African Journal of Biotechnology Vol. 11(65), pp. 12830-12838, 14 August, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.1415
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Construction of a molecular marker linkage map and its use for quantitative trait locus (QTLs) underlying drought tolerance at germination stage in soybean

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Accepted 22 June, 2012

A backcross inbred line (BIL) population of soybean was examined under polyethylene glycol (PEG) and well-watered conditions to identify the quantitative trait locus (QTL) controlling the drought-tolerance at germination stage. The recipient, SNWS0048, was a wild soybean with strong drought tolerance and the donor, Jinda73, was a drought-sensitive variety with superior agronomic traits. The molecular genetic linkage map was produced using the technique of simple sequence repeats (SSR) marker and tool of gene mapping, and 120 SSR markers and 2 morphology markers covering 1655.4 cM were produced. The average genetic distance between markers was 17.68 cM. The range of markers per linkage group was from 2 to 9, and the length was from 2.8 to 230.0 cM. Most of the markers among linkages were well distributed. 17 QTLs with additive effects and/or additive × environment interaction effects, involved in drought tolerance of soybean in germination stage, were found on linkage group G2-A2, G10-D2, G11-E. Out of these QTLs, 9 QTLs only were significant in additive effects, 8 QTLs had additive effect and additive effect by PEG treatment. Four tightly linked QTLs (Sat_199-I on MLG G2-A2, I-Satt327 on G2-A2, Satt528-Sat_365 on MLG G10-D2, Satt573-Satt606 on MLG G11-E) controlling drought tolerant traits in germination stage were revealed, and would be useful in future for marker assisted selection programs (MAS) and cultivar improvement.

Key words: Soybean, molecular marker linkage map, quantitative trait loci (QTLs), drought tolerance, germination stage.

INTRODUCTION

Water shortage is increasingly becoming a major constraint for crop productivity worldwide (Wollenweber et al., 2003). As one of the five major crops in the world, soybean is an important source of high-quality protein and edible oil for mankind. Water stress usually occurs during the whole growth season of soybean, leading to a big loss of both seed yield and quality, particularly in arid and semi-arid regions. Consequently, much attention has been paid to drought-resistant breeding and culturing of soybean since 1980s (Gu et al., 1998).

In the past decade, a great deal of information has been collected from the research on the molecular, biochemical, physiological, anatomical, and morphological responses of plants to drought (Desclaux et al., 2000; Hufstetler et al., 2007). DNA marker technology has been developed and integrated into soybean breeding programs (Boerma and Mian, 1999; Cregan et al., 1999; Kristin et al., 1997; Lv et al., 2010). Verification and confirmation of the quantitative trait locus (QTL) are recommended before the application of marker assisted selection programs (MAS) in a practical breeding program (Boerma and Mian, 1999). Up to date, a series of genetic maps have been established to study QTLs underlying drought tolerance in soybean. Mian et al. (1996, 1998) used the genotypic data from a soybean (*Glycine max* (L.) Merr.) population of 120 F4-derived lines from a cross of 'Young' × PI416937 to identify

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quantitative trait loci (QTL) associated with water use efficiency (WUE) and leaf ash (LASH) in 36-day old, greenhouse-grown plants. Specht et al. (2001) used the genotypic data of Orf et al. (1999) from the Minsoy × Noir 1 population to identify six yields of QTLs under water-stressed conditions. Du et al. (2009b) used a recombinant inbred line (RIL) population derived from a cross between Kefeng 1 and Nannong 1138-2 to identify QTLs for yield per plant across different water regimes and drought susceptibility index (DSI) in field and the greenhouse. Meanwhile, they used the above population to identify QTLs for the leaf pubescence density and water status traits in field and the greenhouse, for the trichome densityon, the upper and lower surface of leaf blade (Du et al., 2009a; 2009c). Liu et al. (2005) used a RIL population derived from Kefeng 1 × Nannong 1138-2 to analyze the inheritance of the three relative root traits by using the segregation analysis of quantitative trait under the major gene plus polygene mixed inheritance model. Li (2011a, 2011b) used a primary backcross introgression soybean population derived from 'Hongfeng11' as recurrent parent and Clark, as donor parent to identify QTLs for water retention curve (WRC) and other drought-tolerance traits. However, most of those studies focused on late vegetative growth and reproductive periods. The study combining soybean breeding and drought tolerance at germination stages is very limited.

Obviously, drought tolerance should include the ability of seeds germination development under limited moisture availability since successful field establishment and vigorous stand directly contribute to higher grain yield (Baalbaki et al., 1999). Germination stage is an important period for final production in soybean. Under water stress, tolerant cultivars had higher germination rate than susceptible ones (Baalbaki et al., 1999) .Therefore, it is very essential to find QTLs affecting drought tolerance at soybean germination stages. The methods of hypertonic solution and repeated drought treatment are widely used to assess the drought tolerance of crop at germination stages (Li, 1993). In this study, phenotypic data were obtained using these two kinds of methods. The objectives of the present study were to identify and map QTLs associated with drought tolerance involved in soybean germination growth, using a backcross inbreed lines (BIL) population resulting from a cross between a cultivated and a wild soybean.

MATERIALS AND METHODS

Plant

A mapping population of 200 BC1F4 lines derived from (JD73 × SNWS0048) × JD73 × JD73 × JD73 × JD73 was employed to test the presence of conferring drought tolerance over the whole genome of soybean. The recipient SNWS0048 was an accession of common wild soybean from Shanxi Province, China, with strong drought tolerance; the donor JD73 was a drought-sensitive variety with superior agronomic traits.

Drought resistance screening

Germination tests were performed in Shanxi Agricultural University, in China. The experiment was designed as a completely randomized factorial of 200 genotypes and 2 treatments. The experimental run included two replications per treatment, for a total of 800 experimental units. The treatments consisted of water and 15% polyethylene glycol (PEG). For each treatment, 25 seeds (the same in size and sterilized by 70% alcohol) were placed on two layers of filter paper in a 9 cM petri dish (disinfected and dried). The controls were established by irrigating each petri dish with 20 ml of distilled water, and the water stress treatments were created with 20 ml of the appropriate osmotic solution. Osmotic potentials were created using 15 polyethylene glycol (PEG 6000, Sigma Chemical Company, St. Louis, MO, USA) and were adjusted for temperature (25°C). Seeds were incubated in an illumination incubator at 25°C. Germination was recorded every 24 h for 6 days. Seeds were regarded as germinated when the radicle appeared normal and had protruded the same as the length of seeds. Germinated seeds were removed from the experiment, and the dishes were recovered immediately after each enumeration.

Germination potential (GP) was calculated as follows: GP = SNm / SN, where SNm and SN mean germinative number in peak time (usually in the third day) and number of all tested seeds, respectively. The rate of germination percentage (GR) was calculated as follows: GR = SNl / SN, where, SNl means germinative number of all tested seeds. Germination index (GI) was calculated as follows: GI = SN1/1 + SN2/2 + SN3/3 +……SNn/n, where, SN1, SN2, SN3 and SNn mean germinative number in the first, second, third and n day. The water absorption (WA) was calculated as follows: WA = (WW - DW) / DW, where WW and DW mean water weight in 12 h and dry weight. The radicle length (RL) was measured using tape. As for the root hair number (RHN), it was few in germination stage and was counted directly. The germinated seeds were put into an oven at 105°C for 20 min and dried to constant weight at 80°C. The dry weight (DW) was measured using electronic loadcell scales. Relative values of traits were calculated using the ratio between the mean value of the traits under PEG conditions and well watered conditions.

Simple sequence repeat marker analysis

Total genomic DNA was isolated from fresh young leaves from individual plant of each family from obtained seedlings, using modified cetyl trimethylammonium bromide (CTAB) extraction method (Eujayl et al., 2002). Polymerase chain reaction (PCR) was carried out in a 20 μl volume containing template DNA 50 to 100 ng, 1× PCR buffer (including $MgCl_2$), 0.2 mmol dNTPs, 0.2 μmol SSR primers and 1 U Taq DNA polymerase. The reaction was denaturing at 95°C for 5 min, 35 cycles each consisting of denaturing at 94°C for 1 min, annealing at 52°C for 1 min, extension at 72°C for 1 min, and a final extension at 95°C for 5 min. The amplification products were separated on non-denaturing polyacrylamide gels (PAGE) according to Wang et al. (2003), and detected by argentation. DNA from the parents of the mapping populations was first screened against 340 SSR markers covering the 20 chromosomes of the soybean genome. The populations were tested with polymorphic markers, which included 120 in the population. The primer sequences of these SSR markers were obtained from the SoyBase, the USDA-ARS sponsored genome database (http://129.186.26.94/SSR.html) and was synthesized by AuGCT Biotechnology.

Data analysis

Phenotypic data for the BC1F4 population was processed using

EXCEL 2003 programs. For the simple sequence repeats (SSR) data, fragments were amplified from the recurrent parent JD73 and non-recurrent SNWS0048 and were scored as 1 and 2, respectively, whereas, hyterozygosity and missing fragments were scored as 3 and 0, respectively. The genetic linkage map was constructed with the software MAPMAKER/EXP ver. 3.0 (Lander et al., 1987; Lincoln et al., 1993a). A threshold LOD score of 3.0 was used in the mapping analysis. CentiMorgan units were calculated using the Kosambi mapping function (Kosambi, 1944). The QTLs were detected using QTL Network 2.0 (Zhejiang University, Hangzhou, China) according to the mixed linear model, and the test standard is a $P < 0.01$ significance (Yang, 2008).

RESULTS

Polymorphism analysis of SSR

A total of 700 SSR markers were tested for polymorphism between JD73 and SNWS0048, 328 of 700 pairs could generate reproducible and unambiguous band patterns, and polymorphic rate is 46.85%. Among 328 markers, to remove severe distorted markers, 193 markers segregated, which accorded with the role of Mendel.

Construction of molecular marker linkage map

Out of the total 193 markers, 122 markers were mapped in the populations. The polymorphic markers were assigned into 24 linkage groups by Mapmaker 3.0, which cover 20 chromosomes of soybean (Figure 1). The total map encompassed 1655.4 cM with an average distance of 13.6 cM between markers. The actual number of markers ranged from 2 for linkage group C1 to 9 for linkage group D1a, which was consistent with other soybean linkage maps in marker alignments and intervals. The characters of linkage groups were summarized in Tables 1 and 2. Phenotypic data of parents and BIL population can be found in Table 3. The values of all traits were lower in PEG treatment than in the control, and the relative values of all traits were higher in PEG treatment than in the control, indicating PEG stress can inhibit the germination of soybean. Non-recurrent parent SNWS0048 presented much higher drought tolerance than recurrent JD73 in PEG treatment, with average germination potential of 0.64 to 0.32, average germination percentage of 0.76 to 0.67, average germination index of 7.47 and 5.47, and average water absorption of 0.96 and 0.89. Similarly, radical length of two parents in PEG treatment was 5.60 and 2.73, and root hair number was 11.67 and 6.67, respectively. Meanwhile, the relative values of traits in non-recurrent parent SNWS0048 were higher than recurrent JD73. The distribution of scores for drought tolerance of the BIL population approximately fitted normal distribution, with the absolute values of skewness and kurtosis close to 1, and a transgressive segregation was found from the minimal value to maximal value (Table 3). Therefore, the distributions of phenotypic data were suitable for QTL analysis.

Correlations between plant type traits

In the PEG treatment, there was a significant correlation between pairs of all traits (Table 4). In the control, there was a significant correlation between pairs of all traits except between DW and GR and between DW and RHN.

QTLs with additive effects and additive × environment interaction effects

QTLs detected with additive effects and additive × environment interaction effects associated with drought tolerance of soybean in BIL population at germination stage are shown in Table 5. 17 QTLs with additive effects and/or additive × environment interaction effects involved in drought tolerance of soybean in germination stage were found. Out of these QTLs, 9 QTLs only were significant in additive effects, which indicted that these QTL might adopt expression patterns in different conditions. 8 QTLs had additive effect and additive effect by PEG treatment, indicating that existence of G × E interaction was trait dependent. These QTLs might be described by their sensibility to the PEG stress, and it is the important cause of phenotypic value change after drought stress. Out of 17 QTLs, 4 QTL namely qGR-E-1, qWA-E-3, qRL-E-1 and qRHN-E-1 had a negative additive effect. In contrast, other QTLs had a positive additive effect and the alleles for increasing drought tolerance from SNWS0048.

2 QTLs detected to be responsible for germination potential showed significant a effects. These two QTLs located on G2-A2 and G10-D2 linkage group were named as qGA-A2-2 and qGA-D2-4, respectively. One QTL of qGA-D2-4, located within the interval Satt528-Sat_365, increased the GA by 0.0889, with corresponding contribution of 6.16%. Another QTL of qGA-A2-2 located within the interval I-Satt327 also increased the GA, but its contribution was small.

3 QTL controlling germination percentage were significant in a and ae effects, and a effect was the opposite of ae effect, indicating that the gene controlling germination percentage was sensitive to moisture movement. The QTL of qGR-D2-4 located within the interval Satt528-Sat_365 increased the GR by 0.0887, with corresponding contribution of 6.11%, and in PEG stress, it reduced the GR by 0.0510 with corresponding contribution of 2.61%. The other two QTLs contributions were small. The QTL of qGR-A2-2 located within the interval I-Satt327 increased the GR by 0.0607, with corresponding contribution of 0.79%, and in PEG stress, it reduced the GR by 0.0396 with corresponding contribution of 0.63%. The QTL of qGR-E-1 located within the interval Satt573-Satt606 increased the GR by 0.0253, with corresponding contribution of 0.59%, and in PEG stress, it reduced the GR by 0.0379 with corresponding contribution of 1.13%. 2 QTLs affecting germination index were significant, which, located on G2- A2 and G10-D2 linkage group,

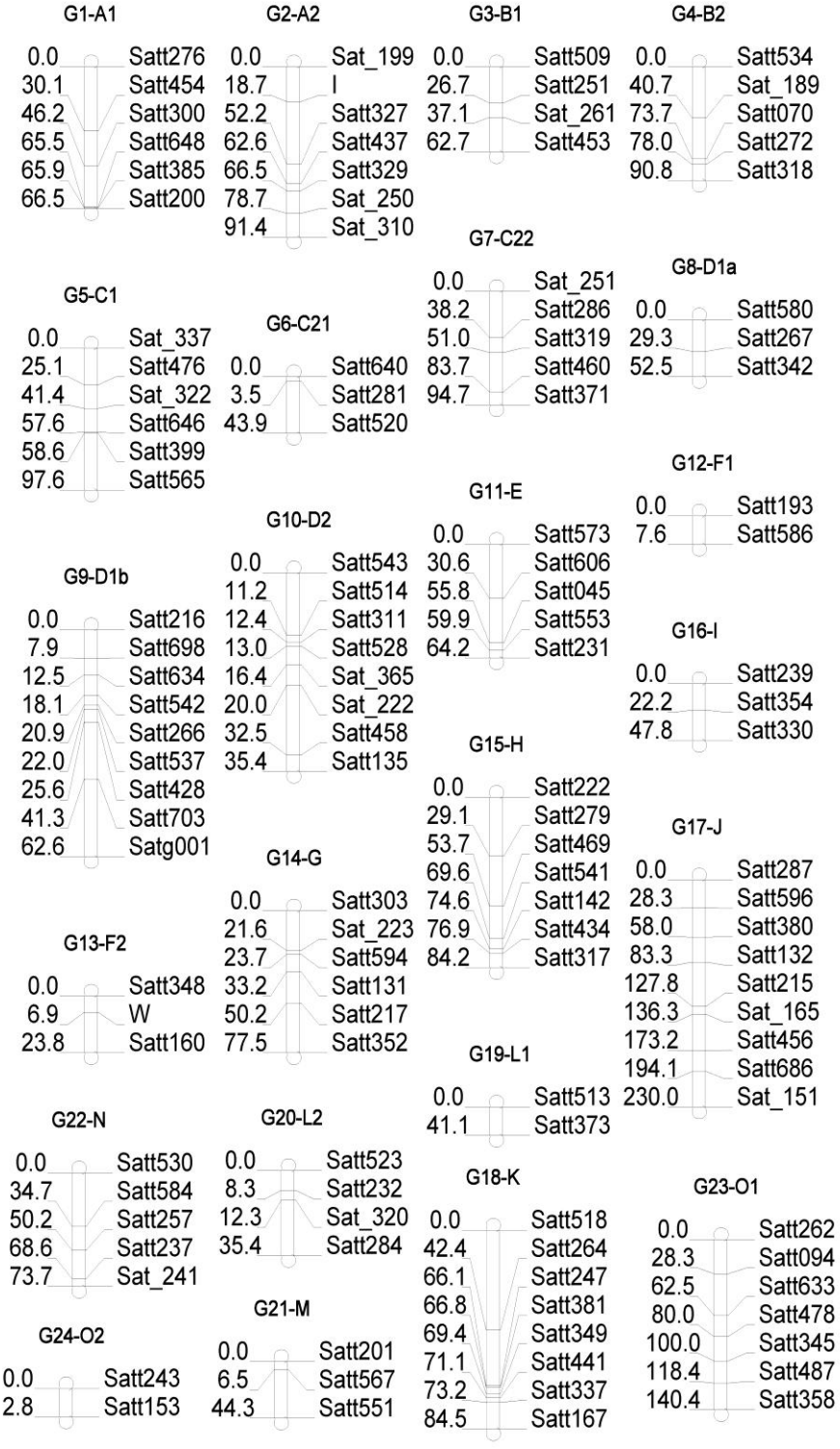

Figure 1. Molecular marker linkage map. Phenotypic data of drought tolerance at germination stage.

were named qGI-A2-1 and qGI-D2-3. One QTL of qGI-D2-3 had significant a and ae effects, which, located within the interval Satt311-Satt528, increased the GI by 1.6580, with corresponding contribution of 17.59%, and in PEG stress, it reduced the GI by 0.1598, with corresponding contribution of 0.44%, suggesting that the

Table 1. The statistics of the linkage map.

Parameter	Minimum	Maximum	Mean	SD	CV (%)	Total
Number of markers	2	9	5.08	2.22	43.76	122
Genetic distance	2.8	230.0	68.98	46.31	67.15	1655.4
Mean distance of markers	2.8	41.1	17.68	8.61	48.71	

Table 2. The distribution of SSR markers on the linkage map.

Linkage group	Length (cm)	No. of markers	Average distance (cm)	No. of intervals (> 20 cm)
G1-A1	66.5	6	13.30	1
G2-A2	91.4	7	15.23	1
G3-B1	62.7	4	20.90	2
G4-B2	90.8	5	22.70	2
G5-C1	97.6	6	19.52	2
G6-C21	43.9	3	21.95	1
G7-C22	94.7	5	23.68	1
G8-D1a	52.5	3	26.25	0
G9-D1b	62.6	9	7.83	0
G10-D2	35.4	8	5.06	0
G11-E	64.2	5	16.05	3
G12-F1	7.6	2	7.60	0
G13-F2	23.8	3	11.90	1
G14-G	77.5	6	15.50	3
G15-H	84.2	7	14.03	2
G16-I	47.8	3	23.90	1
G17-J	230.0	9	28.75	1
G18-K	84.5	8	12.07	1
G19-L1	41.1	2	41.10	2
G20-L2	35.4	4	11.80	1
G21-M	44.3	3	22.15	1
G22-N	73.7	5	18.43	1
G23-O1	140.4	7	23.40	3
G24-O2	2.8	2	2.80	0

expression of this QTL was a little modified by PEG stress. Another QTL of qGI-A2-1 only were significant in addictive, which, located within the interval Sat_199-I, also increased the GI.

2 QTLs affecting water absorption were significant, which located on G10-D2 and G11-E linkage group, were named qWA-D2-1 and qWA-E-3. One QTL of qWA-E-3 had significant a and ae effects, which located within the interval Satt045-Satt553, reduced the WA by 0.0241, with corresponding contribution of 0.02%, and in PEG stress, it increased the WA by 0.0266, with corresponding contribution 0.02%. Addictive effects and additive × environment interaction effects of the QTL were about equal in values, indicating that it is sensitive to PEG stress. Another QTL of qWA-D2-1 only was significant in addictive, which located within the interval Satt543-Satt514, increased the WA. 3 QTL for radical length was identified as being significant in a and/or ae effects, which located on linkage group G2-A2, G10-D2 and G11-E, were named as qRL-A2-2, qRL-D2-4 and qRL-E-1. The QTL of qRL-D2-4 only was significant in addictive, which located within the interval Satt528-Sat_365, increased the RL by 0.6193, with corresponding contribution of 7.03%. The other two QTLs of qRL-A2-2 and qRL-E-1 had significant a and ae effects. One QTL of qRL-A2-2 located within the interval I-Satt327, increased the RL by 0.3736, with corresponding contribution of 1.99%, and in PEG stress, it reduced the RL by 0.3703 with corresponding contribution of 0.93%. Another QTL of qRL-E-1 located within the interval Satt573-Satt606, reduced the RL by 0.6790, with corresponding contribution of 2.76%, and in PEG stress, it increased the

Table 3. Phenotypic scores of drought tolerance in the BIL population and parents of soybean.

Trait	Treatment	Parents		BIL population			
		JD73	SNWS0048	Mean ± SD	Range	Skewness	Kurtosis
GP	CK	0.64	0.93	0.67±0.19	0-1.00	-0.65	0.06
	PEG	0.32	0.64	0.36±0.22	0-0.88	0.34	-0.47
RGP		0.50	0.69	0.55±0.26	0.04-1.00	0	-0.87
GR	CK	0.92	1.00	0.87±0.13	0-1.00	-0.75	0.04
	PEG	0.67	0.76	0.62±0.26	0-1.00	-0.70	-0.47
RGR		0.73	0.76	0.75±0.25	0.04-1.00	-0.95	-0.16
GI	CK	7.77	10.23	9.30±2.43	0-18.50	0.10	0.55
	PEG	5.47	7.47	5.06±2.94	0-11.17	0.23	0.84
RGI		0.70	0.73	0.57±0.20	0.03-1.00	1.22	0.95
WA	CK	1.11	1.20	1.01±0.07	0.77-1.42	0.27	1.09
	PEG	0.89	0.96	0.75±0.08	0.58-0.96	0.08	-0.34
RWA		0.80	0.80	0.72±0.05	0.23-1.00	0.50	1.22
RL	CK	6.33	11.53	6.48±1.61	8.25-18.53	0.38	0.84
	PEG	2.73	5.60	5.67±3.19	2.21-10.23	-0.73	0.95
RRL		0.43	0.48	0.87±0.45	0.36-1.00	-0.95	1.40
RHN	CK	7.55	13.00	13.48±4.63	0-42.5	0.27	0.51
	PEG	6.67	11.67	9.11±5.55	0-28.2	1.30	1.47
RRHN		0.88	0.89	0.90±0.15	0.55-1.00	1.48	1.51
DW	CK	0.11	0.03	0.09±0.02	0-0.14	-0.1	0.80
	PEG	0.09	0.02	0.08±0.02	0-0.13	-0.27	0.78
RDW		0.82	0.84	0.82±0.06	0.47-1.00	0.52	0.96
PI	PEG	0.48	0.73	0.51±0.03	0.02-0.91	-0.51	-0.47

†Plant type traits: GP, Germination potential; RGP, relative germination potential; GR, germination rate, RGR, relative germination rate; GI, germination index; RGI, relative germination index; WA, water absolution; RWA, relative water absolution; RL, radicle length; RRL, relative radicle length; RHL, root hair number; RRHL, relative root hair number; DW, dry weight; RDW, relative dry weight.
‡SD, standard deviation.

Table 4. Correlations of relative values of traits in germination stage.

Traits	AC	GP	GR	GI	RL	RHN	DW
AC		0.2149**	0.1552*	0.2388**	0.2327**	0.2445**	0.1765*
GP	0.4137**		0.7306**	0.8733**	0.5153**	0.4097**	0.1998**
GR	0.4832**	0.8115**		0.6878**	0.3507**	0.2233**	0.1286
GI	0.4825**	0.9129**	0.8983**		0.4870**	0.4539**	0.2537**
RL	0.3309**	0.6181**	0.6321**	0.6190**		0.6177**	0.2611**
RHN	0.2936**	0.5627**	0.5241**	0.5557**	0.8314**		0.1276
DW	0.3025**	0.4109**	0.4171**	0.4225**	0.4563**	0.4044**	

*Significant at $P < 0.05$; **significant at $P < 0.01$. †Abbreviations for plant type traits are the same as in Table 1. ‡The upper angle indicates phenotype in control correlations and the low triangle indicates phenotype in PEG treatment correlations, respectively.

RL by 0.4731, with corresponding contribution of 1.35%.

3 QTL mapped for root hair number were significant in a and/or ae effects, which located on linkage group G2-A2, G10-D2 and G11-E, were named as qRHN-A2-2, qRHN-D2-4 and qRHN-E-1. The QTL of qRHN-D2-4 had significant a and ae effects, which located within the interval Satt528-Sat_365, increased the RHN by 3.0371, with corresponding contribution of 8.09%, and in PEG

Table 5. The QTL locations and estimated effects (additive a and addictive by treatment ae) associated with germination traits of soybean in BIL population.

Traits	QTL	MLG	Marker interval	Location/cm	(a) effects	h^2 (a)/%	(ae) effects	h^2 (ae)/%
GA	qGA-A2-2	G2-A2	I-Satt327	18.7	-0.0446***	0.23		
	qGA-D2-4	G10-D2	Satt528-Sat_365	13.0	-0.0889***	6.16		
GR	qGR-A2-2	G2-A2	I-Satt327	18.7	-0.0607***	0.79	0.0396***	0.63
	qGR-D2-4	G10-D2	Satt528-Sat_365	13.0	-0.0887***	6.11	0.0510***	2.61
	qGR-E-1	G11-E	Satt573-Satt606	16.0	0.0253*	0.59	-0.0379*	1.13
GI	qGI-A2-1	G2-A2	Sat_199-I	16.0	-0.7143***	0.07		
	qGI-D2-3	G10-D2	Satt311-Satt528	12.4	-1.6580***	17.59	-0.1598*	0.44
WA	qWA-D2-1	G10-D2	Satt543-Satt514	1.0	-0.0319***	0.03		
	qWA-E-3	G11-E	Satt045-Satt553	55.8	0.0241***	0.02	-0.0266***	0.02
RL	qRL-A2-2	G2-A2	I-Satt327	19.7	-0.3736**	1.99	0.3703*	0.93
	qRL-D2-4	G10-D2	Satt528-Sat_365	12.4	-0.6193***	7.03		
	qRL-E-1	G11-E	Satt573-Satt606	18.0	0.6790***	2.76	-0.4731*	1.35
RHN	qRHN-A2-2	G2-A2	I-Satt327	18.7	-2.1510***	1.12		
	qRHN-D2-4	G10-D2	Satt528-Sat_365	13.0	-3.0371***	8.09	1.1238*	1.26
	qRHN-E-1	G11-E	Satt573-Satt606	17.0	2.1561***	3.56		
PI	qPI-A2-1	G2-A2	Sat_199-I	17.0	-0.2676***	0.02		
	qPI-D2-4	G10-D2	Satt528-Sat_365	16.0	-0.5399***	0.29		

*, ** and *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively, the same below. a, addictive effect. ae: addictive by PEG treatment, the addictive by control was –ae. Minus indicates the alleles from JD73 increase the phenotype of the trait.

Table 6. The QTL locations and estimated effects (epistasis aa and epistasis by treatment aae) associated with germination traits of soybean in BIL population.

Trait	QTL	Marker interval	Location/cm	(aa) effects	h^2 (aa)/%	(aae) effects	h^2 (aae)/%
RL	qRL-A2-2	I-Satt327	19.7	-0.5893***	1.07		
	qRL-F1-1	Satt193-Satt586	7.0				
	qRL-B2-4	Satt272-Satt318	78.0	0.7816***	3.80	-0.6147**	2.93
	qRL-C1-2	Satt476-Sat_322	40.1				
DW	qDW-B1-1	Satt509-Satt251	12.0	-0.0072***	3.86		
	qDW-N-4	Satt237-Sat_241	70.6				

aa, Addictive × addictive interaction effect, aae: addictive × addictive interaction effect by PEG treatment, addictive × addictive interaction effect by control was -aae.

stress, it reduced the RHN by 1.1238, with corresponding contribution of 1.26%. The other two QTLs of qRHN-A2-2 and qRHN-E-1 were significant in addictive. One QTL of qRHN-A2-2, located within the interval I-Satt327, increased the RHN by 2.1510, with corresponding contribution of 1.12%. Another QTL of qRHN-E-1, located within the interval Satt573-Satt606, reduced the RHN by 2.1561, with corresponding contribution of 3.56%. 2 QTLs detected to be responsible for PI showed significant a effects. These two QTLs located on linkage group G2-A2 and G10-D2 were named as qPI-A2-1 and qPI-D2-4, respectively. One QTL of qPI-D2-4 located within the interval Satt528-Sat_365, increased the PI by 0.5399, with corresponding contribution of 0.29%. Another QTL of qPI-A2-1 located within the interval Sat_199-I, also increased the PI by 0.2676, but its contribution was small.

Epistatic effects on drought tolerance

Three pairs of QTLs, which had interactions with each other, were mapped on linkage group A2, F1, B2, C1, B1 and N (Table 6). Among these epistatic interactions, one pair was identified with aa effects and aae effect, and two

Table 7. Pleitropic or tightly linked QTLs.

MLG	QTL	Interval	Trait
G2-A2	qGI-A2-1, qPI-A2-1	Sat_199-I	GI, PI
G2-A2	qGP-A2-2,qGR-A2-2, qRL-A2-2, qRHN-A2-2	I-Satt327	GA, GR, RL, RHN
G10-D2	qGA-D2-4,qGR-D2-4, qRL-D2-4,qRHN-D2-4, qPI-D2-4	Satt528-Sat_365	GA, GR, RL, RHN, PI
G11-E	qGR-E-1, qRL-E-1, qRHN-E-1	Satt573-Satt606	GR, RL, RHN

pair only exhibited aa effect. For one pair of QTLs, qDW-B1-1 and qDW -N-4, the additive × additive epistatic effect in parent type increased DW by 0.0072. For the pair of QTLs qRL-A2-2 and qRL-F1-1, the aa effect in parent type increased RL by 0.5893, with corresponding contribution of 1.07%. The other pairs of QTLs were modified by environments. For the pair of QTLs qRL-B2-4 and qRL-C1-2, the aa effect in parental type reduced RL by 0.7816, with corresponding contribution of 3.80%, and in the PEG stress, aae effect increased RL by 0.6147 with 2.93%. 1 pair of QTLs controlling dry weight of radical had non-addictive effects and took part in the additive × additive epistatic effect and aae effects and these gene locus, probably through modifying other gene locus.

Pleitropic or tightly linked QTL

The QTL located on MLG G2-A2 and bordered by the marker Sat_199-I had a genetic effect for GI and PI. The QTL located on MLG G2-A2 and bordered by the marker I-Satt327 had a genetic effect for GA, GR, RL and RHN. The QTL located on MLG G10-D2 and bordered by the marker Satt528-Sat_365 had a genetic effect for GA, GR, RL, RHN and PI. The QTL located on MLG G11-E and bordered by the marker Satt573-Satt606 had a genetic effect for GR, RL and RHN. These QTL had a genetic effect on more than one trait, and seems to act as a major gene.

DISCUSSION

In our research, genetic populations mainly derived from *Glycine Soja* and *Glycine max* crosses could produce higher polymorphism. The polymorphic rate between the two parents (46.85%) was relatively high. Two parents are quite different in some traits related to drought resistance. Therefore, this kind of population was suitable for QTLs mapping of drought resistant or tolerant traits. It was found that no common QTLs for drought tolerant traits are across of different types of mapping populations. Li et al. (2009) used a primary backcross introgression line constructed with Hong-feng11 as recurrent parent and Clark as donor parent to find out related steady and repeatable QTLs; 14 QTLs in 4 linkage groups of L, F, K and N for relative germination rate were investigated. Liu et al. (2005) used a RIL population derived from Kefeng 1 × Nannong 1138-2 to analyze the inheritance of the three relative root traits, five, three, and five QTLs located on N6-C2, N8-D1b + W, N11-E, and N18-K linkage groups for relative dry root weight, total root length and root volume, respectively. 15 QTLs controlling velutinous on linkage group A2, D1a, D1b, E and H were found in a recombinant line population of 184 F2:7:11 lines from a cross of Kefeng1 and Nannong1138-2 (Du et al., 2009c). QTLs for germination trait in present paper were also detected on linkage group A2 and E. Moreover, QTLs for germination trait on linkage group D2 in the present paper were not detected in other populations. QTLs associated with Satt573 were detected controlling SCN and oil (Qi et al., 2011; Guo et al., 2005; Kabelka et al., 2005). In this paper the QTL for GR, RL and RHN were also detected near Satt573.

G × E interaction was analyzed based on the different water treatments data. Eight pairs of epistatic G × E interaction QTLs were detected. These QTL were expressed specifically in PEG treatment. The additive-dominance model approach has been often used on QTL mapping for drought tolerant traits in previous studies, in which no epistatic effects and no G × E interactions were assumed. However, because epistatic QTLs may exist in most of complex quantitative traits (Zhu, 1997), analysis of epistatic effect is necessary for these traits. In the present study, QTLs of epistatic effects for drought tolerant traits were obtained based on mixed linear model approaches. The result showed that epistasis was an important genetic component underlying RL and DW. This result indicated the complexity of genetic basis of drought tolerant traits. Some linkage regions between QTLs controlling drought tolerant traits in germination stage were revealed. QTL region of near Sat_199-I on MLG G2-A2 was found to control GI and PI; I-Satt327 on G2-A2 was detected to govern GA, GR, RL and RHN; Satt528-Sat_365 on MLG G10-D2 was detected to control GA, GR, RL, RHN and PI; Satt573-Satt606 on MLG G11-E was also revealed to control GR, RL and RHN. These tightly linked QTLs may be the genetic basis of phenotypic correlations among drought tolerant traits in germination stage. Therefore, these traits will be improved by MAS with these QTL regions.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science

Foundation of China (Grant No. 31171580), The R and D Infrastructure and Facility Development Program of Shanxi Province, China (Grant No. 20110910040103), The Specialized Research Fund for the Doctoral Program of Higher Education (Grant No. 20111403120001), The Shanxi Agricultural University Breeding Fund of China (2011001), The Shanxi Agricultural University Introduced the Talented Person Scientific Research Start Funds Subsidization Project of China (XB2010010).

Abbreviations

GP, Germination potential; **RGP,** relative germination potential; **GR,** germination rate; **RGR,** relative germination rate; **GI,** germination index; **RGI,** relative germination index; **WA,** water absolution; **RWA,** relative water absolution; **RL,** radicle length; **RRL,** relative radicle length; **RHL,** root hair number; **RRHL,** relative root hair number; **DW,** dry weight; **RDW,** relative dry weight; **QTL,** quantitative trait locus; **MAS,** marker assisted selection programs; **WUE,** water use efficiency; **LASH,** leaf ash; **RIL,** recombinant inbred line; **DSI,** drought susceptibility index; **WRC,** water retention curve; **BIL,** backcross inbreed lines; **PEG,** polyethylene glycol; **CTAB,** cetyl trimethylammonium bromide; **PCR,** polymerase chain reaction; **SCN, GA**.

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