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QTL mapping for the number of branches and pods using wild chromosome segment substitution lines in soybean [*Glycine max* (L.) Merr.]

Qingyuan He[1,2†], Hongyan Yang[1†], Shihua Xiang[1], Wubing Wang[1], Guangnan Xing[1], Tuanjie Zhao[1*] and Junyi Gai[1*]

[1]*Soybean Research Institute, National Center for Soybean Improvement, MOA Key Laboratory for Biology and Genetic Improvement of Soybean (General), National Key Laboratory for Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing, Jiangsu 210095, People's Republic of China and* [2]*Life Science College of Anhui Science and Technology University, Fengyang, Auhui 233100, People's Republic of China*

Abstract

Annual wild soybean characterized with more number of branches and pods may contain favourable exotic genes/alleles for improving the yield potential of cultivated soybeans. To evaluate the wild alleles/segments, the chromosome segment substitution line population *SojaCSSLP3* comprising 158 lines with *N24852* (wild) as the donor and *NN1138-2* (cultivated) as the recurrent parent was tested under three environments. The phenotypic data along with 198 simple sequence repeat markers were analysed for qualitative trait loci (QTL)/segments associated with the number of branches on the main stem (BN) and number of pods per plant (PN) using the inclusive composite interval mapping procedure (RSTEP-LRT-ADD model) of ICIM version 3.0. The analysis was carried out for individual environments due to a significant G × E interaction. A total of eight QTL/segments associated with BN and eight QTL/segments associated with PN were detected under the three environments, with all the wild segments having positive effects. Among these, two QTL/segments for each of the two traits could be detected under two or three environments and three QTL/segments could be detected for both traits. Four QTL/segments associated with BN and one QTL/segment associated with PN were identified only in *SojaCSSLP3*, not reported for cultivated crosses in the literature. The detected wild segments may provide materials for further characterization, cloning and pyramiding of the alleles conferring the two traits.

Keywords: chromosome segment substitution lines; number of branches on the main stem; number of pods per plant; QTL/segment detection; wild soybean (*Glycine soja* Sieb. et Zucc.)

Introduction

The wild soybean (*Glycine soja* Sieb. et Zucc.) is known to be the closest undomesticated relative, even wild ancestor, of cultivated soybean [*Glycine max* (L.) Merr.], which is thought to be an important gene source for the improvement of cultivated soybean, especially with respect to stress tolerance and yield-related factors, such as high numbers of nodes, branches and pods (Concibido *et al.*, 2003). It is known that transferring these elite genes/alleles is difficult by using only phenotypic judgement. However, with the assistance of molecular markers, it becomes much easier to pyramid genes/alleles from different sources, even wild relatives.

* Corresponding authors. E-mail: tjzhao@njau.edu.cn; sri@njau.edu.cn
† These authors contributed equally to this study.

The molecular marker-assisted selection for crops has permitted a systematic and efficient approach to introgression experiments of wild germplasm (Tanksley and McCouch, 1997).

A chromosome segment substitution line (CSSL) population is developed from a continuously backcrossing procedure, with each line carrying a single (a few) chromosome segment from a donor genotype in the genetic background of the recurrent parent (Eshed and Zamir, 1994). The establishment of CSSLs has provided materials to study the genetics of individual segments of the donor parent, including detection of the qualitative trait loci (QTL)/alleles on a segment. Wang *et al.* (2013) developed a CSSL population designated as *SojaCSSLP1* using *N24852* (wild) as the donor and *NN1138-2* (cultivated) as the recurrent parent and then used it to detect the wild QTL/segments for seed and growth traits.

In the wild soybean, the number of branches on the main stem (BN) and the number of pods per plant (PN) are greater than those in cultivated soybeans and therefore might be potential merits in the improvement of soybean canopy and yield as the number and distribution of branches can determine the canopy architecture for light interception as well as lodging resistance and the pod number may directly influence the seed yield (Asanome and Ikeda, 1998; Foroutan-pour *et al.*, 1999; Wang *et al.*, 2004; Li *et al.*, 2008a; Hao *et al.*, 2012). The objective of this study was to identify the QTL/segments associated with BN and PN using an improved CSSL population based on Wang's *SojaCSSLP1* line.

Materials and methods

Plant materials and trait evaluation

Through substitution and addition of lines and markers to make the wild segment size and distribution more reasonable, *SojaCSSLP1*, along with its derivatives *SojaCSSLP2*, was improved and renamed as *SojaCSSLP3* comprising 158 lines with 198 simple sequence repeat (SSR) markers. The genetic constitutions of the lines are shown in Fig. S1 (available online). The trials were carried out in three environments, including Jiangpu Station of Nanjing Agricultural University in 2010 (designated as E1) and in 2011 (E2) and Experiment Farm of Anhui Science and Technology University in 2011 (E3). A randomized complete-block design was used for the trials, and all lines were planted with three replications, with each line being planted in three rows per plot and with each row being 200 cm long and 50 cm apart. Two traits, BN and PN, were evaluated at maturity in four plants in the middle row of each plot in all environments.

SSR markers and genetic linkage map

A total of 198 pairs of SSR primers with polymorphism between the two parents were selected from SoyBase (http://soybase.com/resources/ssr.php) and used to determine the genotypes of the 158 lines. The genetic linkage map reported by Song *et al.* (2004) was used in the present study.

Identification of wild QTL/segments for BN and PN

The analysis of variance revealed a very significant genotype × environment interaction; therefore, the QTL/segment detection was carried out for individual environments using the inclusive composite interval mapping method (RSTEP-LRT-ADD model) of ICIM version 3.0 (Li *et al.*, 2007) and checked with multiple comparisons among the lines significantly different from *NN1138-2*. Thresholds for QTL detection were set as $P = 0.05$ and calculated using 1000 permutations.

Results and discussion

Phenotypic analysis of BN and PN

The frequency distributions of BN and PN in *SojaCSSLP3* varied widely within and among the environments (Table 1). The population means were similar to or slightly greater than those of *NN1138-2*, which indicates that most of the genotypes of lines had recovered to the recurrent parent and the individual donor segments had improved the BN and PN of the lines. (Table S1, available online). The analysis of variance revealed that the genotype, environment and genotype × environment interaction were all significant ($P < 0.05$). The performance of certain CSSLs was significantly different from that of *NN1138-2* with respect to BN and PN in different environments, respectively (Fig. S2, available online).

Identification of wild QTL/segments for BN and PN

A total of four, five and five wild QTL/segments associated with BN and five, four and five QTL/segments associated with PN were identified in E1, E2 and E3, respectively. The genotypes of these loci were further analysed through multiple comparisons among the CSSLs significantly different from *NN1138-2* (Fig. S2, available online). Their phenotypic variance explained, additive effect and logarithm of the odd score are given in Table S2 (available online). Over the three environments, a total of eight wild

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Table 1. Comparison of qualitative trait loci (QTL)/segments found to be associated with the number of branches on the main stem (BN) and number of pods per plant (PN) in this study with those reported in the previous literature

QTL	Chr. (LG)	E	Linked marker	Position[a] (cM)	Recurrent	Donor	Reference
BN							
qBn1-1	1 (D1a)	E1, E2 and E3	Sat_160	104.28	*NN1138-2*	*N24852*	This study
			Sat_160–Satt129	104.28–109.27	*NN1138-2*	*N24852*	Wang *et al.* (2012)
qBn2-1	2 (D1b)	E1, E2 and E3	Satt274	116.35	*NN1138-2*	*N24852*	This study
			Satt274	116.35	*NN1138-2*	*N24852*	Wang *et al.* (2012)
qBn4-1	4 (C1)	E1	Satt565	0.00	*NN1138-2*	*N24852*	This study
			Satt396–Satt578	24.10–65.10	–	–	Tan *et al.* (2013)
qBn6-1	6 (C2)	E1	Satt643	94.65	*NN1138-2*	*N24852*	This study
			Satt643–Satt286	94.65–101.75	–	–	Tan *et al.* (2013)
			Satt460	117.77	Charleston	Dongnong594	Liu *et al.* (2007)
			FT1	111.30–113.30	Tokei 758	To-8E	Sayama *et al.* (2010)
qBn6-2	6 (C2)	E2	Satt316	127.67	*NN1138-2*	*N24852*	This study
			Satt307–Satt376	97.83–121.27	*Jidou12*	*Jihuang13*	Jiang *et al.* (2011)
			Satt365–Satt134	111.68–112.84	–	–	Tan *et al.* (2013)
qBn9-1	9 (K)	E3	Satt337	47.38	*NN1138-2*	*N24852*	This study
qBn10-1	10 (O)	E2 and E3	Satt243	119.50	*NN1138-2*	*N24852*	This study
			Satt581–Satt331	93.37–106.02	*PI171451*	*Hwaeomputkong*	Li *et al.* (2008b)
			Satt345–Sat_038	59.43–112.17	–	–	Tan *et al.* (2013)
qBn14-1	14 (B2)	E2 and E3	Satt318	70.12	*NN1138-2*	*N24852*	This study
PN							
qPn1-1	1 (D1a)	E1	Satt531–Satt320	40.87–46.80	*NN1138-2*	*N24852*	This study
			Satt320	46.80	Jinda52	Jinda57	Yang and Li (2010)
			BARC-043191-08550		Multi-parents		Hao *et al.* (2012)
qPn2-1	2 (D1b)	E3	Satt274	116.35	*NN1138-2*	*N24852*	This study
			Satt579–Satt604	74.21–75.94	*JPYSD-5*	*NN06-17*	Kan *et al.* (2012)
qPn4-1	4 (C1)	E1	Satt646	70.52	*NN1138-2*	*N24852*	This study
			Satt139–Satt476	74.50–85.70	*BARC-8*	*Garimpo*	Vieira *et al.* (2006)
			Satt578–Sat_357	65.08–76.43	*Zhong dou 29*	*Zhong dou32*	Wang *et al.* (2007)
qPn10-1	10 (O)	E2 and E3	Satt243	119.50	*NN1138-2*	*N24852*	This study
			Satt173–Satt581	58.40–106.03	Charleston	Dongnong594	Chen *et al.* (2007)
			Satt592–Sat_274	100.38–107.58	Kefeng No.1	NN1138-2	Zhou *et al.* (2010)
			Satt153–Sat_190	118.14–129.80	*JPYSD-5*	*NN06-17*	Kan *et al.* (2012)
			BARC-015003-01948		Multi-parents		Hao *et al.* (2012)
qPn12-1	12 (H)	E1, E2 and E3	Satt434	105.74	*NN1138-2*	*N24852*	This study
			Sat_175	83.19	*Zihua4*	*Yuanbaojin*	Qin *et al.* (2010)
qPn13-1	13 (F)	E2	Satt335	77.70	*NN1138-2*	*N24852*	This study
			BARC-042035-08159		Multi-parents		Hao *et al.* (2012)
qPn14-1	14 (B2)	E1, E2 and E3	Satt318	70.12	*NN1138-2*	*N24852*	This study
			BARC-041815-08101		Multi-parents		Hao *et al.* (2012)
qPn16-1	16 (J)	E1 and E3	Sat_339	27.97	*NN1138-2*	*N24852*	This study
			Satt596–Satt622	39.63–42.25	*Nannong94-156*	*Bogao*	Zhang *et al.* (2010)
			Satt529–Satt604	41.90–74.21	*JPYSD-5*	*NN06-17*	Kan *et al.* (2012)

Chr., chromosome; LG, linkage group; E, environment; E1, Jiangpu, 2010; E2, Jiangpu, 2011; E3, Fengyang, 2011.
[a] Position of the QTL in a linkage group based on the consensus map (Song *et al.*, 2004).

QTL/segments were associated with BN and PN, respectively. For BN, two QTL/segments (*qBn1-1* and *qBn2-1*) were identified in all the three environments, two in two environments and four in one environment. For PN, two QTL/segments (*qPn12-1* and *qPn14-1*) were identified in all the three environments, two in two environments and four in one environment (Table 1). Among the detected QTL/segments, three were associated with both traits (*qBn2-1* and *qPn2-1*, *qBn10-1* and *qPn10-1*, and *qBn14-1* and *qPn14-1* in Table 1 and Fig. 1). However, the total contribution of the detected QTL/segments in an environment was only 8.08–13.81% for BN and 5.72–11.61% for PN, while the corresponding heritability was 68.10–75.66% and 82.24–95.47%, respectively. There is a large part of the genetic variation that is not explained by the detected QTL/segments, and this might be due to undetected minor QTL on other segments.

Comparisons of the detected QTL/segments with those reported in the literature

The previous QTL mapping results in soybeans were mostly obtained from intraspecific populations. In the present study, *SojaCSSLP3*, an interspecific CSSL population, was used for identifying allelic variation in *G. soja*, not present in cultivated soybeans. On comparing the results of the present study with those reported in the literature, four (*qBn1-1*, *qBn2-1*, *qBn9-1* and *qBn14-1*) of the eight QTL/segments associated with BN were found to be not yet reported and to be newly detected in *SojaCSSLP3*. Among the eight QTL/segments associated with PN, *qPn2-1* might also be a new one in *SojaCSSLP3*, as its location was far from that reported in the literature (Table 1). In the present study, some loci were found to express allelic differentiation only in the

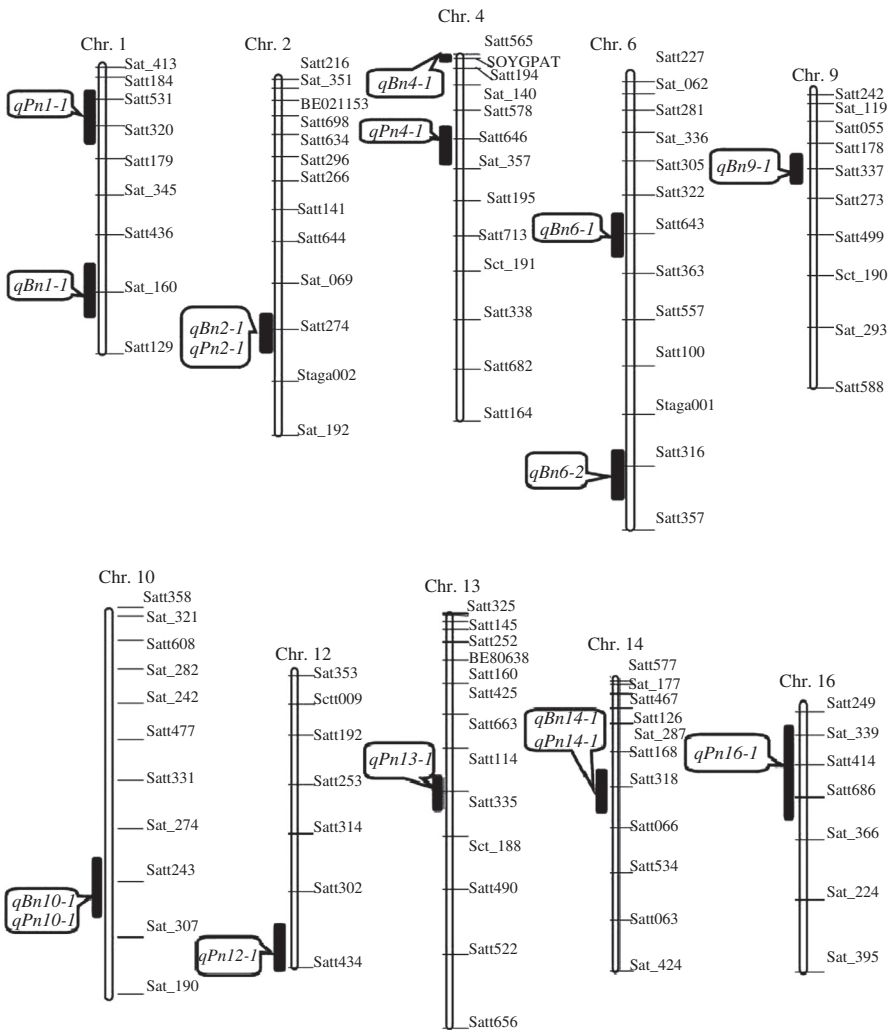

Fig. 1. Distribution of qualitative trait loci (QTL)/segments associated with number of branches per plant (BN) and number of pods per plant (PN).

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cross between *G. max* and *G. soja* and some loci in crosses between *G. max* and *G. soja* and between *G. max* and *G. max*; this implies that the former loci have not differentiated among *G. max* lines yet and remain at the early stage of domestication.

Potential utilization

Previous studies have demonstrated that the high-yielding soybean has more branches and pods than ordinary cultivars under both high and low planting densities (Ao *et al*., 2013). We expected to identify a positive allelic variation in BN and PN in *G. soja* to improve the canopy structure, plant growth and yield of the cultivated soybeans by using this CSSL population. Fortunately, all the detected wild alleles of BN and PN exerted positive effects (Table S2, available online) and there were potential choices for selecting elite wild alleles for breeding programmes as these varied greatly in the population. Among the detected QTL/segments, the utilization of those repeatedly identified over environments and conferring both traits should be prioritized. Furthermore, the detected wild segments may provide materials for further characterization, cloning and pyramiding of the alleles of the two traits.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262114000495

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