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Dissection of genetic overlap of drought and low-temperature tolerance QTLs at the germination stage using backcross introgression lines in soybean

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Abstract Northeast of China is the main soybean production area, drought and low-temperature tolerance are both main factors involved in reducing soybean yield and limiting planting regions, the most effective way to solve this problem is to breed cultivars with drought and low-temperature tolerance. A set of the BC_2F_3 lines was constructed with Hongfeng 11 as recurrent parent and Harosoy as donor parent, and screened in drought and low-temperature condition at the germination stage. Related QTLs were obtained by Chi-test and ANOVA analysis with genotypic and phenotypic data. Eighteen QTLs of drought tolerance and 23 QTLs of low-temperature tolerance were detected. Among them, 12 QTLs were correlated with both drought and low-temperature tolerance, which showed a partial genetic overlap between drought and low-temperature tolerance at the germination stage in soybean. Among the 12 genetic overlap QTLs, Satt253, Satt513, Satt693, Satt240, Satt323, and Satt255 were detected by at least one method for both drought and low-temperature tolerance. Satt557, Satt452, Sat_331, Satt338, Satt271, and Satt588 were detected by only one analysis method. The QTLs detected above were significant loci for drought or low-temperature tolerance in soybean. This will play an important role in MAS for development of both drought and low-temperature tolerance variety.

Keywords Soybean · Backcross introgression lines · Genetic overlap · QTLs · Drought and low-temperature tolerance

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Introduction

Plant growth is greatly affected by abiotic stresses such as drought and low-temperature. Soybean [*Glycine max* (L.) Merr.] is an important crop planted in many countries. Drought stress is one of the major causes that limit the yield instability [1], and great efforts have been made for breeding drought-tolerant variety. Specht et al. [2] mapped three drought-related quantitative trait loci (QTLs) in drought condition with a population of 236 RILs. Mian et al. [3] identified four QTLs that conditioned water use efficiency (WUE) in a F_2-derived soybean population. Low-temperature is a critical environmental factor that limits agricultural production worldwide. Therefore, a large number of studies focused on low-temperature in soybean with the final goal of

improving chilling/freezing tolerance. Extensive studies at the molecular level have strengthened our understanding of the regulatory mechanisms underlying response and tolerance to low-temperature [4]. QTL analysis has also been conducted to unravel the genetic basis for low-temperature tolerance and identify molecular markers useful for marker-assisted selection (MAS) [5]. However, there were only two reports on QTLs analysis of low-temperature tolerance in soybean till now [5].

Most QTLs could not be directly used for molecular aided selection, and the materials for QTL mapping were not suitable in traditional breeding. One effective way to improve this problem was to obtain QTLs with backcross introgression lines (BILs). BILs, termed isogenic lines (ILs), or near isogenic lines (NILs), or chromosome segment substitution lines (CSSLs), can be accomplished through interspecific crosses, phenotypic selection, and multiple backcrosses. In the ideal situation, BILs within each genetic background are phenotypically similar to their recurrent parent but each carries one or a few traits introgressed from a known donor. Therefore, all the phenotypic variation in these lines is associated with the introduced segment. The introduction of new alleles into crops is the foundation for improvement of yield, quality, stress tolerance, disease resistance, and other characters [6, 7]. At present, BILs have been application in many crops, such as tomato [8], rice [9], wheat [10], soybean [11, 12], cotton [13], brassicaceae [14]. BILs mostly were used on abiotic stress in rice, including salt tolerance [15], drought tolerance [16], low-temperature tolerance [17]. And for other crops, drought tolerance effects on root and shoot growth of wild barley [18], low boron stress at seedling stage of brassica napus [19]. However, little studies were used in soybean. Therefore, it is very meaningful to study stress tolerance by BILs in soybean.

With large quantity QTLs developed, many QTLs associated with related traits were focused on the same chromosome interval, so genetic overlap existed among those QTLs. The genetic overlap means that a large number of function-related sites in the genome showed trend of centralized distribution. The overlap is likely the basis of traits phenotypic, for example, between yield and the components [20] and all kinds of biological resistance [21]. One reason leading genetic overlap is pleiotropy—the single locus affects multiple traits simultaneously, and the other reason may be linkage disequilibrium (LD). There was lots of evidence showed that some metabolic pathways also involved different stresses in plant in common [22]. With rice introgressive lines, Zheng Tianqing et al. [23] identified genetic overlap between sheath blight resistance and drought tolerance from directional selection, and Zang Jinping et al. [24] obtained QTLs for salt tolerance related traits at the seedling and tillering stages. However, in soybean, there was nothing reported on genetic overlap.

In this study, QTLs on drought and low-temperature tolerance at germination stage in soybean were analyzed using BILs derived from a cross between a China variety, Hongfeng 11 and an American variety, Harosoy. The objective was to compare the genetic relationship of drought and low-temperature tolerance in soybean, revealed the genetic overlap between them, which provides a basis on mapping of multiple-trait QTLs by BILs.

Materials and methods

Plant materials

The population was developed with Hongfeng 11 as recurrent parent and Harosoy as donor parent. Hongfeng 11 is a major cultivar of Heilongjiang Province, and exogenous Harosoy were an elite cultivar from the United States. F_1 was obtained by crossing Hongfeng 11 with Harosoy in 2004, then backcrossed with the Hongfeng 11 to produce BC_1, BC_2 in 2005 and 2006. Finally, 95 BC_2F_3 lines were obtained by selfing from 2007 to 2008. The selected population (SP) and random population (RP) were all come from the BC_2F_3 lines.

Identification of drought tolerance material

The concentration of PEG-6000 was set to 25% (w/v) according to pre-experiment and the results of Yang Jianping [25]. Seed pretreatment was surface-sterilized with 1% sodium hypochlorite solution for 30 s and rinsed well with sterilized water. Twenty seeds were placed on a filter paper soaked with sterilized water in sterile petri dishes. The plates were placed in an incubator and maintained at 20°C for 12 h. The drought treatment was that seeds were placed on a filter soaked with 25% (w/v) PEG-6000 solution and the plates were maintained at 20°C, and the solution was replaced every other day. The germination stage was investigated every day after 2-day treatment. The transgressive materials were chose because their germination stage was shorter than the recurrent parent, Hongfeng 11, and then were planted in pot.

Identification of low-temperature tolerance material

Combined with the methods of Shan Caiyun [26] and Hu Guoyu [27], the germination stage was chose to indicate low-temperature tolerance. The temperature was set to 6°C according to pre-experiment and the results of Jiang Hongwei [12]. Seed pretreatment were same as that in drought tolerance identification. Then the plates were maintained at 6°C and water was replaced every other day. The germination stage was investigated every day after 2-day treatment. The transgressive materials were selected and planted in pot.

Genotype analysis

One thousand of pairs of soybean SSR primers were synthesized according to soybase (http://soybase.org) [28], and 346 pairs of SSR primers were screened out for further study, which accounted for 34.6% of total primers, then 67 pairs have been chose to analyze the SP of drought tolerance and its RP, 61 pairs have chose to analyze the SP of low-temperature tolerance and its RP. There were 25 pairs of overlap primers, accounted for 37.31 and 40.98%, individually.

Data analysis

The introduced frequency of donor allele in SP, expected frequencies in BC_2 and the introduced frequency of donor allele in RP were used to analyze the deviation by Chi-test, and the probability level was $P < 0.05$.

Phenotypic data of BILs obtained from both drought and low-temperature tolerance was used to identify QTL by ANOVA using SAS PROC GLM. The probability level of $P < 0.05$ was used as the threshold for claiming the presence of QTLs. When a QTL was detected by two or more linked markers, the one with the highest F value was presented.

Results

QTL mapping for drought tolerance at germination stage in soybean

Introduced frequency of donor allele

The SP and RP were both used to analysis the introduced frequency of donor allele. Sixty-seven pairs with better polymorphism among 346 pairs of SSR primers were selected for genotyping. Due to directional selection, the introduced frequency of SP (0.338) were higher than that of RP (0.278), so there was a more deviation in the introduced frequency of donor allele of SP than RP (Table 1).

Chi-test analysis

According to the introduced frequency of donor allele of SP and RP, fifteen regions on thirteen LGs for drought tolerance in germination were detected by Chi-test, and the probability level was $P < 0.05$ (Table 2). They were Sat_271, Sat_331, Satt338, Satt640, Satt271, Satt452, Satt253, Satt529, Satt693, Satt588, Satt513, Satt323, Satt255, Satt237, and Sat_108, distributed on LG A1, B1, C1, C2, D1b, E, H, J, K, L, M, N, and O, respectively. The introduced frequency of donor allele was higher than that of RP obviously. Because of directional selection, ultra-introgressive alleles were obtained from donor parent in most detected loci, so these loci may show close correlation with drought tolerance.

ANOVA analysis

Germination stage for drought tolerance was analyzed by ANOVA with individual genotype, 7 QTLs were detected, distributed on LG A1, B2, C2, H, L, and K. The probability level was $P < 0.05$ (Table 2). Among them, the R^2 value of the QTL Sat_271 on LG A1 was 10.7%, and that of the Satt640 on LG C2 was 8.7%, Satt253 on LG H was 9.3%, and Satt513 on LG L 14%. These 4 QTLs were all detected by Chi-test. However, the introduced frequencies of donor allele from Satt577, Sat_113, and Satt240 didn't reach significant level between SP and RP, so they were not detected by Chi-test.

QTL mapping for low-temperature tolerance at germination stage in soybean

Introduced frequency of donor allele

Sixty-one pairs among 346 pairs of SSR primers were selected to genotype the SP and RP for low-temperature tolerance (Table 1). Higher mean in the introduced

Table 1 Introduced frequency of donor alleles for drought and low-temperature tolerance in introgression lines

Trait	Population	No[c]	Mean ± SD		Range	
			Freq	χ^2	Freq	χ^2
Drought tolerance	Selected population (SP[a])	46	0.338 ± 0.144	22.490 ± 26.065	0–0.756	0–143.585
	Random population (RP[b])	46	0.278 ± 0.121	13.389 ± 17.631	0–0.622	0–93.222
Low-temperature tolerance	Selected population (SP[a])	42	0.324 ± 0.176	23.985 ± 31.389	0–0.725	0–126.228
	Random population (RP[b])	50	0.301 ± 0.159	22.376 ± 37.282	0–0.830	0–220.811

Freq was the introduced frequency of donor allele; χ^2 value of allelic deviation with one degree of freedom ($df = 1$)

[a] Selected population which was selected under low-temperature tolerance

[b] Random population which was not selected

[c] The number of the population

Table 2 Distribution of drought tolerance loci by Chi-test and analysis of ANOVA

QTL		RP[b]		SP[c]		Selected/ random[d]	ANOVA of SP[e]	*P* value
Marker	LG[a]	Freq	χ^2	Freq	χ^2	χ^2	*F* value	
Sat_331	B1	0.29	6.86	0.51	46.29	8.10	–	0.004427
Satt338	C1	0.21	2.10	0.50	51.91	20.85	–	4.98E-06
Satt271	D1b	0.32	14.25	0.53	51.06	6.20	–	0.012779
Satt452	E	0.22	2.80	0.42	31.26	9.08	–	0.002578
Satt529	J	0.23	3.89	0.42	26.60	6.12	–	0.013335
Satt693	J	0.22	2.79	0.37	19.60	4.20	–	0.040509
Satt588	K	0.23	3.62	0.46	37.37	10.21	–	0.001397
Satt323	M	0.50	50.63	0.68	112.67	4.67	–	0.030754
Satt255	N	0.39	27.44	0.56	69.85	4.35	–	0.037033
Satt237	N	0.18	0.83	0.35	16.51	6.52	–	0.010685
Sat_108	O	0.05	1.87	0.40	27.55	116.83	–	3.13E-27
Sat_271*	A1	0.10	0.03	0.24	4.27	7.06	4.92	0.007866
Satt640*	C2	0.10	0.12	0.39	25.16	39.79	4.19	2.83E-10
Satt253*	H	0.27	7.01	0.44	35.39	5.63	4.19	0.017657
Satt513*	L	0.20	1.22	0.34	15.64	4.50	7.01	0.03384
Satt577	B2	0.36	18.63	0.33	10.29	0.03	4.58	0.86249
Satt240	K	0.27	7.48	0.30	9.66	0.04	4.73	0.841481
Sat_113	L	0.36	20.78	0.50	50.63	2.81	6.64	0.093678

The loci with * are both detected by Chi-square test and analysis of variances ($P < 0.05$)

[a] Where the QTL on the linkage groups

[b] Random population which was not selected

[c] Selected population which was selected about low-temperature tolerance

[d] The χ^2 which was obtained by the introduced frequency of donor allele between SP and RP

[e] The *F* value which was obtained by phenotypic data and genotyping of SP

frequency of donor allele showed in SP than that in RP by directional selection, which demonstrated the same tendency as drought tolerance.

Chi-test analysis

Nineteen regions on fourteen LGs for low-temperature tolerance in germination were detected by chi-test, and the probability level was $P < 0.05$ (Table 3). They were Satt577, Satt338, Satt457, Satt271, Satt669, Satt651, Satt142, Satt253, GMGLPSI, Satt693, Satt588, Satt240, Sat_020, Satt513, Satt540, Satt323, Satt549, Satt255, and Satt_331, distributed on LG B2, C1, C2, D1b, D2, E, H, I, J, K, L, M, N, and O, respectively. These show close correlation with low-temperature tolerance.

ANOVA analysis

Germination stage for low-temperature tolerance was also analyzed by ANOVA, 10 QTLs were detected, distributed on LG D1b, D2, E, F, J, K, M, and N. The probability level was $P < 0.05$ (Table 3). Among them, the R^2 value of the QTL Satt651 on LG E was 9.96%, and that of the Satt693 on LG J was 12.49%, Satt240 and Sat_020 on LG K were 11.95% and 19.02%, respectively, Satt323 on LG M was 12.03%, and Satt255 on LG N was 15.22%. These 6 QTLs were all detected by Chi-test. In addition, the introduced frequencies of donor allele from Satt041, Sat_262, Satt458, and Satt452 were not detected by Chi-test.

Genetic overlap between drought and low-temperature tolerance

Eighteen QTLs for drought tolerance and 23 QTLs for low-temperature tolerance were detected at the germination stage by two analysis method. Among them, 12 QTLs were both detected by drought and low-temperature tolerance, distributed on 11 linkage groups, including LG B1, B2, C1, D1b, E, H, J, K, L, M, and N (Fig. 1). These could be considered as the genetic overlap loci.

Twelve genetic overlap loci were divided into two groups based on the detecting results. The first group means that QTLs for one trait were detected by both methods and for the other trait were detected by one analysis method. Six QTLs were classified into this group. Satt253 on LG H and Satt513 on LG L were detected by two analysis methods for drought tolerance, but only detected by Chi-test for low-temperature tolerance. Satt693 on LG J, Satt240 on LG K, Satt323 on LG M, and Satt255 on LG N were all detected by two analysis methods for low-temperature tolerance, but were detected by Chi-test for drought tolerance. These QTLs affecting drought and low-temperature tolerance located in the same regions and might be closely linked.

The other group means that QTLs for two traits were detected by only one analysis method. The remained 6 QTLs were included. Satt557 on LG B2 was detected by Chi-test for low-temperature tolerance, but by ANOVA for drought tolerance. Satt452 on LG E was detected by Chi-test for

Table 3 Distribution of low-temperature tolerance loci by Chi-test and analysis of ANOVA

QTL		RP[b]		SP[c]		Selected/random[d]	ANOVA of SP[e]	*P* value
Marker	LG[a]	Freq	χ^2	Freq	χ^2	χ^2	*F* value	
Sat_331	B1	0.19	1.19	0.32	13.07	4.11	–	0.042756
Satt577	B2	0.33	16.40	0.51	54.00	5.75	–	0.016515
Satt338	C1	0.20	2.13	0.60	78.23	36.20	–	1.78E-09
Satt457	C2	0.44	40.05	0.64	98.30	6.39	–	0.011496
Satt271	D1b	0.31	12.84	0.49	46.08	5.37	–	0.020529
Satt669	D2	0.15	0.14	0.34	14.45	9.07	–	0.002601
Satt142	H	0.18	0.93	0.40	27.55	12.89	–	0.00033
Satt253	H	0.27	8.33	0.52	57.48	5.12	–	0.023703
GMGLPSI	I	0.28	9.61	0.12	0.01	4.63	–	0.031451
Satt588	K	0.06	1.29	0.20	1.43	11.10	–	0.000865
Satt513	L	0.18	0.93	0.38	22.87	10.17	–	0.001428
Satt540	M	0.43	37.58	0.24	4.27	5.33	–	0.020999
Satt549	N	0.43	39.78	0.21	1.59	6.34	–	0.011798
Satt651*	E	0.23	4.43	0.40	26.37	5.52	4.31	0.018766
Satt693*	J	0.31	13.09	0.49	40.96	4.85	5.00	0.027657
Satt240*	K	0.67	129.80	0.51	54.00	4.28	5.43	0.038614
Sat_020*	K	0.40	27.48	0.58	67.50	4.48	8.46	0.03428
Satt323*	M	0.50	55.76	0.73	126.23	7.23	5.20	0.00719
Satt255*	N	0.33	12.86	0.08	0.31	8.95	6.28	0.002769
Satt041	D1b	0.40	29.76	0.29	7.68	1.23	8.26	0.004053
Satt458	D2	0.37	24.08	0.42	21.94	0.45	5.15	0.023246
Satt452	E	0.23	4.43	0.19	1.10	0.24	4.60	0.031972
Sat_262	F	0.13	0.00	0.10	0.04	0.87	4.69	0.030339

The loci with * are both detected by Chi-square test and analysis of variances. ($P < 0.05$)

[a] Where the QTL on the linkage groups

[b] Random population which was not selected

[c] Selected population which was selected about low-temperature tolerance

[d] The χ^2 which was obtained by the introduced frequency of donor allele between SP and RP

[e] The *F* value which was obtained by phenotypic data and genotyping of SP

drought tolerance, and by ANOVA for low-temperature tolerance. In addition, there were 4 QTLs (Sat_331 on LG B1, Satt338 on LG C1, Satt271on LG D1b, and Satt588 on LG K) which was all detected by Chi-test in two traits. These QTLs detected by one method should contribute to drought and low-temperature tolerance.

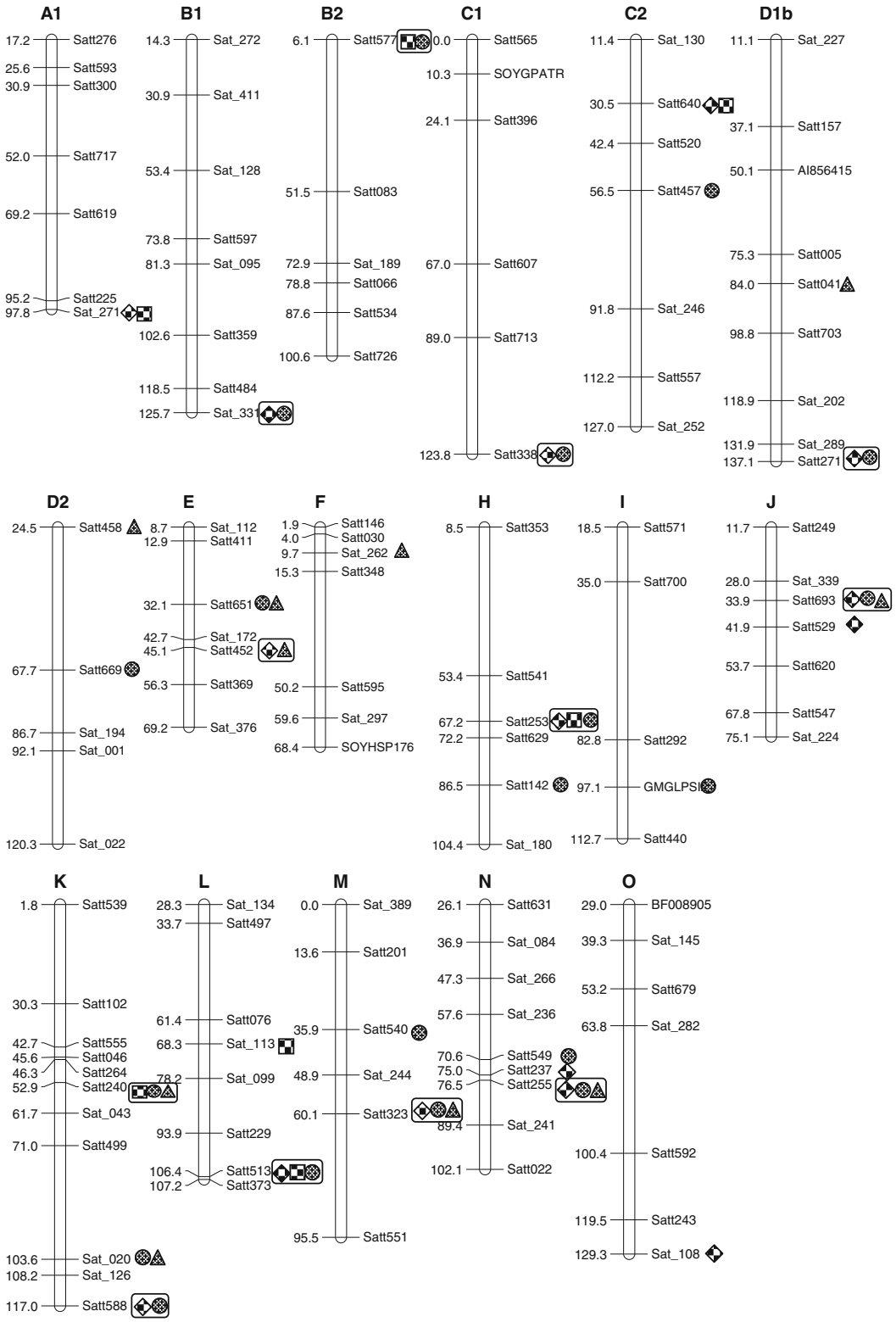
Discussion

Advantage of QTL analysis using BILs

Since the pioneer work by Eshed et al. [29, 30] and the theoretical landmark laid by Tanksley and Nelson [31], BIL progeny and similar materials such as CSSLs and NILs have been increasingly used in gene/QTL mapping studies of crop plants [32]. Although analogous to the AB-QTL (Advanced backcross QTL analysis) analysis approach [30], the forward genetics strategy of BILs was more time-saving and cost-effective than the conventional QTL mapping approach in at least two aspects [33]. First, selecting extreme phenotypes was much easier than phenotyping the whole population practiced in typical QTL mapping experiments. Second, a small number of BILs with extreme phenotypes selected from each BILs population reduces the genotyping efforts considerably. The BILs not only provide a powerful tool to identify QTLs, but also offer an efficient strategy to overcome the difficulty of QTL fine-mapping, that is reducing the noise of genetic background [34]. Two methods can be used QTL fine-mapping by BIL. One is a substitution-map-ping approach [35]. The second strategy employed that the BILs, the phenotype of whose donor parent and receptor parent exist significant difference, backcross with the receptor parent, then fine-mapping is accomplished by the obtained F_2 [30, 36]. In this study, BILs were used as the experimental material for abiotic-tolerance identification, a set of transgressive plants were screened out, and the related QTLs were analyzed by genotyping. It makes sure the fragments related to stress primarily and the BILs valuable for large-scale trait identification, QTL fine-mapping, and gene cloning in soybean.

Comparison of QTLs between drought and low-temperature tolerance with reported researches

In this study, 18 QTLs for drought tolerance and 23 QTLs for low-temperature tolerance were detected at the



QTL detected by chi-test about drought tolerance at the germination stage;

QTL detected by ANOVA about drought tolerance at the germination stage;

QTL detected by chi-test about low-temperature tolerance at the germination stage;

QTL detected by ANOVA about low-temperature tolerance at the germination stage

◀ **Fig. 1** Distribution of QTLs for drought and low-temperature tolerance on the soybean linkage map at germination stage. QTLs in rectangles mean genetic overlapping loci underlying drought and low-temperature tolerance. At the appeared QTLs there, fifteen and seven QTLs for drought tolerance in germination were detected by Chi-test and ANOVA, respectively. Nineteen and ten QTLs for low-temperature tolerance in germination were detected by Chi-test and ANOVA. Among them, 12 QTLs were both obtained by drought and low-temperature tolerance, distributed on 11 linkage groups

germination stage, respectively. The results showed some QTLs detected located in the same regions with QTLs previously reported. In this study, Satt237 on LG N which at germination stage was mapped for drought tolerance by Li Cangdong [37] and low-temperature tolerance by Jiang Hongwei [12] at same stage. Sat_271 on LG A1, affected drought tolerance, were detected for total seed weight in the chilling environment [5]. Satt253 on LG H was detected to affect low-temperature tolerance at germination stage and mapped by chilling tolerance in early stage [38]. Both Satt323 and Satt540 on LG M were located for low-temperature tolerance in this study and formal research. These QTLs regions for the related traits mentioned above, which detected in different mapping populations and various environments, were significant genomic regions for drought and low-temperature tolerance at germination stage in soybean. This will play an important role in MAS for development of drought and low-temperature tolerance variety.

Genetic overlap detection by Chi-test and ANOVA

Drought and freezing impose osmotic stress on plants. Upon exposure to osmotic stress, plants exhibit a wide range of responses at the molecular, cellular and whole plant levels [39, 40]. Therefore, there is no difficult to understand that an overlap to a considerable degree of is existed in its resistance mechanism [41]. According to the QTLs (18 for drought tolerance and 23 for low-temperature tolerance) detected by the Chi-test and ANOVA in this study, 12 QTLs were correlated with drought and low-temperature tolerance commonly, which showed a partial genetic overlap between drought and low-temperature tolerance in soybean. The similar research on genetic overlap occurred in rice. Four same or adjacent regions harboring salt tolerance QTLs were detected at the seedling and tillering stages, suggesting that partial genetic overlap of salt tolerance across the two stages occurs [24]. Three loci (QSbr6, QSbr8, and QSbr10) overlapped with the same ILs between sheath blight resistance and drought tolerance [23].

Directional selecting in BILs would lead to small population size, allele segregation distortion, so linkage mapping in traditional analysis could not be analyzed. In this study, two methods of genetic analysis were used to analyze QTLs about drought and low-temperature tolerance in BILs. One is Chi-square analysis based on "genetic hitch-hiking" effect. According to the theory of population genetics, the genetic-hitchhlking effects will cause the rise in frequencies of selection-favored alleles and the related alleles at closely linked loci. The genetic overlap are caused by both genetic hitchhiking and pleiotropy [42]. With this principle, elite materials and the related QTL should be obtained more quickly and efficiently. But for some complex trait, QTLs could not be detected thoroughly by Chi-test, which can be obtained by the analysis between genotype and phenotype by ANOVA. In this research, three more QTLs for drought tolerance and four more QTLs for low-temperature tolerance were added by ANOVA. Among the 12 genetic overlap QTLs 6 QTLs were detected by two analysis methods.

The mechanism of drought and low-temperature tolerance in crops was complicated, two analysis methods were used to detect QTLs in order to improve the reliability of the results. So the loci detected by both methods may show close correlation with drought and low-temperature tolerance of the germination stage in soybean, which lay the foundation for fine mapping of genetic overlap QTL and molecular pyramiding breeding.

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