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Quantitative trait locus (QTL) analysis of pod related traits in different environments in soybean

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Soybean is an important crop, whose most agronomic traits are quantitative inherited. Mapping of these quantitative trait loci in soybean genes is importance for various applications. A $F_{2:15}$ RIL population containing 149 lines derived from a cross between Charleston as female and Dongnong 594 as male parent were used for mapping of the QTL of pod related traits. Three agronomic traits showing clear phenotypic variations between parents were investigated and relevant QTLs were analyzed with software WindowsQTL Cartographer V2.5. The pod related traits are podwall thickness, weight of podwall, and ratio of podwall to pod (weight to weight). A total of 67 QTLs were mapped for 3 agronomic traits. Some QTLs identified under all environments tend to be valuable for soybean molecular marker assistant breeding selection.

Key words: Soybean, pod traits, QTL, different environments.

INTRODUCTION

Yield related traits in soybean are generally controlled by multiple genes and environmental dependent (Kwon and Torrie, 1964). Epigenetics of genes controlling these traits also affect the yield. Yield is complex trait; many components of yield had been studied, which assist to the selection of yield breeding (Fehr, 1987; Specht et al., 1999; Chapman et al., 2003). Genetic maps have been useful for soybean genome analysis (Zhang et al., 2004). Maps have allowed the identification of many economically important soybean genes underlying quantitative trait loci (QTL), including those for yield and disease resistance (Walker et al., 2004; Yuan et al., 2002). Use of high throughput QTL marker in assistance of breeding can shorten breeding procedure and promote the efficiency.

The pod related traits are main component of soybean yield. Except for few reports, no promising progress has achieved in this field for the past 10 years. QTLs of podwall weight and podwall thickness were studied in one introgression lines and two primary backcross populations (Li et al., 2008). Path coefficient analysis indicated that the seed number per pod had highest direct positive effect on seed yield (Oz et al., 2009). The order in terms of the effect on soybean yield is pod weight per plant > seed weight per plant > seed number per plant > 100 seed weight > podwall weight based on path coefficiency analysis (Arshad et al., 2006). Pods are involved in photosynthesis with highest efficiency at R7 (Yang et al., 2008). QTLs of one-seed pod, two-seed pod, three-seed pod and four-seed pod were studied in a RIL population line having 255 lines in two environments (Wang et al., 2007). And the QTL of seed dehiscence and hard seededness also studied in a RIL population which parent is *G. max* ssp. *Soja* (Liu et al., 2007).

In this study, one RIL (recombination inbred line) population of 149 lines was used to map three pod related traits in five years and to identify stable and major QTLs. This information will facilitate molecular breeding, molecular marker development and cloning of genes underlying yield.

MATERIALS AND METHODS

A single-seed-descent recombinant inbred lines (RILs) mapping

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Table 1. Statistical analyses of pod traits for the derived RIL population in five years.

Trait	Year	Maximum	Minimum	Mean	SD	Kurtosis	Skewness
	2006	0.65	0.22	0.50	0.09	1.21	-1.17
	2007	0.60	0.27	0.50	0.04	6.46	-1.21
podwall thickness (cm)	2008	0.46	0.28	0.41	0.02	8.70	-2.26
	2009	0.49	0.17	0.34	0.05	0.81	-0.67
	2010	0.37	0.15	0.27	0.03	0.60	-0.19
	2006	16.15	1.95	7.98	2.66	0.20	0.36
	2007	15.56	2.90	6.90	1.80	5.93	1.81
podwall weight (g)	2008	7.48	1.25	3.68	1.11	0.17	0.51
	2009	15.19	2.15	7.75	1.99	1.29	0.54
	2010	12.19	1.95	6.89	2.08	0.08	0.24
	2006	0.45	0.14	0.33	0.06	2.89	-1.38
	2007	0.46	0.15	0.32	0.03	5.96	-0.33
ratio of podwall to pod	2008	0.40	0.16	0.32	0.03	1.81	-0.45
	2009	0.48	0.21	0.35	0.05	0.89	-0.50
	2010	0.47	0.22	0.36	0.04	1.58	-0.24

population of 149 $F_{2:15}$ lines was derived from a cross between 'Charleston' (provided by Dr. Lijuan Qiu, Chinese Agricultural Academy of Science, Beijing, China) and 'Dongnong594' (developed by Northeast Agricultural University, Harbin, Heilongjiang, China). During 2006 to 2010, RILs were planted in the field of the Crop Research and Breeding Center of Land-Reclamation, Harbin (short for Harbin, longitude 126°38′ E, latitude 45°45′ N). During 2009 to 2010 (confusing for 2006 to 2010), RILs were grown in the field in Hongxinglong, 129°55′ - 134°35′ E, 45°35′ - 47°17′ N). Plants were randomly arranged in a single-row plot (1 m in length and 0.5 m in width), and trials were duplicated or triplicated local regular farming practices were followed. The RIL population was designed in duplicate during 2006 to 2007, while in triplicate during 2008 to 2010.

Measurement of soybean pod traits

Five plants in the middle of each row were randomly selected for a line. Measurement of podwall weight was performed for five plants; podwall thickness was measured thickness of ten two-seed pod wall with a vernier callipers, the average value was used to calulate the QTL.

Phenotype variance

The linkage map of RIL population was constructed by Chen (Chen et al., 2005). QTL analysis was conducted by using the QTLMapper V2.0 program based on a mixed model approach with composite interval mapping module with a LOD threshold of 2.5 (Wang et al., 1999). The linkage group was drawn by the software MapChart 2.1.

RESULTS

Phenotypic variation

All three pod related traits, pod thickness, podwall weight, and ratio of podwall to pod varied to a great extent (Table

1 and Figure 1). And all three traits were in agreement with a normal distribution by the analysis efficiency, kurtosis, and skewness. From 2006 to 2008, the value of kurtosis and skewness for podwall thickness was higher than 1.0, and reduce to less than 1.0 form 2009 to 2010; thus, suggesting that podwall thickness might be sensitive to the environments. And the value of skewness for podwall weight and ratio of podwall to pod were mainly less than 1.0, suggesting that the segregation of these two traits was normally distributed.

QTL analysis of pod traits by CIM

By the analysis of CIM, 12 QTLs of podwall thickness were identified in five linkage groups of A1, B1, C2, N, and J (Table 2 and Figure 2). The explain variance of these five QTLs ranged from 8 to 39%, with the additive effect range from -0.04 to 0.04 (Figure 1). Three QTLs were identified in 2006, a biggest one in A1 (Sat_105-Satt390) could explain 13% variance with additive effect of -0.04; other two LG C2 (Satt460-Satt202 and Satt202-Satt277) located QTLs could explain 8 and 13% variance with additive effect of 0.03 and 0.04, respectively. In 2007, one QTL on A1 (Satt300-Satt276), could explain 13% variance, with additive effect of 0.02. Two QTLs were found in 2008 on linkage group A1 and C1 (Sat_105-Satt270 and Satt509-Satt5211), which could explain 8 and 21% variance with both additive effect of 0.01. Four QTLs were found in 2009, two located on linkage group B1 (Satt197-Satt251 and Satt251-Satt229) could explain 38 and 39% variance with same additive effect of 0.04. Another two QTLs were located on linkage group C2 (Satt460-Satt134) and N (Satt551-GMABAB),
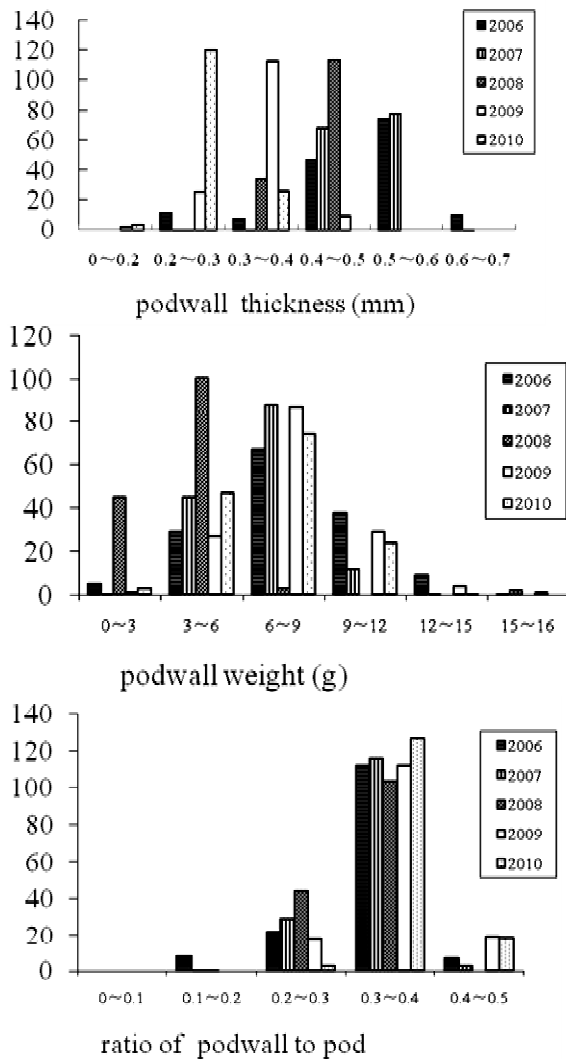
Figure 1. The distribution of pod related traits in five years

and could explain 9 and 12% variance with additive effect of 0.02 and -0.02, respectively. In 2010, two QTLs were found on linkage group A1 (Satt300-Satt587) and J (Satt457-Satt431) could explain 8 and 11% variance with both additive effect of 0.01, respectively.

Six QTLs were identified for podwall weight, located on linkage group C2, D1a, F, and G. The explain variance range from 7 to 13% and additive range from 0.31 to

1.49. In 2006, three QTLs were found on linkage group D1a (Sat_062-Satt370; Satt370-Satt198) and G (Satt505-Satt288), could explain 17, 13 and 8% variance and additive effect were 0.35, 1.49 and 0.80, respectively. Only one QTL was found in 2007, 2008 and 2010, respectively. Three QTL located on linkage group C2 (Sat_188-Satt335), D1a (Satt383-Satt402) and F (Satt269-Satt538), could explain 7, 7 and 10% variance

Table 2. QTL (CIM) analysis of pod traits in soybean.

Trait	Year	QTLs	Chromosome Interval		LOD	Explainable variation (%)	Additive effect
		Qspwt1	A1	Sat_105(2.3)-Qspwt1-(5.1)Satt390	5.1	13	-0.04
	2006	Qspwt2	C2	Satt460(0)-Qspwt2-(2.9)Satt202	3.5	8	0.03
		Qspwt3	C2	Satt202(2.5)-Qspwt3-(0.9)Satt277	4.2	10	0.04
	2007	Qspwt4	A1	Satt300(3.0)-Qspwt4-(2.7)Satt276	4.5	13	0.02
	2008	Qspwt1	A1	Sat_105(4.0)-Qspwt1-(0.9)Satt270	2.7	8	0.01
podwall thickness (cm)		Qspwt5	B1	Satt509(6.4)-Qspwt5-(26.5)Satt5211	5.3	21	0.01
		Qspwt6	B1	Satt197(31.3)-Qspwt6-(16.6)Satt251	2.6	38	0.04
	2009	Qspwt7	B1	Satt251(13.5)-Qspwt7-(43.8)Satt229	2.7	39	0.04
		Qspwt2	C2	Satt460(1.8)-Qspwt2-(2.3)Satt134	3.4	9	0.02
		Qspwt8	N	Satt551(17.2)-Qspwt8-(2.6)GMABAB	3.9	12	-0.02
	2010	Qspwt4	A1	Satt300(3.4)-Qspwt4-(3.5)Satt587	3.2	8	0.01
		Qspwt9	J	Satt457(11.7)-Qspwt9-(4.0)Satt431	2.7	11	0.01
		Qspww1	D1a	Sat_062(6.8)-Qspww1-(2.1)Satt370	5.7	17	0.35
	2006	Qspww2	D1a	Satt370(5.3)-Qspww2-(2.9)Satt198	5.3	13	1.49
		Qspww3	G	Satt505(6.9)-Qspww3-(16.1)Satt288	3.1	8	0.80
podwall weight (g)	2007	Qspww4	C2	Sat_188(2.3)-Qspww4-(0)Satt335	2.6	7	0.64
	2008	Qspww5	D1a	Satt383(0.2)-Qspww5-(3.7)Satt402	2.5	7	0.31
	2010	Qspww6	F	Satt269(3.2)-Qspww6-(18.2)Satt538	3.6	10	0.80
	2006	Qsrpwp1	D1a	Satt203(9.6)-Qsrpwp1-(13.6)Satt273	2.6	7	0.02
	2008	Qsrpwp2	F	Satt252(10.6)-Qsrpwp2-(14.4)Satt538	4.5	13	-0.01
ratio of podwall to pod		Qsrpwp3	D1b	Satt141(6.7)-Qsrpwp3-(14.9)Satt428	2.5	6	-0.01
	2009	Qsrpwp4	N	Satt551(9.7)-Qsrpwp4-(1.5)GMABAB	5.4	13	-0.02
		Qsrpwp5	N	GMABAB(3.9)-Qsrpwp5-(4.1)Sat_091	4.6	17	-0.02
	2010	Qsrpwp6	H	Satt191(8.1)-Qsrpwp6-(17.3)Satt293	2.5	12	0.01

and additive were 0.64, 0.31 and 0.80. No QTL was found in 2009.

Six QTLs were identified for ratio podwall to pod, these QTLs located on linkage group D1a, D1b, F, H, and N. The explain variance ranged from 6 to 17% and additive effect ranged from -0.02 to 0.01.

In 2006, 2008 and 2009 year, only one QTL was identified, respectively. These three QTLs were located on linkage group D1a (Satt203-Satt273), F (Satt252-Satt538) and H (Satt191-Satt293), explain 7, 13 and 12% variance and additive effect were 0.02, -0.01 and 0.01, respectively. In 2009 year, three QTLs were identified on linkage group D1b (Satt141-Satt428 and Satt551-GMABAB) and N (GMABAB-Sat_091) could explain 6, 13 and 17% variance and additive effect were -0.01, -0.02 and 0.02, respectively.

QTL analysis of pod traits by MIM

By the analysis of CIM, 6 QTLs of podwall thickness were identified in linkage group A1, B1, C2, and N, additive effect ranged from -0.04 to 0.04 (Table 3; Figures 1 and 2). In 2006, three QTLs were found, located on the linkage
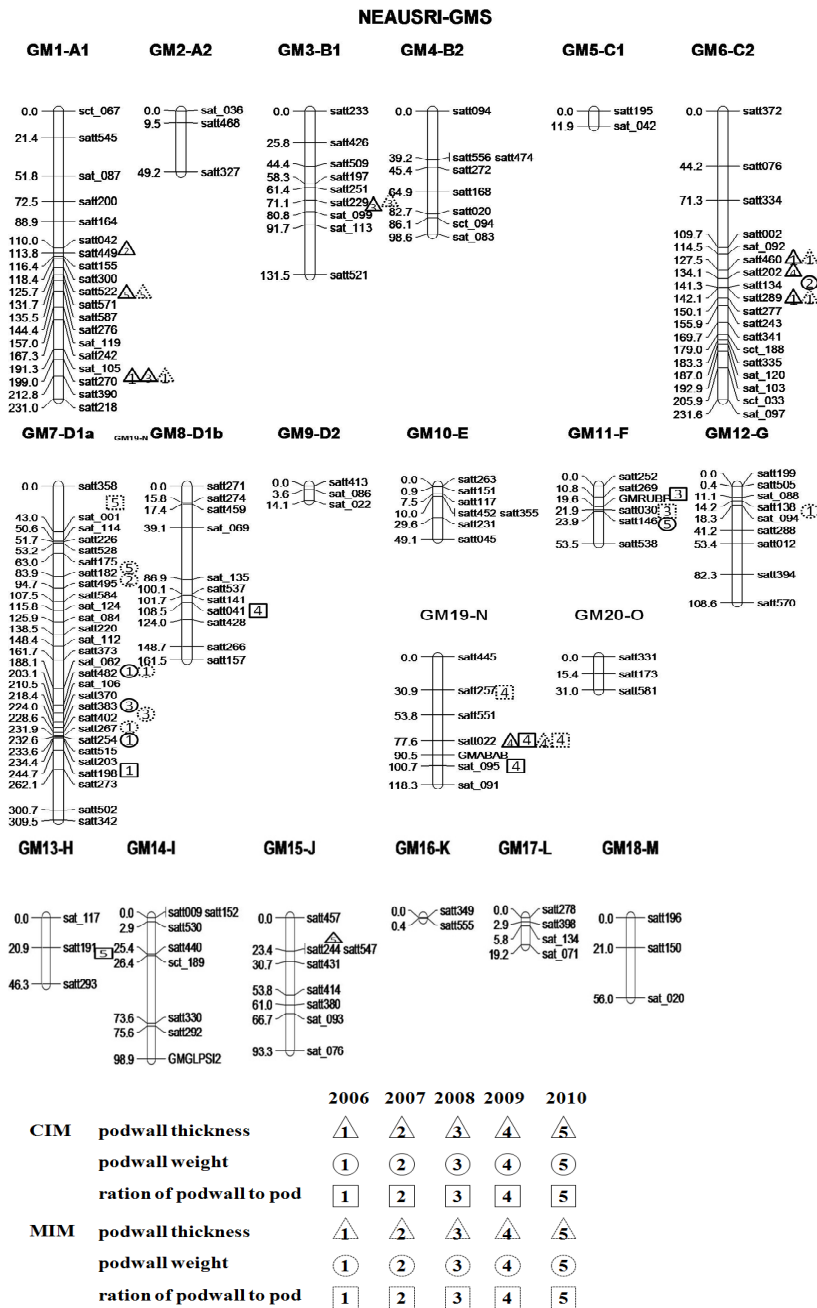
Figure 2. The distribution of QTLs of pod related traits in different linkage group.

Table 3. QTL (MIM) analysis of pod traits in soybean.

Traits	Year	QTLs	Chrom	Interval	LOD	Additive effects
podwall thickness (cm)	2006	Qspwt1	A1	Sat_105(1.6)-Qspwt1-(4.1)Satt390	5.5	-0.04
		Qspwt2	C2	Satt202(3.0)-Qspwt2-(0.6)Satt277	4.0	0.04
		Qspwt3	C2	Sat_092(10.6)-Qspwt3-(4.6)Satt202	2.7	0.03
	2008	Qspwt4	B1	Satt426(30.0)-Qspwt4-(43.3)Satt197	11.2	0.04
	2009	Qspwt5	N	Satt551(15.0)-Qspwt5-(4.4)GMABAB	5.1	-0.04
	2010	Qspwt6	A1	Satt300(1.7)-Qspwt6-(0.7)Satt587	4.2	0.01
podwall weight (g)	2006	Qspww1	D1a	Sat_062(10.3)-Qspww1-(3.7)Satt370	4.6	-1.41
		Qspww2	D1a	Satt370(2.4)-Qspww2-(1.0)Satt198	5.3	1.60
		Qspww3	G	Satt505(6.4)-Qspww3-(15.1)Satt288	3.2	-0.73
	2007	Qspww4	A1	Satt200(11.7)-Qspww4-(17.7)Satt042	0.8	-4.20
	2008	Qspww5	D1a	Satt383(1.3)-Qspww5-(0.7)Satt267	2.7	0.40
	2010	Qspww6	D1a	Satt175(5.2)-Qspww6-(5.3)Satt182	2.7	-0.92
ratio of podwall to pod	2008	Qsrpwp1	F	Satt030(1.0)-Qsrpwp1-(1.0)Satt146	2.5	-0.01
	2009	Qsrpwp2	N	Satt445(22.7)-Qsrpwp2-(12.4)Satt551	3.6	-0.02
		Qsrpwp3	N	Satt551(10.7)-Qsrpwp3-(0.5)GMABAB	4.6	0.01
	2010	Qsrpwp4	D1a	Sat_106(5.2)-Qsrpw 4-(4.1)Satt383	2.8	-0.01

group A1 (Sat_105-Satt390 and Satt202-Satt277) and C2 (Sat_092-Satt202), the additive effect were -0.04, 0.04 and 0.04, respectively. There had no QTL was identified in 2007, but in 2008, 2009 and 2010 year, one QTL was identified in each year. In 2008, the QTL found on linkage group B1 (Satt426- Satt197), additive effect was 0.04. In 2009 and 2010, these two QTLs were located on linkage group N (Satt551- GMABAB) and A1 (Satt300-Satt587), additive effect were -0.04 and 0.01.

For the podwall weight, six QTLs were identified on linkage group A1, D1a, and G, additive effect ranged from -4.2 to 1.6. Three QTLs were identified on linkage group D1a (Sat_062-Satt370 and Satt370-Satt198) and G (Satt505-Satt288), additive effect were -1.41, 1.60 and -0.73, respectively. Only one QTL was identified in each year of 2007, 2008 and 2010, located on linkage group A1 (Satt200-Satt042 and Satt383-Satt267) and D1a (Satt175-Satt182), additive effect were -4.2, 0.40 and -0.92, respectively.

Four QTLs of ratio podwall to pod were identified on linkage group D1a, F and N, additive effect were -0.02 to 0.01. In 2008 and 2010 year, only one QTL was identified in each year on linkage group F (Satt030-Satt146) and D1a (Sat_106-Satt383), additive effect both was -0.01.

Two QTLs were identified in 2009 on linkage group N (Satt445-Satt551 and Satt551-GMABAB), additive effect were -0.02 and 0.01.

DISCUSSION

In this study, the pod related traits were studied in five years. The pod traits are key component of soybean yield, and have strong effect on insect resistance, especially for the podwall thickness (Wang et al., 2007). It was the first time; these traits were analyzed by QTL in five years. These QTL sites that we found could supply for valuable information for molecular assistant breeding for soybean pod and selection during breeding. For soybean, podwall thickness also significantly negatively correlated with the degree of pod-shattering (Tiwari et al., 1995). So, using the QTLs of pod thickness to assist, solve the problem of pod-shattering, would open a new way for breeding.

Several stable QTLs sites were mapped for the podwall thickness in different environments, mainly located on linkage group A1, where some QTLs of yield also located in these sites (Han et al., 2008). Some QTL is environ-

mental dependent, major genetically controlled QTL could be identified easily (Huang et al., 1997). Some major QTL could be found in different environments while some QTLs only can be detected in one or two specific environment. Genotype×environment interactions are very important to the expression of QTL, QTLs with these characters are important for assistant breeding in different environments (Zhuang et al., 1997). The QTL on the linkage group N (Satt022- GMABA) was identified in two different environments, where QTLs of podwall thickness and ratio podwall to pod, and the QTL of seed length also were identified (Salas et al., 2006). Key QTL position could provide promising evidence for gene mining. Two QTLs of podwall thickness on B1 (Satt197-Satt251 and Satt251-Satt229) had a high explain efficiency of 38 and 39%, respectively. In the proximate positons, QTLs of oil content, seed weight, chilling resistance and yield also were located (Cui et al., 2008; Funatsuki et al., 2005; Qi et al., 2011). In these regions, the key gene was found or the gene cluster underlying various QTLs.

Analysis of QTLs in different environments at the same time could increase repeatability and accuracy for the QTLs identified. By analysis of the QTLs of podwall thickness, podwall weight and ratio podwall to pod, total 67 QTLs have been identified. On linkage group D1a, ten QTLs were found by CIM and MIM method at the same time, and on linkage group A1, D1b and H only one QTL was identified, respectively. Some linkage groups which had no QTL was identified, that is A2, B2, C1, D2, E, I, K, L, M and O.

QTLs of podwall weight and podwall thickness had the character of agminated distribution, which is helpful in identifying the true QTLs. One explanation to this phenomenon may be due to the major gene effect making it independent of environment. Another explanation may be due to a cluster of genes in this region underlying the pod-related traits. More experiment is needed to understand this phenomenon precisely. The parents used in this study were semi-dwarf cultivar Charleston and high protein line Dongnong594, with great phenotypic variations in terms of quality, yield and agronomic traits, which might be beneficial to QTL identification (Chen et al., 2007).

Acknowledgments

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