought. Among the tested accessions, the genetic coefficient of variation (*GCV*) of drought tolerance was about 42.6%–43.3%, indicating that a great genetic variation existed and the tested population was potential for drought tolerance breeding. Five accessions, i.e. Code 1, 7, 10, 11, and 17 in Table 1, were ranked 1 (highly tolerant) in drought tolerance.

3.1.2 Aluminum toxin tolerance

The membership index values for each trait (data omitted) were pretty close to each other, which indicated that the mean membership index value averaged over the four traits (plant height, number of leaves, above ground dry weight and under ground dry weight) could be used as an appropriate indicator for all the traits in tolerance to aluminum toxin. Among the tested accessions, the genetic coefficient of variation (*GCV*) of aluminum toxin tolerance at the seedling stage was about 41.1%, indicating a great genetic variation existed and the tested population was potential for aluminum toxin tolerance breeding. Seven accessions, i.e. Code 19, 21, 23, 24, 33, 57 and 61 in Table 1, were ranked 1 (highly tolerant) in aluminum toxin tolerance.

3.1.3 Low phosphorus stress tolerance

Three accessions, i.e. Code 19, 21 and 57 in Table 1, were ranked 1 (highly tolerant) in low phosphorus stress tolerance at seedling stage. It was very interesting that all the three accessions were also tolerant to aluminum toxin. The materials are relevant to breeding for cultivars in the southern acid red soil area.

3.1.4 Correlation among rhizo-spheric stress tolerances

The correlation results showed that drought tolerance was significantly correlated with aluminum toxin tolerance ($r = 0.23^*$) and with low phosphorus tolerance ($r = 0.29^*$), while much higher and very significant correlation existed between tolerance to aluminum toxin and that to low phosphorus stress ($r = 0.74^{**}$).

Table 2 showed the selected accessions highly tolerant to at least one of the abiotic stresses (Rank 1), from which it was impressed that the accessions with high aluminum tolerance more likely had higher tolerance to low phosphorus stress, while the accessions with high drought tolerance more likely had medium rank of tolerance to aluminum toxin and low phosphorus stress and the accessions with high tolerance to aluminum toxin and low phosphorus stress more likely had sensitivity to drought stress. The situation is coincided with the above correlation results and might be due to that both aluminum toxin and low phosphorus stresses are caused by acidic soil while drought stress is sometimes related with clay soil, but not necessary related to acidic soil.

3.2 Root traits related to tolerance to rhizo-spheric stresses

3.2.1 Correlation between drought tolerance and root traits

Table 3 showed that tolerance to drought (mean membership index) was not correlated with the three first order root traits, i.e. dry root weight, total root length, and root volume, but very significantly correlated with the three second order root traits or the relative root values, i.e. dry root weight relative

Table 2 The selected accessions with high tolerance to stress(es)

Name	Code	Maturity group	Source	Stress tolerance (Rank)		
				D	A	P
Yuanheidou	10	III	Shanxi	1		
Jindou 14	7	III	Shanxi	1	2	2
Jindou 16	8	III	Shanxi	3		
HZ Bayuehuang	1	VI	Shaanxi	1	2	3
Kefeng 1	11	III	Beijing	1	3	2
NQ Laoshupi	3	IV	Shaanxi	3	3	
6-13	14	III	Hebei	2		
GY Heidou	31	V	Anhui	2	2	2
XC Liuyuedou	61	IV	Jiangxi	4	1	2
You 91-11	33	V	Hubei	4	1	3
CF Xiaotianedan	23	V	Anhui	2	1	2
RN Pingdingdou	24	IV	Henan	4	1	3
WM Tiezhugan	19	V	Shandong	3	1	1
Qihuang 1	21	IV	Shandong	2	1	1
SH Daqingdou (xuan)	57	VII	Shanghai	4	1	1

Note: D, A, and P represent drought tolerant, aluminum toxin tolerant, and low phosphorus tolerant, respectively. Rank 1 means highly tolerant and Rank 5 means highly sensitive.

to dry plant weight, total root length relative to dry plant weight and root volume relative to dry plant weight. It might be due to the difference of the development of the first order root traits among eco-types or groups. Therefore, the relative values were better than the first order root traits, and could be used as under ground indicators of drought tolerance.

Table 3 Correlation between drought tolerance and root traits under drought stress in Experiment 1

Year	Dry root weight	Total root length	Root volume	Dry root weight /plant weight	Total root length /plant weight	Root volume /plant weight
2001	-0.21	0.10	-0.05	0.63**	0.81**	0.77**
2002	-0.23	0.17	0.07	0.66**	0.79**	0.81**

Note: ** represents significant at 0.01 level.

Table 4 Correlation between aluminum toxin tolerance and root traits in Experiment 2

Number of lateral roots	Tap root length	Total root length	Root volume	Dry root weight	Ratio of number of lateral roots	Ratio of tap root length	Ratio of total root length	Ratio of root volume	Ratio of dry root weight
-0.26*	0.55**	0.34**	0.20	-0.01	0.67**	0.74**	0.81**	0.81**	0.93**

Note: ** represents significant at 0.01 level.

Table 5 Estimates of genetic parameters of the RIL population and results of segregation analysis under drought stress in Experiment 4

Root trait	Kefeng 1	1138-2	RIL				Segregation analysis				
			Mean	Range	<i>GV</i>	<i>GCV</i> /%	<i>h</i> ² /%	Optimum model	Major gene	Additive polygene	<i>Re</i> /%
Dry root weight/plant weight	0.30	0.24	0.28	0.18–0.39	0.0015	13.64	90.60	E-1-0	2	+	
Total root length/plant weight	875.32	586.26	756.10	407.75 1220.12	15 098.75	16.25	79.11	E-2-5	2 linked	+	4.30
Root volume/plant weight	0.70	0.55	0.67	0.37–0.93	0.008 7	13.71	77.00	E-2-4	2 linked	+	1.93

Note: All the root traits were significantly different between parents and among RILs at 0.01 level. *GV* = genetic variance; *GCV* = genetic coefficient of variation; + = yes; *Re* = recombination value.

3.2.2 Correlation between aluminum toxin tolerance and root traits

Table 4 showed the very significant and close correlations between aluminum toxin tolerance (mean membership index) and the five second order root traits, i.e. the stress to non-stress ratios of number of lateral roots, tap root length, total root length, root volume, and dry root weight. There were some correlations between aluminum toxin tolerance and some of the first order root traits, but not close enough except that with tap root length. Therefore, the ratio values were better than the first order root traits, used as under ground indicators of aluminum toxin tolerance.

3.3 Inheritance of root traits related to tolerance to rhizo-spheric stresses

3.3.1 Inheritance of the three root traits related to drought tolerance

Table 5 showed that there existed very significant differences between the two parents, Kefeng 1 and 1138-2, and among the RILs in dry root weight relative to dry plant weight, total root length relative to dry plant weight and root volume relative to dry plant weight. Therefore, the segregation data fitted the requirement for further inheritance analysis of the involved traits.

By using the segregation analysis of quantitative traits under the mixed major gene plus polygene genetic model, the optimum genetic models for the three relative root traits were detected through the comparison of *AIC* values (Akaike's Information Criterion) and a set of tests for goodness of fit (data omitted, refer to Gai and Wang, 1998 and Gai et al., 2003 for the analytical procedures). The results were indicated at the right side in Table 5. The models showed that between the two parents, all the three relative root traits, dry root weight relative to dry plant weight, total root length relative to dry plant weight and root volume relative to dry plant weight, were controlled by two major genes plus polygenes, with the latter two traits having the two major genes linked each other at a recombination value 4.30% and 1.93%, respectively. The sample distribution, fitted population distribution and component distributions of the three root traits, indicating a good fit, were shown in Figs. 1, 2 and 3, respectively.

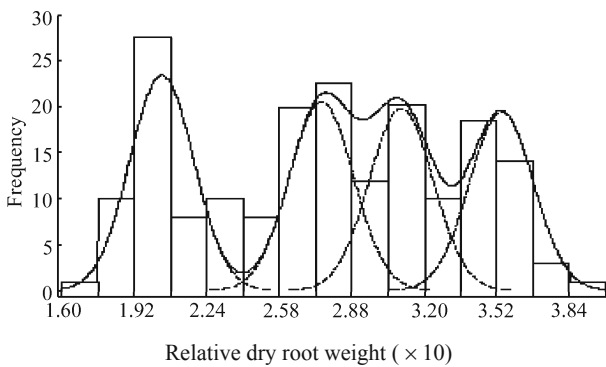


Fig. 1 Sample distribution and fitted population and component distributions of relative dry root weight (Model E-1-0) in Experiment 4

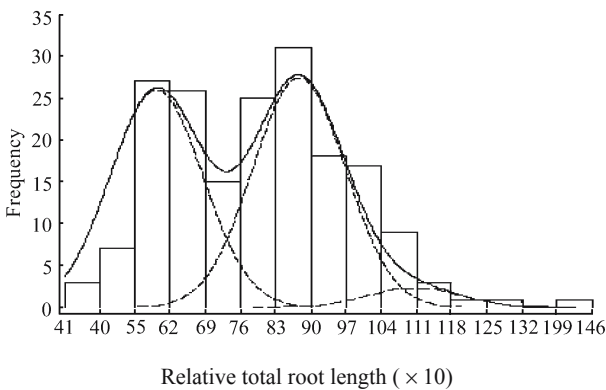


Fig. 2 Sample distribution and fitted population and component distributions of relative total root length (Model E-2-5) in Experiment 4

The estimates of genetic effects of major genes and heritability values of both major genes and polygenes were listed in Table 6. For all the three relative root traits, between their two

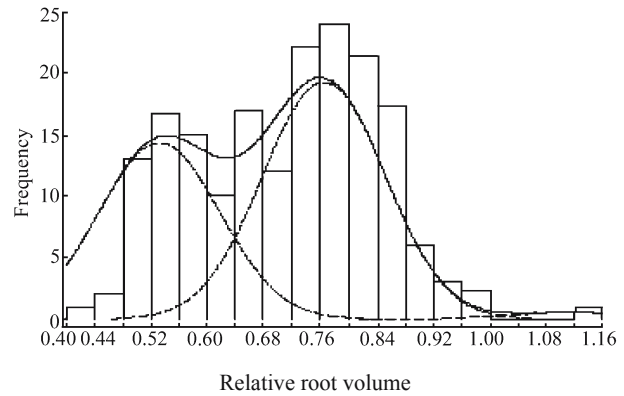


Fig. 3 Sample distribution and fitted population and component distributions of relative root volume (Model E-2-4) in Experiment 4

major genes, the additive effects of one were much larger than the other one. There existed pretty small additive \times additive interaction effect between the two major genes for relative dry root weight, but not for the other two root traits. The major gene heritability value of relative dry root weight was 91.81%, much larger than that of any other two traits (75.35% and 62.26%), but the polygene heritability value of relative dry root weight was only 2.99%, much smaller than that of any other two traits (11.15% and 24.75%), indicating the relative importance of major genes for the former trait and the relative importance of minor genes for the latter two traits.

Table 6 Estimates of genetic parameters of root traits related to drought tolerance under major gene plus polygene model in Experiment 4

Genetic parameter		Dry root weight /plant weight	Total root length /plant weight	Root volume /plant weight
Additive effect	d_a	0.023	199.47	0.029
	d_b	0.014	114.95	0.148
Epistasis effect	i	-0.007	-	-
Genetic variance of major gene	σ_{mg}^2	0.001	25944.71	0.012
Heritability of major gene	$h_{mg}^2/\%$	91.81	75.35	62.26
Genetic variance of polygene	σ_{pg}^2	0.000	3840.16	0.005
Heritability of polygene	$h_{pg}^2/\%$	2.99	11.15	24.75

3.3.2 Inheritance of the five ratios of root traits related to aluminum toxin tolerance

Table 7 showed that there existed very significant differences between the two parents, Bogao (Rank 4) and NG94-156 (Rank 2), and among the RILs in lateral root number ratio, tap root length ratio, total root length ratio, root volume ratio, and dry root weight ratio. Therefore, the segregation data fitted the requirement for further inheritance analysis of the involved traits.

By using the segregation analysis, the optimum genetic models for the five ratios of root traits were detected through

Table 7 Estimates of genetic parameters of the RIL population and results of segregation analysis under aluminum toxin stress in Experiment 5

Root trait	Parent		RIL				Segregation analysis			
	Bogao	NG94-156	Mean	Range	<i>GV</i>	<i>GCV</i> /%	<i>h</i> ² /%	Optimum model	Major gene	Additive polygene
Lateral root number ratio	0.53	0.77	0.66	0.36–0.96	0.010	15.43	77.72	G-2	3	+
Tap root length ratio	0.52	0.80	0.66	0.35–0.99	0.011	15.94	83.94	G-2	3	+
Total root length ratio	0.54	0.79	0.66	0.36–0.91	0.010	15.31	89.65	G-1	3	+
Root volume ratio	0.55	0.77	0.65	0.38–0.91	0.011	16.26	88.52	F-1	3	–
Dry root weight ratio	0.56	0.74	0.66	0.36–0.96	0.015	18.59	90.32	G-2	3	+

Note: All the root traits were significantly different between parents and among RILs at 0.01 level, respectively. *GV* = genetic variance; *GCV* = genetic coefficient of variation; + = yes; – = no.

the comparison of *AIC* values and a set of tests for goodness of fit (data omitted). The results were indicated at the right side in Table 7. The models showed that between the two parents, four root traits, lateral root number ratio, tap root length ratio, total root length ratio, and dry root weight ratio were controlled by three major genes plus polygenes, while root volume ratio was controlled by three major genes without any polygene detected.

The estimates of genetic effects of major genes and heritability values of both major genes and polygenes were listed in Table 8. For all the five ratios of root traits, among their three major genes, the additive effects of one were much larger than the other two. There existed additive \times additive first order and second order interaction effects among major genes for total root length ratio and root volume ratio, but not for the other three root traits. The major gene heritability values of the five ratios of root traits were 80.22%–93.44%, much larger than their polygene heritability values, 3.52%–11.39%, indicating the relative importance of major genes in comparison with the minor genes.

3.4 QTL mapping of root traits related to drought tolerance

Table 9 showed the results of QTL mapping of the three root traits related to drought tolerance, i.e. dry root weight relative to dry plant weight, total root length relative to dry plant weight and root volume relative to dry plant weight from the

data of the (Kefeng No.1 \times 1138-2) RIL population by using composite interval mapping procedure with QTL Cartographer V 2.0. The QTLs of the three traits were mapped on four linkage groups, i.e. N6-C2, N8-D1b+W, N11E and N18-K (Table 9, Fig. 4).

For relative dry root weight, five loci were located on Wang et al.'s linkage group N6-C2, N8-D1b+W, N11E. Among them, *Dw1* located on N6-C2 was of the largest genetic contribution (24.7%), while the other four loci were located on N8-D1b+W and N11E with much less genetic contribution.

For relative total root length, three loci were located on linkage group N6-C2 and N8-D1b+W. Among them, *R11* located on N6-C2 was of the largest genetic contribution (22.9%), while the other two loci were located on N6-C2 and N8-D1b+W with much less genetic contribution.

For relative root volume, five loci were located on linkage group N6-C2, N11E, and N18-K. Among them, *Rv1* located on N6-C2 was of the largest genetic contribution (22.0%), while the other four were located on N6-C2, N11E6 and N18-K. with much less genetic contribution.

From the above results, there existed one major locus for each of the three root traits, i.e. *Dw1*, *R11* and *Rv1* all located on N6-C2, while the others scattered on the four linkage groups. Furthermore, in Table 9, *Dw1*, *R11*, and *Rv1* were even located in the same region STAS8_3T-STAS8_6T with the same distance to the flanking markers on N6-C2. Therefore, *Dw1*, *R11*, and *Rv1* even might be on the same locus and performed as pleiotropism of the same gene.

Table 8 Estimates of genetic effects and heritability values of major gene and polygene of aluminum tolerance related root traits in Experiment 5

Genetic parameter		Number of lateral root ratio	Tap root length ratio	Total root length ratio	Root volume ratio	Dry root weight ratio
Major gene additive effect	d_a	–0.095	–0.030	–0.074	–0.067	–0.100
	d_b	–0.026	–0.043	0.002	–0.028	–0.019
	d_c	–0.028	–0.097	0.056	–0.066	–0.067
Major gene epistasis effect	i_{ab}	–	–	0.008	0.024	–
	i_{ac}	–	–	0.013	–0.010	–
	i_{bc}	–	–	0.003	0.025	–
	i_{abc}	–	–	0.048	0.020	–
Major gene genetic variance	σ_{mg}^2	0.014	0.013	0.013	0.012	0.014
Major gene heritability	h_{mg}^2	81.02	87.13	91.81	93.44	80.22
Polygene genetic variance	σ_{pg}^2	0.001	0.001	0.001	–	0.002
Polygene heritability	h_{pg}^2	7.79	6.18	3.52	–	11.39

Table 9 QTL mapping of root traits related to drought tolerance of the (Kefeng No.1 × 1138-2) RIL population in Experiment 4

Root trait	Linkage group	Locus	Marker	Distance /cM	LOD	Variance explained/%	Additive effect
Dry root weight/plant dry weight	N6-C2	<i>Dw1</i>	STAS8_3T-STAS8_6T	1.7–0.7	18.7	24.7	0.028
		N8-D1b+w	<i>Dw2</i>	LC5T-Rn1	10.2–5.6	3.1	4.0
	<i>Dw3</i>		Rn3-Rsa	16.1–5.4	3.0	3.9	-0.011
	N11E		<i>Dw4</i>	LC20B-Sat_112	0.7–12.9	4.6	5.9
		<i>Dw5</i>	A86H	0.0	4.2	5.2	0.014
Total root length/plant dry weight	N6-C2	<i>R11</i>	STAS8_3T-STAS8_6T	1.7–0.7	13.6	22.9	85.98
		<i>R12</i>	A676I-Satt316	3.8–15.9	3.0	6.8	53.07
	N8-D1b+w	<i>R13</i>	LC5T-Rn1	12.2–3.6	3.5	4.1	-45.04
Root volume/plant dry weight	N6-C2	<i>Rv1</i>	STAS8_3T-STAS8_6T	1.7–0.7	14.7	22.0	0.164
		<i>Rv2</i>	A131I-K455D	17.9–0.4	3.1	3.6	0.031
		<i>Rv3</i>	A676I	0.0	5.4	6.5	0.046
	N11E	<i>Rv4</i>	LC20B-Sat_112	0.7–12.9	4.5	6.2	0.038
		N18-K	<i>Rv5</i>	K401H	0.0	3.2	4.2

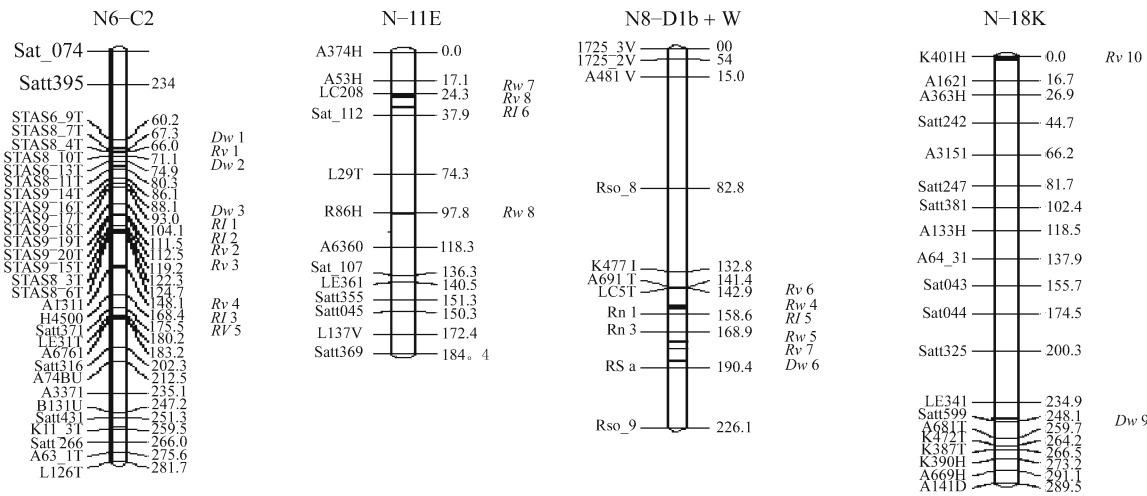


Fig. 4 Linkage groups with QTLs of three root traits related to drought tolerance in Experiment 4

4 Discussion

4.1 Further consideration on evaluation and identification for tolerance to rhizo-spheric stresses

The present study indicated that there existed potential genetic variation in tolerance to rhizo-spheric stresses among the landraces of soybeans and that there showed some tendency in the geographical source of the tolerant materials, i.e. the materials tolerant to drought being mainly from northern arid and semi-arid areas, while those tolerant to aluminum toxin and low phosphorus stress mainly from the southern acid red soil area. It implied that the stress factor(s) of the geographical environment caused historical adaptive variation, which provided some hint of where to find the respective tolerance materials.

Fifteen accessions highly tolerant to one or two of the three kinds of rhizo-spheric stresses were screened out, which should be elite parental materials for the respective breeding purposes. However, they are basically landraces and might be in lack of elite yield potential. Accordingly, it is especially

important to choose the other parent to be complementary to the stress tolerant parent.

In fact, the present study was our first step to start a research program on tolerance to rhizo-spheric stresses. After the study, it has been realized that the work on evaluation and identification of the tolerances needs to be further improved, including the following three aspects.

The membership index as the indicator of tolerance is a measure, relative to the tested population of material and will vary from population to population and from time to time. For comparisons among accessions from different populations, the most tolerant and most sensitive standard checks should be determined and be tested as checks in each population, each time.

The testing stage for tolerances in the present study was at the seedling stage only, from which the tolerance might not last as long as the whole growth period. For example, in another experiment on drought tolerance study, the correlation between drought tolerance at the seedling stage and that at reproductive stage was only $r = 0.56^{**}$, and the accessions with high tolerance at the seedling stage are not necessary with high tolerance at the reproductive stage. Therefore, the

consistency versus differential response of the tolerances at different developmental stages should be further studied.

Furthermore, the materials used in the present study were only a small sample in comparison to the whole germplasm collection with more than 20 thousand accessions in China. Therefore, for a thorough and effective evaluation and identification of resources with tolerance to rhizo-spheric stresses, some further work plan including sampling strategy should be designed based on the improvement of evaluation technology.

4.2 Further exploration of root traits related to stress tolerances

It is interesting that the first order measurement of root traits, dry root weight, total root length, and root volume, was not correlated with drought tolerance, but the three second order root traits or the relative root values were very significantly correlated with drought tolerance. The same situation was for the correlation between ratio values of root traits and aluminum toxin tolerance. Unfortunately, the two sets of relative root traits were obtained in different ways; the set for drought tolerance was the values relative to the whole plant dry weight under stress condition, while the set for aluminum toxin tolerance was the stressed values relative to their corresponding unstressed values. However, both kinds of the relative root traits were correlated with stress tolerances, which might be due to the relative values well reflecting the whole plant performance and the first order values were not comparable among accessions, especially among the different eco-types of accessions involved.

Scientists have characterized a great number of above ground traits, but very few underground traits have been explored. However, the underground part is really difficult to be measured due to not only being hidden in the soil and difficult to be dug out without damage but also having less morphological variation. Even people realize already the importance of the root system. In the present study, the scanning technique combined with utilization of the software WinRhizo was used so that the total root length, root volume, number of lateral roots, etc. could be measured. But the measurable traits are still limited. Therefore, further exploration of measurable root traits, as well as the corresponding measurement technology, especially from two-dimensional extended to three-dimensional technique is needed. Based on it, more second order or relative root traits are also to be explored for stress tolerance study.

4.3 Implication of the genetic study and QTL mapping of drought tolerance and aluminum toxin tolerance

In the present study, it was revealed that the genetic bases of the three relative root traits related to drought tolerance between the two parents of Kefeng 1 \times Nannong 1138-2 were respectively composed of two major genes (linked or not linked) and a number of polygenes, with the major gene

heritability values 62.26%–91.98% and the polygene heritability values 2.99%–24.75%, and that those of the five ratio root traits related to aluminum toxin tolerance between the two parents of Bogao \times NG94-156 were respectively composed of three major genes (all five traits) and a number of polygenes (four traits), with the major gene heritability values 82.22%–91.98% and the polygene heritability values 0%–11.39%.

The results of inheritance of drought tolerance were relatively consistent with those from QTL mapping for the three root traits related to drought tolerance. Both procedures detected a major gene with obviously larger effect than any one of the others for each of the traits. In segregation analysis, the two major genes for relative dry root weight as well as relative root volume were tightly linked with each other, respectively, while in QTL mapping, *R11* and *R12* as well as *Rv1* and *Rv3* were located on a same linkage group N6-C2. Therefore, in this case, the results from both procedures were comparable at certain degree. Since the segregation analysis procedure of quantitative trait costs much less, it can be used broadly, especially when the genetic map is not saturated enough and could not offer accurate mapping information.

Based on the above, the existence of two or three major genes for the two traits implied that transferring or pyramiding a few major genes should be the main breeding strategy in the improvement of the two traits. It must be rather easier to be done than those traits with larger polygene heritability values (more polygenes involved for the trait).

In addition, the results obtained above can only explain the genetic difference or genetic structure between the two parents, here between Kefeng 1 and 1138-2 for drought tolerance and between Bogao and NG94-156 for aluminum toxin tolerance, since only a single cross for each trait was studied. For a breeding plan, more parental materials are involved and elite genes may be separated in different materials, therefore, in the further study, multiple parents and multiple genetic or mapping populations should be used to explore all the possible loci or genes or the whole genetic system of the traits.

Acknowledgements The project was supported by the IAEA Coordinated Research Project No. 302-D2-CPR-10815, the Natural Science Foundation of China (No. 30490250), the National Key Basic Research Program (No. 2002CB111304, 2004CB7206, 2006CB101708), the National “863” Program (No. 2002AA211052, 2006AA100104), and the Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT).

References

- Carter T E Jr, Ruffy T W (1993). Soybean plant introductions exhibiting drought and aluminum tolerance. In: Kuo C G, ed. *Adaptation of Food Crops to Temperature and Water Stress*. Shanhua: Asian Vegetable Research and Development Center, 335–346
- Gai J (2006). Segregation analysis on genetic system of quantitative traits in plants. *Front Biol China*, 1: 85–92
- Gai J, Wang J (1998). Identification and estimation of a QTL model and its effects. *Theoretical and Applied Genetics*, 97(7): 1162–1168

- Gai J Y, Wang Y J, Wu X L, Chen S Y (2007). A comparative study on segregation analysis and QTL mapping of quantitative traits in plants —with a case in soybean. *Front Agric China*, 1(1): 1–7
- Gai J Y, Zhang Y M, Wang J K (2000). A joint analysis of multiple generations for QTL models extended to mixed two major genes plus polygene. *Acta Agronomica Sinica*, 26(4): 385–391 (in Chinese)
- Gai J Y, Zhang Y M, Wang J K (2003). *Genetic System of Quantitative Traits in Plants*. Beijing: Science Press
- Garay A F, Wilhelm W W (1983). Root system characteristics of two soybean isolines undergoing water stress conditions. *Agron J*, 75(6): 973–977
- Hoogenboom G M, Huck C M, Peterson R P (1987). Root growth rate of soybean as affected by drought stress. *Agron J*, 79: 607–614
- Hudak C M, Patterson R P (1995). Vegetative growth analysis of a drought-resistant soybean plant introduction. *Crop Sci*, 35: 464–471
- Hudak C M, Patterson R P (1996). Root distribution and soil moisture depletion pattern of a drought-resistant soybean plant introduction. *Agronomy J*, 88: 478–486
- Liu F L, Andersen M N, Jensen C R (2004). Root signal controls pod growth in drought-stressed soybean during the critical, abortion-sensitive phase of pod development. *Field Crops Research*, 85: 159–166
- Liu G Z, Gai J Y, Ma Y H (1989). Evaluation of drought tolerance of soybean germplasm from lower Yangtze and Huai Valleys. *J Nanjing Agric Univ*, 12(1): 15–21 (in Chinese)
- Sartain J B, Kamprath E J (1998). Aluminum tolerance of soybean cultivars based on root elongation in solution culture compared with growth in acid soil. *Agron J*, 70: 17–23
- Sloane R J, Patterson R P, Carter T E (1990). Field drought tolerance of soybean plant introduction. *Crop Science*, 30: 118–123
- Spehar C R (1994). Aluminum tolerance of soybean genotypes in short term experiments. *Euphytica*, 76: 73–80
- Von Uexkull H R (1995). Global extent, development and economic impact of acid soil. *Plant and soil*, 171(1): 1–15
- Wang J K, Gai J Y (1998). Identification of major gene and polygene mixed inheritance model of quantitative traits by using joint analysis of P1, F1, P2, F2 and F2:3. *Acta Agronomica Sinica*, 24(6): 651–659 (in Chinese)
- Wang S, Basten C J, Zeng Z B (2001–2004). *Windows QTL Cartographer 2.0*. Department of Statistics, North Carolina State University, Raleigh, NC
- Wang Y J, Dongfang Y, Wang X Q, Yang Y L, Yu D Y, Gai J Y, Wu X L, He C Y, Zhang J S, Chen S Y (2004). Mapping of five genes resistant to SMV strains in soybean. *Acta Genetica Sinica*, 31(1): 87–90 (in Chinese)
- Wang Y J, Wu X L, Yu D Y, Zhang Y M, Chen S Y, Gai J Y (2004). Method of evaluation and adjustment of recombinant inbred line population and its application to the soybean RIL population NJRIKY. *Acta Agronomica Sinica*, 30(5): 413–418 (in Chinese)
- Yan X, Zhang F S (1997). *Plant Nutrition Genetics*. Beijing: China Agriculture Press, 22, 40–41, 193–196 (in Chinese)
- Zhang Y M, Gai J Y (2000a). Identification of mixed major-gene and polygene inheritance model of quantitative traits by using DH or RIL population. *Acta Genetica Sinica*, 27(7): 634–640 (in Chinese)
- Zhang Y M, Gai J Y (2000b). The IECM algorithm for estimation of component distribution parameters in segregating analysis of quantitative traits. *Acta Agronomica Sinica*, 26(6): 699–706 (in Chinese)
- Zhang, Y M, Gai J Y, Yang Y H (2003). The EIM algorithm in the joint analysis of quantitative traits. *Genetical Research*, 81: 157–163