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A genetic composition analysis of soybean sibling varieties Jidou17 and Ji nf58

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Abstract

The purpose of this study was to reveal the genetic compositions of soybean varieties under large-scale cultivation and their cornerstone breeding lines and to provide information for the more effective utilization of the soybean germplasm in Chinese breeding programs. We examined the genetic compositions of the sibling varieties Jidou17 and Ji nf58, the maternal line Hobbit, and the paternal line Zao5241 and evaluated the genetic contributions from the parental lines to the offspring varieties. The genetic backgrounds of Jidou17, Ji nf58, and the parental lines were analyzed by using 408 pairs of simple sequence repeat (SSR) primers that were distributed among 20 soybean linkage groups. Our study showed that 197 loci were polymorphic between the parental lines, whereas 211 loci were non-polymorphic. Differences in the genetic backgrounds between Jidou17 and Ji nf58 were primarily reflected by genetic loci variations in the linkage groups A1, B2, and K. The genetic contribution rates of the maternal line Hobbit and the paternal line Zao5241 to Jidou17 were 79.3% and 20.7%, respectively. In 17 of the 20 linkage groups, the maternal line Hobbit contributed more genetic loci to Jidou17 than the paternal line Zao5241. The large genomic segments that mapped to linkage groups G and J were passed down from Hobbit to Jidou17. The genetic contribution rates of Hobbit and Zao5241 to Ji nf58 were 58.9% and 41.1%, respectively. Hobbit contributed more genetic loci to Ji nf58 than did Zao5241 in 12 linkage groups. Large genomic segments belonging to linkage groups C1 and J were passed down from Hobbit to Ji nf58, whereas large fragments in linkage group A1 were passed down from Zao5241. These genetic segments were located in previously mapped quantitative trait loci (QTL) that controlled important soybean traits, including yield, quality, adaptability, and disease resistance. Our results explain the reasons for agronomic trait formation in Jidou17 and Ji nf58 at the molecular level.

Keywords: soybean, Jidou17, Ji nf58, SSR, genetic composition.

Abbreviations: SSRs_simple sequence repeats; RAPD_random amplified polymorphic DNA; iTRAQ_isobaric tags for relative and absolute quantitation; QTL_quantitative trait locus; LG_linkage group; SDS_sodium dodecyl sulfate; PH_plant height; NN_number of nodes on main stem; SNP_seed number per plant; 100-SW_100-seed weight; SWP_seed weight per plant; YP_yield per ha; O_oil content; P_protein content.

Introduction

Jidou17 and Ji nf58 are elite soybean cultivars bred by crossing the maternal Hobbit line with the paternal line Zao5241 (Li and Zhang, 2006). In comparison with the parental lines, Jidou17 and Ji nf58 exhibit significantly increased yields, oil content, and resistance to abiotic stresses. In Chinese national regional trials, Jidou17 has achieved an average yield greater than 3750 kg per Ha for three consecutive years, and it is the highest yielding soybean variety in the history of the national soybean regional trials. During the regional trial in Hebei Province, the average yield for Jidou17 has increased by 33.11% in two years compared with control varieties, which is the largest yield increase in the history of the regional trials. High-yield experts have found that Jidou17 produces a yield of 4510 kg per Ha, which exceeds the national standards for super high-yielding varieties. Ji nf58 is the only high-oil soybean variety in the national regional trials that has had a stable fat content greater than 23% for 3 consecutive years. An investigation of the genetic contribution from Hobbit and Zao5241 to Jidou17 and Ji nf58 at the molecular level is necessary to guide future breeding projects. In previous studies, researchers have examined the genetic contributions of soybean parental varieties to their offspring with regard to aspects of pedigree lineage, molecular markers, and protein expression. For example, Gai et al. (1998a) analyzed the parental compositions of Chinese soybean cultivars released from 1923–1995 on the basis of coancestry coefficients. These authors examined 348 parental ancestors for the 651 soybean cultivars, including 347 nuclear and 214 cytoplasmic ancestors. Among these ancestors, 38 were shown to provide the most significant genetic contributions to the cultivars in China and in the three large ecological zones of China (Gai et al., 1998b). Xiong et al. (2008) examined the pedigrees of 1300 Chinese soybean cultivars released between 1923 and 2005, retrospectively tracked their ancestors, calculated the values of the nuclear and cytoplasmic genetic contributions of each ancestor, and identified 46 important ancestors of the soybean cultivars released during this period. Zhang et al. (2003) examined the genetic diversity and relationships between 12 award-winning soybean varieties and their ancestors by using 60 pairs of simple sequence repeats (SSRs) and found that the coancestry coefficients calculated based on pedigree analysis were significantly correlated with the genetic similarity coefficients acquired using SSRs. Guan et al. (2009) examined the genetic composition of the elite soybean variety Hefeng 25 using SSR markers and analyzed the mechanism by which parental varieties contributed to the genetic compositions and high purities of the progenies. Qin et al. (2006a) investigated the genetic recombination of Suinong14 and its ancestors by using 139 pairs of SSR markers (Qin et al., 2006b) and discovered that certain agronomic traits could be disseminated between generations (Qin et al., 2006a; Qin et al., 2010a; Qin et al., 2010b). Zheng et al. (2000) analyzed the genetic compositions of progeny lines derived from crossing of wild soybeans with cultivated soybeans by using random amplified polymorphic DNA (RAPD) technology to examine the proportion of parental genetic materials in the genomes of the progenies, which provided theoretical guidance for soybean breeding. Using iTRAQ combined with an antibody method, Qin et al. (2013a) studied the similarities in protein expression patterns between Jidou17 and its parental lines. These investigators showed that the similarities in protein expression patterns between Jidou17 and the maternal Hobbit line were greater than those between Jidou17 and the paternal Zao5241 line and those between Hobbit and Zao5241. They also showed that the maternal Hobbit line had greater genetic contributions to Judou17 (Hu et al*.*, 2008). Hobbit is a semi-dwarf soybean variety that is specifically used in the American solid-seeded semi-dwarf soybean system. Hobbit has high yield and high oil content characteristics. Zao5241 is an elite soybean variety with early maturation traits. Both varieties are elite parental lines with high combining abilities, and both have been bred into a number of varieties and strains (Hu et al., 2009; Wang et al., 2003; Guo et al., 2004; Guo et al., 2005; Hu et al., 2001; Hu, 2007; Wang et al., 2010; Wu et al., 2010). However, research regarding parental contributions during breeding is scarce, and there has been no report on the genetic contributions of Hobbit and Zao5241 to offspring varieties at the molecular level. SSRs possess a number of attractive features, including their abundance, wide distribution throughout the genome, high polymorphic information content, and simple and convenient detection. SSRs are widely used in the genome mapping of animals and plants, linkage analyses, kinship identification, genetic diversity assessments, phylogenetic tree construction, marker-assisted selection, and many other applications. In the present study, we examined Jidou17, Ji nf58, and their parental lines by using 408 pairs of SSRs distributed among 20 soybean linkage groups; analyzed the genetic backgrounds of the maternal Hobbit line, the paternal Zao5241 line, and the sibling lines Jidou17 and Ji nf58; explored the genetic differences between the sibling lines; tracked the origins of the genetic components in the sibling lines Jidou17 and Ji nf58; identified the genetic contributions of the parental lines Hobbit and Zao5241 to the sibling lines; and provided guidance for the cultivation of new soybean varieties.

Results

Differences in the genetic compositions and genetic backgrounds of Jidou17 and Ji nf58

Jidou17, Ji nf58, the maternal Hobbit line, and the paternal Zao5241 line were examined by using 408 pairs of SSR primers. The results showed that there were 197 polymorphic loci between the parental lines, and the average rate of polymorphism among the 20 linkage groups was 48.3%. The polymorphism rates in the linkage groups ranged from 20.0% to 72.7%. The polymorphic loci in linkage group J reached 72.7%, whereas only 20% of loci were polymorphic in linkage group H. The average rate of conserved loci in the 20 linkage groups was 51.7%. To determine whether the conserved loci were randomly distributed in each linkage group, we conducted a $\chi 2$ test. The result showed that the distribution of conserved loci in each linkage group was not random ($\chi 2 = 32.81$, $P \leq 0.05$). The level of conserved loci in each linkage group ranged from 27.3% to 77.5%. The level of conserved loci in linkage group C2 reached 77.5%, whereas the level in linkage group J was only 27.3% (Table 1). Moreover, there was no correlation between the polymorphic loci and conserved loci in each linkage group. However, there was a relationship between the non-randomness of the polymorphic loci and the conserved loci among linkage groups and trait selection during the breeding process (Li et al., 2000). The genetic similarity coefficient between the soybean sibling lines Jidou17 and Ji nf58 was 0.7623, indicating that there are genetic differences between these two lines. The percentages of loci with identical allelic variations in Jidou17 and Ji nf58 ranged from 47.6% to 94.4%. The percentages in 15 of the 20 linkage groups were greater than 60.0%, and the percentages in linkage groups F, H, and L were 94.4%, 93.3%, and 93.3%, respectively (Table 2). Relatively higher percentages were found in linkage groups A1, K, and B2, which had values of 52.4%, 46.7%, and 42.9%, respectively (Table 2). These results indicated that there were significant differences in the above linkage groups between Jidou17 and Ji nf58. A total of 197 polymorphic loci were analyzed, and the results showed that the genetic loci in linkage group A1 in Jidou17 were primarily descended from the maternal Hobbit line (84.6%), whereas the proportion of loci that descended from Zao5241 was only 15.4%. In contrast, the genetic loci in linkage group A1 in Ji nf58 were completely descended from the paternal line Zao5241, and no genetic loci in this group were descended from Hobbit (Table 3). These results showed that the genetic loci in linkage group B2 in Jidou17 were primarily from Hobbit (72.7%), and those descended from Zao5241 accounted for only 27.3%, whereas the genetic loci in linkage group B2 in Ji nf58 were primarily from Zao5241 (63.6%), and those descended from Hobbit accounted for 36.4%. In addition, the genetic loci in linkage group K of Jidou17 were completely descended from the paternal Hobbit line. The genetic loci in linkage group K of Ji nf58 were primarily descended from Zao5241 (87.5%), and those descended from Hobbit accounted for only 12.5% (Tables 3 and 4, respectively).

The genetic contributions of the parental lines to Jidou17

Of the 197 polymorphic loci examined in this study, the number of loci passed down from Hobbit and Zao5241 to Jidou17 was 153 and 40, respectively, and the genetic contribution rates were 79.3% and 20.7%, respectively. The genetic contribution rate of the maternal Hobbit line to Jidou17 was higher than that of the paternal line Zao5241 (Table 3).

The distributions of loci that Jidou17 inherited from different parental lines differed considerably among the linkage groups (Table 3). The percentages of loci passed from Hobbit to the different linkage groups ranged from 47.1% to 100.0%. A greater number of genetic loci were contributed by Hobbit than by Zao5421 in 17 of the 20 linkage groups. In linkage group J, 28 (87.5%) loci were passed down from Hobbit to Jidou17, and only 12.5% of the loci were from Zao5241. In linkage group G, 11 loci were passed down from Hobbit to Jidou17, whereas no loci were passed down from Zao5241. In linkage group A1, 11 (84.6%) loci were passed down from Hobbit to Jidou17, whereas only 15.4% of the loci came from Zao5241. The $\chi 2$ test indicated that the loci passed down from Zao5241 to Jidou17 were not randomly distributed among the 20 linkage groups ($\chi 2 = 32.80$, $P \leq 0.05$). The percentages of loci that were

Table 1. Conserved and polymorphic loci in the parental lines.

Linkage group	Markers	Monomorphic loci	Polymorphic loci
A1	21	8 (38.1%)	13 (61.9%)
A2	20	11 (55.0%)	9 (45.0%)
B1	10	5 (50.0%)	5 (50.0%)
B2	21	10 (47.6%)	11 (52.4%)
C1	42	24 (57.1%)	18 (42.9%)
C2	40	31 (77.5%)	9 (22.5%)
D1a	17	10 (58.8%)	7 (41.2%)
D1b	21	11 (52.4%)	10 (47.6%)
D2	18	9 (50.0%)	9 (50.0%)
E	13	9 (69.2%)	4 (30.8%)
F	18	10 (55.6%)	8 (44.4%)
G	21	10 (47.6%)	11 (52.4%)
H	15	12 (80.0%)	3 (20.0%)
I	14	7 (50.0%)	7 (50.0%)
J	44	12 (27.3%)	32 (72.7%)
K	15	7 (46.7%)	8 (53.3%)
L	15	7 (46.7%)	8 (53.3%)
M	21	8 (38.1%)	13 (61.9%)
N	5	2 (40.0%)	3 (60.0%)
O	17	8 (47.1%)	9 (52.9%)
Total	408	211 (51.7%)	197 (48.3%)

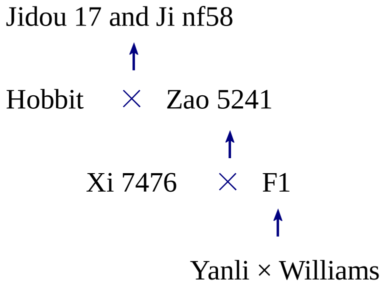

Fig 1. Pedigree of Jidou17 and Ji nf58.

passed down from Zao5241 among the different linkage groups ranged from 0% to 52.9%.

The genetic contributions of the parental lines to Ji nf58

Of the 197 polymorphic loci in Ji nf58 that were examined in this study, 116 loci were descended from Hobbit, whereas 81 came from Zao5241. The genetic contribution rates of Hobbit and Zao5241 were 58.9% and 41.1%, respectively. The genetic contribution rate of the maternal Hobbit line to Ji nf58 was higher than that of the paternal line Zao5241. The loci distributions among the linkage groups passed down by the two parental lines to Ji nf58 varied considerably (Table 4). In 12 of the 20 linkage groups, a greater number of genetic loci were contributed by Hobbit than by Zao5421. In linkage group J, 28 (87.5%) loci were passed down from Hobbit to Ji nf58, and only 12.5% of the loci came from Zao5241. In linkage group M, 8 (61.5%) loci were passed down from Hobbit to Ji nf58, whereas 38.5% of the loci were descended from Zao5241. In linkage group C1, 16 (88.9%) loci were passed down from Hobbit to Ji nf58, whereas 11.1% of the loci were from Zao5241. The χ2 test showed that the distribution of loci that were passed down from Zao5241 to Ji nf58 was not random among the 20 linkage groups ($\chi2 = 75.58$, $P \leq 0.01$). A greater number of genetic loci were contributed by Zao5421 than by Hobbit in 8 linkage groups. In linkage group A1, all of the loci were descended from Zao5421, whereas no locus was descended from Hobbit.

Discussion

The contribution of the US soybean germplasm Hobbit during breeding

The US soybean cultivar Hobbit is widely used as a parental line in the three large ecological zones of China, and it has been bred to produce a series of popular soybean varieties (Hu et al., 2007; Hu et al., 2009; Guo et al., 2004; Wang et al., 2010; Zhang et al., 2010). Based on a theoretical analysis, Hobbit is prone to producing elite soybean varieties and germplasms because it carries numerous superior genes related to agronomic traits, and it exhibits a high degree of heritability (Hu et al., 2008). Therefore, Hobbit is easily selected and preserved by breeders. Because Hobbit possesses beneficial allelic variations of genes related to important agronomic traits on the molecular level, it is likely to be selected by plant breeders, thereby passing the variations to the offspring. Therefore, because of the hitchhiking effect, certain chromosomal segments of "conservative heredity" exist between Hobbit and its derived cultivars. In this study, the identification of 197 SSR loci showed that the genetic contribution of the American soybean germplasm Hobbit to the offspring Jidou17 and Ji nf58 was greater than that of Zao5241. In addition to the Hobbit genetic contributions described in Fig.

Table 2. Differences in genetic loci between Jidou17 and Ji nf58.

Linkage group	Markers	Number of same loci	Number of different loci
A1	21	10 (47.6%)	11 (52.4%)
A2	20	16 (80.0%)	4 (20.0%)
B1	10	9 (90.0%)	1 (10.0%)
B2	21	12 (57.1%)	9 (42.9%)
C1	42	32 (76.2%)	10 (23.8%)
C2	40	36 (90.0%)	4 (10.0%)
D1a	17	10 (58.8%)	7 (41.2%)
D1b	21	14 (66.7%)	7 (33.3%)
D2	18	14 (77.8%)	4 (22.2%)
E	13	10 (76.9%)	3 (23.1%)
F	18	17 (94.4%)	1 (5.6%)
G	21	13 (61.9%)	8 (38.1%)
H	15	14(93.3%)	1 (6.7%)
I	14	12 (85.7%)	2 (14.3%)
J	44	38 (86.4%)	6 (13.6%)
K	15	8 (53.3%)	7 (46.7%)
L	15	14 (93.3%)	1 (6.7%)
M	21	14 (66.7%)	7 (33.3%)
N	5	3 (60.0%)	2 (40.0%)
O	17	15 (88.2%)	2 (11.8%)
Total	408	311 (76.2%)	97 (23.8%)

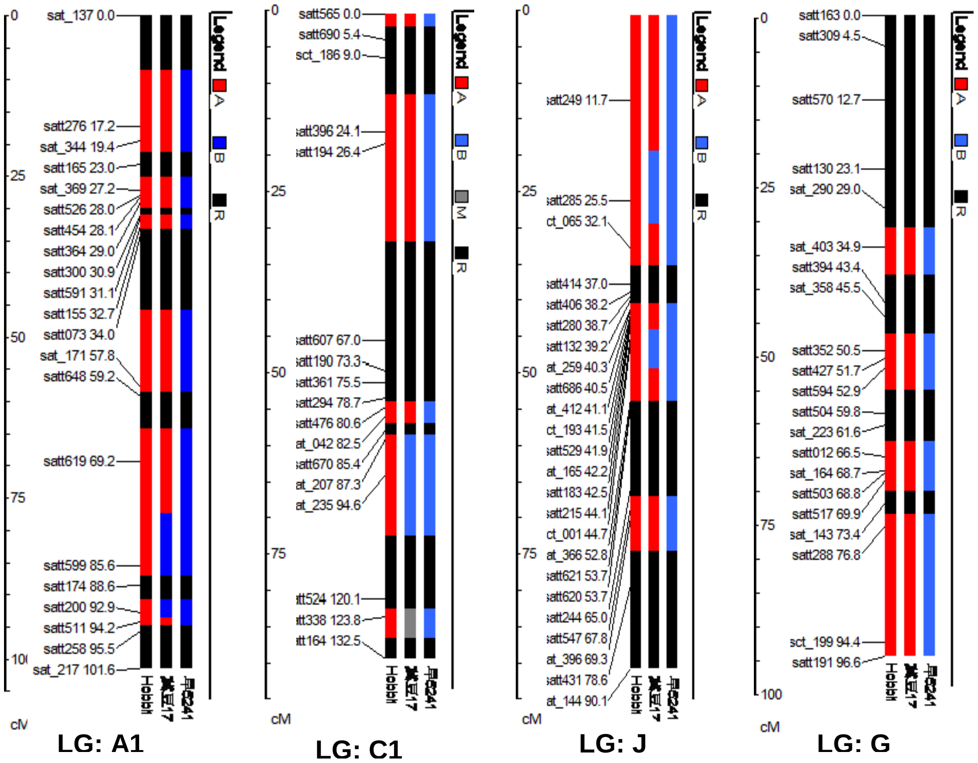

Fig 2. Diagram of the genetic composition of Jidou17 for linkage groups A1, G, J, and C1.The loci in Jidou 17 that contain the same allelic variations as both parents are shown in black; the loci in Jidou 17 that contain the same allelic variations as Hobbit are shown in red; the loci in Jidou 17 that contain the same allelic variations as Zao5241 are shown in blue; and the loci in Jidou 17 that contain allelic variations that are different from both parents are shown in gray.

1 and Fig. 2, a relatively large chromosomal segment (Sat_259 - Satt686 - Sat_412 - Sat_193 - Satt529 - Sat_165 - Satt183) (Genomic Map Position 40.5-42.51) in linkage group J of Hobbit was passed down to both Jidou17 and Ji nf58. This segment belongs to a previously reported yield-related quantitative trait locus (QTL) (Sd yld23-14) (Guzman et al., 2007). By using iTRAQ in conjunction with an antibody method, Qin et al. (2013a) confirmed that Hobbit made greater genetic contributions to Jidou17 and that the protein expression patterns of Hobbit and Jidou17 were similar. Therefore, the research results at the genomic level and at the proteomic level are in agreement. In future studies, related varieties derived from Hobbit will be collected to identify and analyze chromosomal segments of "conservative heredity" to determine the mechanism by which Hobbit produces elite varieties and germplasms. The results will provide supporting data for the selection and use of soybean parental lines during breeding and for the foundation of molecular-based breeding.

Table 3. Origin of the genetic loci in Jidou17.

Linkage group	Markers	Polymorphic loci		Genetic loci from Monomorphic loci
		Genetic loci from Hobbit	Genetic loci from Zao5241	
A1	21	11 (84.6%)	2 (15.4%)	8 (38.1%)
A2	20	8 (88.9%)	1 (11.1%)	11 (55.0%)
B1	10	4 (80.0%)	1 (20.0%)	5 (50.0%)
B2	21	8 (72.7%)	3 (27.3%)	10 (47.6%)
C1	42	8 (47.1%)	9 (52.9%)	24 (57.1%)
C2	40	7 (77.8%)	2 (22.2%)	31 (77.5%)
D1a	17	6 (85.7%)	1 (14.3%)	10 (58.8%)
D1b	21	5 (50.0%)	5 (50.0%)	11 (52.4%)
D2	18	9 (100.0%)	0 (0.0%)	9 (50.0%)
E	13	2 (50.0%)	2 (50.0%)	9 (69.2%)
F	18	8 (100.0%)	0 (0.0%)	10 (55.6%)
G	21	11 (100.0%)	0 (0.0%)	10 (47.6%)
H	15	3 (100.0%)	0 (0.0%)	12 (80.0%)
I	14	7 (100.0%)	0 (0.0%)	7 (50.0%)
J	44	28 (87.5%)	4 (12.5%)	12 (27.3%)
K	15	8 (100.0%)	0 (0.0%)	7 (46.7%)
L	15	5 (71.4%)	2 (28.6%)	7 (46.7%)
M	21	6 (54.5%)	5 (45.5%)	8 (38.1%)
N	5	2 (66.7%)	1 (33.3%)	2 (40.0%)
O	17	7 (77.8%)	2 (22.2%)	8 (47.1%)
Total	408	153 (79.3%)	40 (20.7%)	211 (51.7%)

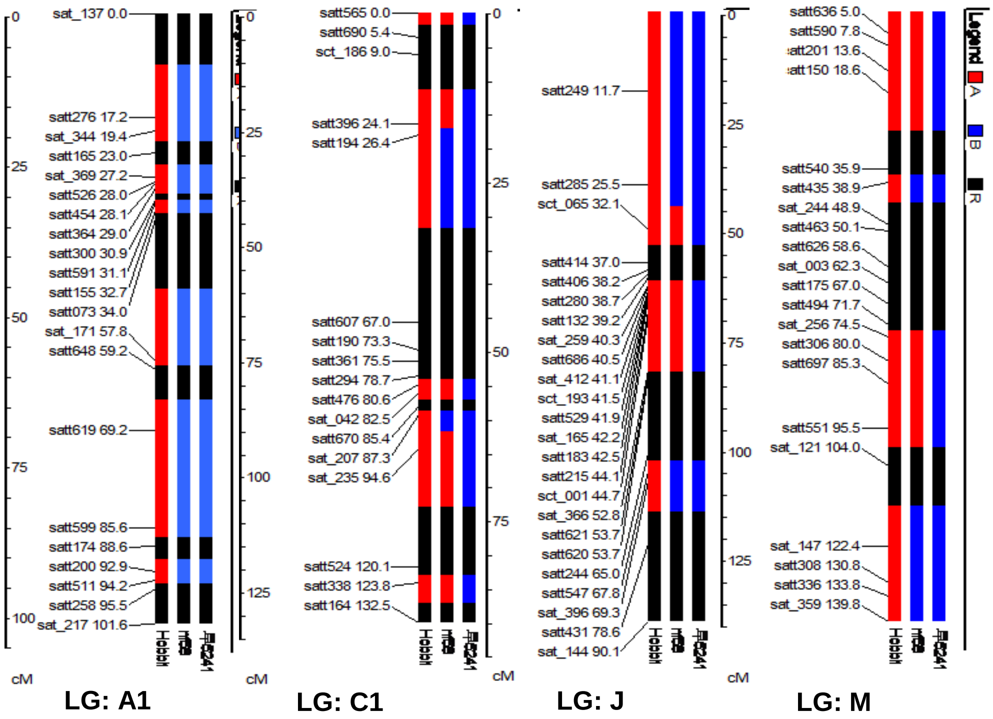

Fig 3. Diagram of Genetic composition of Ji nf58 for linkage groups A1, C1, J, and M. The loci in Ji nf58 that contain the same allelic variations as both parents are shown in black; the loci in Ji nf58 that contain the same allelic variations as Hobbit are shown in red; and the loci in Ji nf58 that contain the same allelic variations as Zao 5241 are shown in blue.

The relation between conserved chromosomal segments and artificial selection

The percentages of conserved loci in linkage groups C2 and H reached 77.5% and 80.0%, respectively, between the parental lines Hobbit and Zao5241, but the rate of genetic recombination was relatively low. As a result of the hitchhiking effect, these regions were subject to strong selection, which results in the fixation of an effective allelic variation and the reduction of genetic diversity at these loci (Rieseberg et al., 2002; Andolfatto, 2001). In addition, these regions are subject to a genetic linkage effect, which reduces the genetic diversity of the loci flanking sequences (Nurminsky et al., 2001), increases the genetic linkage disequilibrium of the loci in the genomic segments (Huttley et al., 2000; Kohn et al., 2000), and changes the distribution patterns of the allelic variations at each loci, causing segregation distortion. Previous studies have shown that the selection of genes related to important agronomic traits results in stronger hitchhiking effects (Yan et al., 2012). Therefore, it is believed that a gene controlling important agronomic traits or a main-effect QTL exists in or near this region in sibling lines Jidou17 and Ji nf58. This gene

Table 4. Origin of the genetic loci in Ji nf58.

Linkage group	Markers	Polymorphic loci		Monomorphic loci
		Genetic loci from Hobbit	Genetic loci from Zao5241	
A1	21	0 (0.0%)	13 (100.0%)	8 (38.1%)
A2	20	6 (66.67%)	3 (33.3%)	11 (55.0%)
B1	10	5 (100.0%)	0 (0.0%)	5 (50.0%)
B2	21	4 (36.4%)	7 (63.6%)	10 (47.6%)
C1	42	16 (88.9%)	2 (11.1%)	24 (57.1%)
C2	40	6 (66.7%)	3 (33.3%)	31 (77.5%)
D1a	17	1 (14.3%)	6 (85.7%)	10 (58.8%)
D1b	21	4 (40.0.0%)	6 (60.0%)	11 (52.4%)
D2	18	5 (55.6%)	4 (44.4%)	9 (50.0%)
E	13	1 (25.0%)	3 (75.0%)	9 (69.2%)
F	18	7 (87.5%)	1 (12.5%)	10 (55.6%)
G	21	3 (27.3%)	8 (72.7%)	10 (47.6%)
H	15	2 (66.7%)	1 (33.3%)	12 (80.0%)
I	14	5 (71.4%)	2 (28.6%)	7 (50.0%)
J	44	28 (87.5%)	4 (12.5%)	12 (27.3%)
K	15	1 (12.5%)	7 (87.5%)	7 (46.7%)
L	15	5 (62.5%)	3 (37.5%)	7 (46.7%)
M	21	8 (61.5%)	5 (38.5%)	8 (38.1%)
N	5	0 (0.0%)	3 (100.0%)	2 (40.0%)
O	17	9 (100.0%)	0 (0.0%)	8 (47.1%)
Total	408	116 (58.9%)	81 (41.1%)	211 (51.7%)

Table 5 Trait t-test for significance between Jidou 17 and Ji nf58

Traits	years	Jidou17 means	Ji nf58 means	Difference standard errors	t-values
Yield per ha	3	3750.00	3341.00	102.28	4.00 *
Plant height	3	103.50	101.17	4.38	0.53
Number of nodes on main stem	3	19.97	20.30	0.25	-1.35
Seed number per plant	3	102.87	109.80	9.82	-0.71
Seed weight per plant	3	15.79	13.51	1.71	1.33
100-seed weight	3	18.88	18.67	1.42	0.15
Fat content	3	22.98	23.63	0.28	-2.31
Protein content	3	37.99	35.77	0.73	3.02 *

* Significant at the 0.05 level, $t_{0.05}$=2.776.

is intensively selected for, causing a strong hitchhiking effect. As a result, the selection pressure on linkage groups C2 and H is enhanced, and the percentage of conserved loci in these linkage groups is increased. In contrast, the percentages of polymorphic loci in linkage groups M, A1, and J reached 72.7%, 62.9%, and 61.9%, respectively, and the rates of genetic recombination were relatively high. Previous studies have shown that if a high frequency of allelic variation is found at a locus, then the distribution of allelic variations is extremely uneven; this high allelic variation also indicates that important genes related to ecological adaptability or agronomic traits are located in this region (Yang et al., 2011). The highly conserved and highly polymorphic segments identified in the present study are likely to be valuable for mapping the natural population of soybeans in Huang-Huai-Hai and for identifying future research projects.

The relation between genetic loci and agronomic traits

According to existing data, more than 1700 QTL that are currently associated with more than 140 agronomic traits in soybeans have been integrated into the soybean genetic map (*http://soybase.org/pmd/index.php*). The QTL information has been used to analyze soybean pedigrees and to track the origin of disease-resistance genes (Ha et al., 2004; Ha et al., 2007). An analysis of 84 loci passed down from Hobbit to Jidou17 and Ji nf58 showed that these loci were related to the QTL that reportedly control more than 50 traits, including yield, plant height, oil content, and maturity. An analysis of 12 loci passed down from Zao5241 to Jidou17 and Ji nf58 showed that these loci were related to the QTL that reportedly control more than 30 traits, including yield, plant height, and flower number (*http://soybase.org/pmd/index.php*). The yields of Jidou17 and Ji nf58 were significantly greater than the yields of the parental lines Hobbit and Zao5241 because of the beneficial recombination of the parental genes and because the relevant traits of the different parents were reserved and optimized. Jidou17 and Ji nf58 yields were also analyzed. Both Jidou17 and Ji nf58 were tested in the regional trials in the same group (Huang-Huai-Hai Middle Group) at the same test location during different years. Except for the yield, protein content, and adaptability, there were no significant differences in traits between Jidou17 and Ji nf58 (Table 5). Compared with control variety Lu 99-1, the increased yield of Jidou17 was 5-6% greater than that of Ji nf58. The genetic contributions from the maternal Hobbit line to these two varieties were greater than the contributions of the paternal line Zao5241, and the genetic contribution from Hobbit to Jidou17 was greater than that to Ji nf58. Therefore, Hobbit likely has a higher hereditary transmissibility for the yield trait than Zao5241. The results of this study showed that the loci that were passed down from Hobbit to Jidou17 and Jinf58, including Sd yld3-2 and Sd yld21-2 (Satt079) in linkage group C2 (Cregan et al., 1999), Sd yld15-11 (Satt191) in linkage group G (Cregan et al., 1999), Sdyld 14-1 (Satt239) and Sdyld 21-7 (Satt354) in linkage group I (Cregan et al., 1999), Sd yld20-2 (Satt529) in linkage group J

(Cregan et al., 1999), Sd yld23-7 (Satt313) in linkage group L (Cregan et al., 1999), and Sdyld 6-1,11-7 (Satt150) in linkage group M (Cregan et al., 1999), belonged to a previously reported yield-related QTL. Thus, the dissemination of these loci may be correlated with the yield of the offspring. In all regional trials conducted over a large number of areas, including the central and southern parts of the summer-seeding zones of Huang-Huai-Hai, the spring soybean-planting areas of the southwest mountainous region and the Yangtze River Basin, and the spring soybean-planting areas of northwestern and northern China, Jidou17 displayed a wide range of adaptability that exceeded the current adaptation range of domestic and foreign high-oil soybean varieties (Qin et al., 2013b). Qin et al. (2013a) found that some up-regulated proteins, such as LOX, were densely distributed in the jasmonic acid synthesis pathway among the differentially expressed proteins in Jidou17 and the parental lines. This observation was closely associated with abiotic stress resistance, providing a proteomic basis for the extensive adaptability of Jidou17. The present study also showed that loci passed down from Hobbit to Jidou17 were associated with stress resistance (*http://soybase.org/pmd/ index.php*). For example, the drought index-related QTL was distributed in the 57.79-64.73 cM section of linkage group A1 (Du et al., 2009). In addition, certain loci located in other linkage groups, including SCN33-2 (Satt632) (Song et al., 2004), SCN17-3 (Satt371) (Cregan et al., 1999), SCN16-1 (Satt082) (Cregan et al., 1999), Sclero2-12 (Satt114) (Cregan et al.,1999), Sclero2-14 (Satt191) (Cregan et al., 1999), Sclero2-19 (Satt143) (Arahana et al., 2001), and SDS7-3 (Satt570) (Iqbal et al., 2001), were associated with disease resistance. In summary, Jidou17 and Ji nf58 retained the important traits of the parental lines, and they developed superior genomes by optimizing their combinations of the parental lines. The regions related to the agronomic traits described above provide starting points for breeding new soybean varieties based on the Huang-Huai-Hai cultivars and for future in-depth research.

Materials and Methods

Plant materials

The soybean varieties used in this study included Jidou17, Ji nf58, Hobbit, and Zao5421 (Fig. 1). Jidou17 and Ji nf58 are progeny of the Hobbit×Zao5241 cross. Hobbit, the female parent of Jidou17 and Ji nf58, is an elite soybean variety that was imported from the USA. Zao5421, the male parent of Jidou17 and Ji nf58, is a progeny of the Xi 7476×F1 (Yanli×Williams) cross. Xi 7476 is also an elite early maturing soybean variety from northwestern China. Another exotic germplasm, Yanli from Japan, has a short stem internode. Williams (from the USA) possesses strong stalk traits, good adaptability, and a desirable growth habit.

Fields

The study fields were flat and level, and the fertilization levels were representative of the local area. The fields had convenient irrigation, had been planted with the same crop in the previous season, and were located away from tall buildings and tall crops. The experimental area was buffered by protective plantings of six rows or more. The fields were randomly assigned with advanced cultivars and breeding lines and each trial was conducted in triplicate. There were 12 field blocks (12 = 4 x 3). Each block area was 18 m^2 with 6 columns and 6 rows, and the distance between the rows was 0.5 m. The density was 225 thousand plants per ha. Immediately before the harvest, 10 plants were randomly chosen from the central 4 rows from which to take detailed measurements in the lab, and the seeds of these plants were included in the yield calculation. During the harvest, the surrounding two rows were reaped before the central 4 rows, and the calculated yield included the central 4 rows, which were 12 m^2 in area. Data related to 8 agronomic traits were collected, including PH, NN, SNP, PNP, 100-SW, SWP, YP, O and P.

SSR marker analysis

The sodium dodecyl sulfate (SDS) method was used to extract DNA before purifying by phenol-chloroform extraction. Four hundred and eight SSR markers were selected from the 20 soybean linkage groups established by Song et al. (2004, 2010). A polymerase chain reaction (PCR) was performed with a PE9600 thermocycler (PerkinElmer, Inc., USA). The 20-μL reaction system contained 30 ng of soybean genomic DNA as a template, 2 μL of 10X PCR buffer, 0.15 mmol/L dNTPs, 1 U of Taq DNA polymerase, and 0.15 μmol/L of primers. The amplification cycle was as follows: 94°C pre-denaturation for 5 min; 94°C denaturation for 30 s, 47°C annealing for 30 s, and 72°C extension for 30 s for 35 cycles; and 72°C extension for 5 min. The products were electrophoresed in agarose gels and dried before the bands were statistically analyzed or photographed (Supplementary Table 1-2).

Statistical methods

In Ji nf58 and Jidou17, the SSR banding patterns derived from Hobbit were denoted by A and those derived from Zao5241 were denoted by B. The banding patterns that differed between the parental lines were marked as M (mutated), and the banding patterns that were non-polymorphic between the parental lines were labeled as R (reserved). Genetic contributions were calculated according to the polymorphic loci (Guan et al., 2009). Data were analyzed by using SPSS17.0 software, and chromosomal segment diagrams were plotted with GGT software (Van Berloo R., 1999).

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