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Molecular Genetic Analysis on Soybean Cyst Nematode Resistance in Heilongjiang Province, China

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Abstract: The soybean cyst nematode(SCN), *Heterodera glycines*, is a major threat to soybean production around the world. In order to explore the molecular genetic characteristics of soybean germplasms resistant to SCN and screen SCN resistance related genes, we analyzed the field trials, identified the resistance with SLAF-seq technology and identified the loci related to the resistance to SCN race 3 *via* genome-wide association mapping based on the genetic and genomic features of the SCN resistant soybean cultivars widely planted in Heilongjiang province, China. The results showed that the genetic sources of SCN resistance were Fliklin and Peking. Kangxian 2, Kangxian 6 and Kangxian 10 were genetically close, with a genetic resistance of 0.24 between Kangxian 2 and Kangxian 6, 0.213 between Kangxian 2 and Kangxian 10. Kangxian 2 was genetically distant from Fengdou 3, with a genetic distance of 0.799. A total of 105 563 SNP loci were amplified from Kangxian 2 and its derived lines, among which 4 352 loci were genetically conserved, accounting for 4.12% of the total. The percentage of identical allelic variants ranged from 56% to 96.3% among the tested soybean cultivars, which differed between cultivars and chromosomes. Above 65% of the genetic information in different chromosomes of Kangxian 2 was passed on to Kangxian 4 and Kangxian 6. Between Kangxian 2 and Kangxian 4, Kangxian 6, the percentage of identical allelic variants was more than 95% on chromosome 4, more than 91% on chromosome 10, indicating that there may be particular genome sections on chromosomes 4 and 10 related to the main agronomic traits, SCN resistance, Phytophthora resistance, drought resistance, virus resistance, root morphology, hilum color, 100-seed weight and so on, which are the genetic basis of the SCN resistant cultivars planted in western Heilongjiang province, China. In addition, four loci associated to the resistance to SCN race 3 were detected on chromosome 11, and among them Glyma11g35700.1 has the greatest synergistic effect, and can be used for molecular marker assisted selection of soybean.

Keywords: Soybean cyst nematode resistance; Allelic variation; Genetic contribution; Heilongjiang

黑龙江省抗胞囊线虫大豆的分子遗传和相关基因挖掘

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摘　要:大豆胞囊线虫是世界大豆生产的一种毁灭性病原,为解析抗胞囊线虫大豆种质的分子遗传特征,挖掘相关基因,采用田间试验、接种鉴定和 SLAF-seq 技术相结合的方法,对黑龙江省主推的抗线虫品种的遗传性状和基因组遗传特性进行解析,并应用关联分析方法确立抗胞囊线虫 3 号小种的相关基因位点。研究结果表明:抗线品种的抗原来源于 Fliklin 和 Peking 小黑豆;抗线 2、抗线 6、抗线 10 的遗传距离较近,品种间的遗传距离为 0.24 和 0.213,抗线 2 与丰豆 3 的遗传距离为 0.799,亲缘关系较远. 抗线 2 号及其抗线品种的进化 SNP 位点有105 563个,而品种间遗传保守位点4 352个,占进化标记位点的 4.12%;品种间相同等位变异在不同品种和不同染色体上存在差异;相同等位变异为 56% ~96.3%;抗线 2 对抗线 4 和抗线 6 的遗传贡献表现在不同染色体上的遗传信息传递在 65% 以上,在 4 号染色体上,抗线 2 与抗线 4、抗线 6 比较的相同等位变异比例达 95% 以上,在 10 号染色体上相同等位变异比例超过 91%,推测抗线虫大豆在 4、10 号染色体上有一些特殊与主要农艺性状、胞囊线虫抗性、疫霉菌抗性、抗旱性、病毒病 1

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号抗性、根系形态、脐色、百粒重等相关基因位点的遗传成为黑龙江省西部地区抗线大豆的生态遗传基础。在 11 号染色体上找到了抗胞囊线虫 3 号小种的关联位点 4 个,其中 Glyma11g 35700.1 的增效作用较大,可用于大豆育种的分子标记辅助选择。

关键词:抗胞囊线虫大豆;等位变异;遗传贡献;黑龙江

Western Heilongjiang province has long suffered from sandstorms, drought, saline stress and the prevalence of soybean cyst nematode (SCN, *Heterodera glycines*). Soybean was not planted here until 1992. New soybean cultivars that could be planted in western Heilongjiang were generated by introducing Flinlklin, a USA soybean cultivar with SCN resistance, and genetically improving Peking, a Chinese black soybean cultivar. Among them, Kangxian 2 which was developed in 1995, was used as an important parental line in the selection of SCN resistant soybean cultivars in this area. Characterizing the phenotypic and genotypic characteristics of the excellent parents will help to develop new cultivars with expected traits. There are mainly three physiological races of SCN in western Heilongjiang.

Numerous studies have been conducted on the selection and innovation of soybean germplasms[1-3], QTL mapping is one of the most effective methods to screen genes related to resistance. Whole genome sequencing and QTL mapping are important approaches to screen candidate genes. The QTLs related to the resistance to SCN race 4 have been mapped using SSR markers[4-6]. The infection mechanism and control of SCN race 3 have also been reported[7-8]. Sixteen studies on SCN resistance related QTLs have been published, and in these studies 360 QTLs have been mapped on 17 genetic linkage groups: A1, A2, B, B2, C1, C2, D1, D2, E, F, G, H, I, J, L, M and N [9]. Reduced-representation genome sequencing [also know as specific-locus amplified fragment sequencing(SLAF-seq)] is an efficient and high-throughput technique used to analyze the genetic difference between verities based on genomic GC analysis. In 2017, Cao,et al[10] created a high-density genetic map for plant height and flowing time by using SLAF-sequencing. And Li,et al[11], in the same year, using the same method, created another map of an RIL group for oil quality. However, the genomic and genetic differences between SCN resistant soybean cultivars have not been analyzed using SNP markers.

In the present study, the genetic and genomic features of the SCN resistant soybean cultivars widely planted in Heilongjiang province, China were analyzed by field trials, resistance identification and SLAF-seq technology, and the loci related to the resistance to SCN race 3 were identified *via* genome-wide association mapping, to reveal the genetic basis of SCN resistant soybean cultivars, screen the loci and genes related to SCN resistance, and thus to provide technical support for molecular marker assisted selection of soybean.

1 Materials and Methods

1.1 Materials

Three hundred and twelve soybean cultivars from Northeast China and 14 cultivars those are genetically related to Kangxian 2 and widely planted in western Heilongjiang: Kangxian 11, Kangxian 6, Kangxian 9, Kangxian 4, Kangxian 8, Kangxian 2, Nenfeng 14, Kangxian 7, Nenfeng 20, Kangxian 10, Kangxian 13, Kangxian 5, Fengdou 3 and Fengyuan 3 were selected as the experimental materials in the present study.

We planted the 14 cultivars in the field from 2012 to 2014, and each cultivar was sown in three rows, with three replicates. The agronomic traits, yield traits, photosynthetic characteristics, resistances to SCN, root rot, leaf spot and virus diseases of each cultivar were analyzed.

1.2 Methods

1.2.1 Measurement of photosynthetic characteristics The photosynthetic parameters of functional leaves at R5 stage were measured using LI-6400XT portable photosynthesis system [12-13].

1.2.2 Identification of diseases The main disease in soybean seedlings were identified as previously described[1,14].

1.2.3 SLAF-seq (specific-locus amplified fragment sequencing) Reduced-representation genome sequencing in soybean was carried out. In brief, the genomic DNA was extracted from each sample, digested with designed combination of restriction endonucleases. The digestion product was end-repaired, phosphorylated at the 5′ end, and adenylated to add a single A at the 3′ end, which was complementary to the T at the 5′ end of Solexa adapter. After Solexa adapter was ligated,

the DNA fragments were loaded into flow cell channels for bridge amplification. The amplification products were separated *via* agar gel electrophoresis. The entire library was PCR-amplified to increase the total amount of DNA, and then sequenced using Illumina HiSeq 2 500 System. Finally, 2 230 890 reads were obtained from each sample on average, and mapped to reference sequences using SOAP aligner[15-16]. As a result, a total of 312 398 SLAFs were obtained, with a sequencing depth of 4. 12. The SNPs were detected using the SLAFs, and as a result 432 222 SNPs were found. 56. 5% of the SLAFs were polymorphic. Genetic components and principal component analysis was performed according to SNPs of each cultivar[17-20]. The genetic distances between the soybean cultivars were computed using neighbor-joining (N5) and the maximum composite likelihood methods in Mega 5. 0[21-22], and the identical allelic variations were calculated using custom Perl scripts.

2　Results and Analysis

2. 1　Cluster analysis and genetic improvement of Kangxian 2 and other cultivars of Kangxian series

Kangxian 2 which was developed by Heilongjiang Academy of Agricultural Sciences is an indeterminate line with high resistance to race 3 SCN, high yield, resistances to drought and salt, and widely planted in the areas of Heilongjiang province suffered from sandstorm, drought and salt. The genes responsible for resistance to SCN race 3 in Kangxian 2 were from Flenklin. Cluster analysis of Kangxian 2 and its derived lines (Fig. 1) showed that Kangxian 2 had a close genetic relationship with Kangxian 10 and Kangxian 6, and with genetic distances of 0. 213 and 0. 24, respectively. Kangxian 2 was genetically distant from Fengdou 3, and their genetic distance was 0. 799. Kangxian 10 and Kangxian 6, which were genetically close to Kangxian 2, still had the resistance to SCN. However, Kangxian 10 was a semi-determinate line, and Kangxian 6 had increased oil content, but was susceptible to leaf spot. Kangxian 8 and Kangxian 9 had similar genetic basis, and their genetic distance was 0. 064. Kangxian 8 was moderately resistant to SCN race 3, and Kangxian 9 was susceptible to root rot, and the leaf photosynthetic capacity of the former was lower than that of the latter. Kangxian 2 was genetically distant from Fengdou 3 and Kangxian 11, and the genetic distances were 0. 799 and 0. 554 respectively, indicating that Fengdou 3 had abundant genetic basis (Table 1) and thus could be used for genetic improvement in soybean breeding. Fengdou 3 was genetically distant from all the tested cultivars, especially the genetic distance between Fengdou 3 and Kangxian 8 was up to 0. 921.

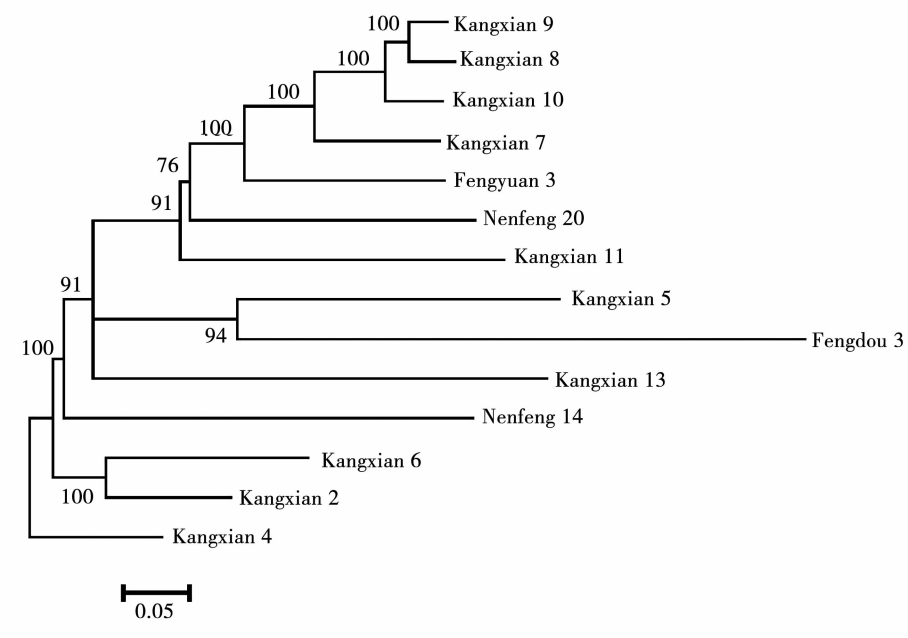

Fig. 1　Cluster analysis of Kangxian 2 and its derived lines

Table 1 Genetic distance between Kangxian series cultivars

KX11	KX6	KX9	KX4	KX8	KX2	NF14	KX7	NF20	KX10	KX13	KX5	FD3	FY3
KX6	0.622												
KX9	0.446	0.441											
KX4	0.530	0.243	0.334										
KX8	0.455	0.434	0.064	0.348									
KX2	0.554	0.247	0.306	0.318	0.304								
NF14	0.558	0.468	0.548	0.479	0.598	0.445							
KX7	0.309	0.498	0.201	0.387	0.195	0.396	0.631						
NF20	0.504	0.587	0.423	0.451	0.401	0.452	0.667	0.362					
KX10	0.495	0.406	0.090	0.330	0.095	0.253	0.585	0.189	0.413				
KX13	0.632	0.531	0.600	0.522	0.630	0.555	0.623	0.606	0.568	0.646			
KX5	0.589	0.575	0.692	0.487	0.673	0.572	0.516	0.604	0.497	0.685	0.680		
FD3	0.662	0.711	0.912	0.655	0.921	0.799	0.885	0.803	0.743	0.891	0.749	0.672	
FY3	0.478	0.473	0.294	0.334	0.300	0.333	0.639	0.350	0.444	0.267	0.573	0.662	0.749

2.2 Distribution of genetic information from Kangxian 2 on the chromosomes of its derived lines

A total of 105 563 SNPs were amplified from Kangxian 2 and its derived lines, among which, 4 352 (accounting for 4.12% of the total) were genetically conserved among the cultivars. The identical allelic variations were different on all the chromosomes between the cultivars (Table 2). The percentage of identical allelic variants on chromosome 14 was up to 96.2% between Kangxian 4 and Kangxian 2, but only 59.5% between Kangxian 13 and Kangxian 2. The percentage of identical allelic variants in all the chromosomes ranged from 55% to 96.3% between Kangxian 2 and its derived lines. In the genome of Fengdou 3, the percentage of identical allelic variants was more than 80% on chromosome 4, more above 70% on other five chromosomes, and only 58%, 55% and 59% on chromosomes 4, 6 and 9. The results indicated the genetic information of Kangxian 2 was differentially passed on to its derived lines.

Table 2 Percentage of identical allelic variants on different chromosomes between Kangxian 2 and its derived lines

Chromosomes	KX13	KX10	KX8	KX9	KX7	FY3	NF20	KX6	KX4	NF14	KX11	FD3	KX5
1	0.74356	0.93599	0.92605	0.93529	0.92635	0.92621	0.6273	0.9232	0.87396	0.847250	0.839	0.62	0.62
2	0.80833	0.81264	0.75410	0.79736	0.80905	0.78189	0.7817	0.8237	0.78992	0.688687	0.799	0.71	0.74
3	0.78725	0.61967	0.62708	0.61899	0.61658	0.78229	0.8426	0.8067	0.76886	0.893989	0.865	0.72	0.70
4	0.92421	0.88681	0.89167	0.88808	0.88605	0.87886	0.8700	0.9627	0.94879	0.905471	0.869	0.58	0.88
5	0.82760	0.84633	0.81490	0.83004	0.90294	0.82288	0.7366	0.7110	0.76932	0.862344	0.805	0.64	0.88
6	0.78488	0.81975	0.85496	0.86232	0.62762	0.66572	0.6048	0.9226	0.66631	0.893776	0.635	0.55	0.71
7	0.76168	0.88070	0.89577	0.90361	0.89640	0.71434	0.9113	0.8469	0.82713	0.874098	0.806	0.67	0.79
8	0.81948	0.87998	0.84826	0.88808	0.87397	0.87145	0.8086	0.8756	0.82090	0.828105	0.860	0.78	0.82
9	0.77029	0.68468	0.69932	0.67177	0.74259	0.64189	0.8294	0.9463	0.72696	0.749919	0.692	0.59	0.87
10	0.59792	0.95974	0.94689	0.95684	0.66904	0.81814	0.5942	0.9408	0.91420	0.885006	0.621	0.65	0.56
11	0.84229	0.85870	0.93849	0.90751	0.88719	0.85892	0.8561	0.8861	0.88210	0.851233	0.783	0.78	0.88
12	0.74642	0.95722	0.96315	0.95290	0.93187	0.87537	0.8779	0.8918	0.83714	0.890129	0.900	0.77	0.88
13	0.69482	0.78916	0.69483	0.69033	0.72713	0.72520	0.5928	0.8795	0.82590	0.804468	0.597	0.80	0.73
14	0.59531	0.96227	0.85569	0.77239	0.78340	0.84713	0.8484	0.9563	0.85537	0.679895	0.655	0.65	0.83
15	0.70088	0.94708	0.95767	0.95370	0.95945	0.94820	0.9345	0.8758	0.86648	0.645023	0.941	0.88	0.78
16	0.67497	0.68843	0.69602	0.68120	0.69913	0.68513	0.6539	0.8190	0.71706	0.704694	0.722	0.62	0.70
17	0.73940	0.89849	0.74365	0.82346	0.57182	0.92625	0.6992	0.6533	0.66868	0.669239	0.580	0.63	0.66
18	0.77292	0.91506	0.90569	0.90210	0.85427	0.87377	0.8726	0.8102	0.83882	0.837357	0.789	0.83	0.88
19	0.89146	0.91983	0.89938	0.90576	0.90585	0.80645	0.8993	0.8295	0.88397	0.697671	0.567	0.67	0.56
20	0.84176	0.94479	0.94010	0.91814	0.91372	0.91033	0.9165	0.7683	0.81615	0.822936	0.894	0.81	0.84

2.3 Introgression of genetic material among SCN resistant cultivars

Kangxian 4 and Kangxian 6 were two soybean cultivars widely planted in western Heilongjiang. Kangxian 4 which was more widely planted in eastern Heilongjiang, was developed by hybridization and pedigree selection using SCN resistant 8105-5 as the female parent, and Jiufeng 1 as the male parent, which was a genetically improved line derived from the cross between Kangxian 2 and Heinong 37. The base substitution coefficient between Kangxian 2 and Kangxian 4 was 0.334, indicating close genetic relationship between them. The genetic information of Kangxian 2 was distributed unevenly on the chromosomes of Kangxian 4, as the percentage of identical allelic variants was more than 91% on chromosomes 4 and 10, more than 81% on chromosomes 1, 7, 8, 11, 12, 13, 14, 15, 18, 19 and 20, more than 71% on chromosomes 2, 3, 5, 9 and 16, and more than 68% on chromosomes 6 and 17, indicating that more than 66.8% of the genetic information of Kangxian 2 was passed on to its offsprings, which possessed high resistance to stresses, but changed in stem termination type, plant height, growth period (Table 3 and Fig. 3), which was also proved by the introgression of genetic material between Kangxian 2 and Kangxian 4 (Fig. 2, Fig. 3).

Kangxian 6 was a SCN resistant line generated through the introduction of exogenous DNA *via* pollen tube pathway, with the genomic DNA of a Hainan soybean cultivar as the donor, and Kangxian 2 as the receptor. The genetic distance between Kangxian 2 and Kangxian 6 was 0.47. More than 65.3% of the genetic information of Kangxian 2 was passed on to Kangxian 6, and differentially distributed on the chromosomes. In detail, the percentage of identical allelic variants was 92% on chromosomes 1, 4, 6, 9, 10 and 14, above 80% on chromosomes 2, 3, 7, 8, 11, 12, 13, 15, 16, 18 and 19, above 70% on chromosomes 5 and 2, and 65.33% on chromosome 17. The two cultivars had similar phenotypic traits, increased oil content and decreased resistance to leaf spot, in addition, the introgression of genetic material showed significant differences between them (Fig. 2, Table 3 and Fig. 3).

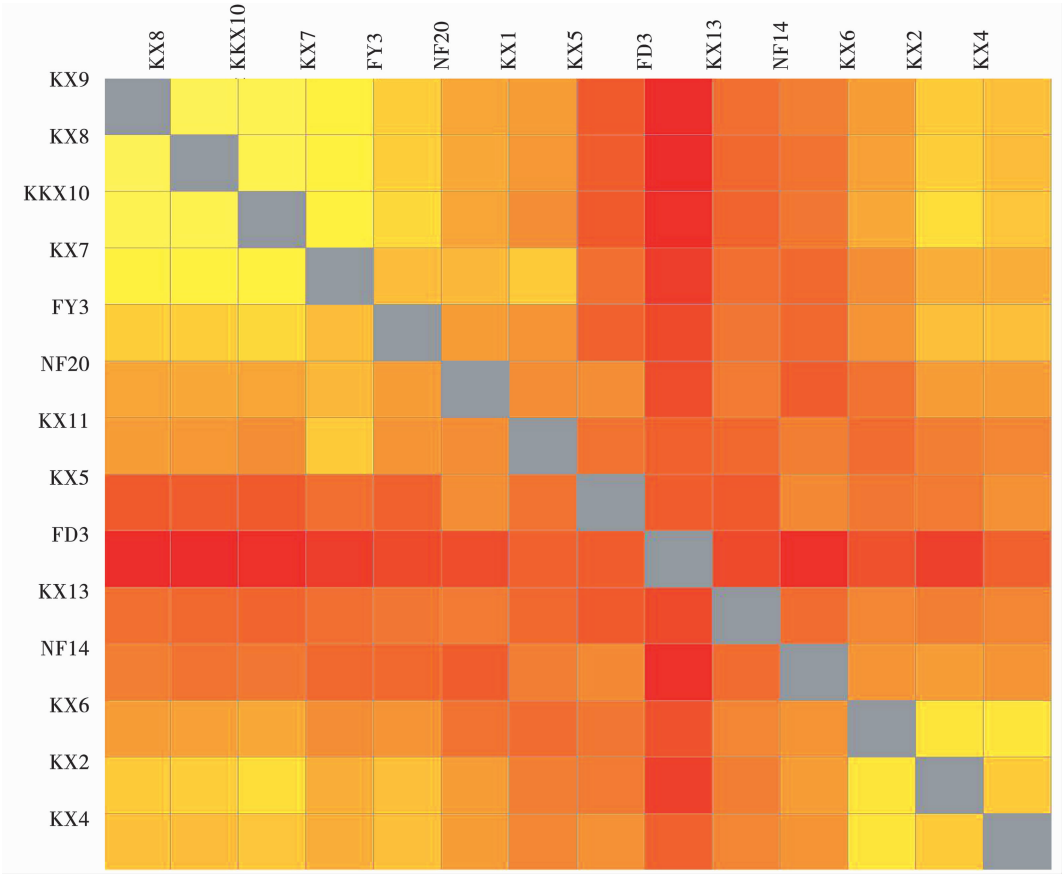

Fig. 2　Schematic diagram of introgression of genetic material among SCN resistant cultivars

Table 3 Identical variants of SCN resistance soybean cultivar

Cultivar	Parents	Stem termination	Plant height /cm	Leaf shape	Flower color	Seed coat color	Hilum color	100-seed weight /g	Accumulated temperature /℃	Days of growth period /d	Yield /(kg·ha^{-1})	Oil content /%	Protein content /%	Characteristics	SMV1 disease index/%	Resistance	SMV3 disease index/%	Resistance	SCN disease index/%	Resistance	Mortality rate caused by root rot	Resistance	Average value of resistance to leaf spot	Resistance	*Pn*
Kangxian 13	Heikang 002-24/Nongda 5129	Semi-determinate	90	Round	Purple	Yellow	Light brown	21.4	2550	122	2681.0	20.65	40.77	Resistant to SCN race 3 and drought	21.74	MR	39.26	MS	4.20	R	64.35	MR	52.00	MR	29.161 ± 1.75
Kangxian 10	Derived from the cross between Hefeng 33 (the female parent) and Kangxian 3 (the male parent)	Semi-determinate	85	Round	White	Yellow	Brown	21.0	2550	123	2289.6	19.22	42.30	Resistant to SCN race 3, drought and salt	21.60	MR	10.00	R	2.25	R	54.63	MR	42.80	MR	28.294 ± 0.39
Kangxian 8	Developed by hybridization and pedigree selection using Dongnong 690 as the female parent, An 95-1409 as the male parent	Semi-determinate	85	Round	White	Yellow	Brown	21.0	2500	120	2530	20.37	40.35	Highly resistant to SCN	22.31	MR	29.09	MR	21.95	MR	69.15	MR	53.60	MR	28.267 ± 2.47
Kangxian 9	Developed by hybridization and pedigree selection using Heinong 37 as the female parent, An 95-1409 (Nenfeng 10 / Frenklin) as the male parent	Semi-determinate	85	Round	White	Yellow	Brown	20.0	2500	121	2106.8	21.22	40.09	Moderately resistant to SCN	23.85	MR	28.42	MR	8.30	R	74.23	S	57.40	MR	23.644 ± 0.32
Kangxian 7	Developed by hybridization and pedigree selection using Hefeng 36 as the female parent, Kangxian 3 as the male perant	Indeterminate	85	Round	White	Yellow	Brown	20.0	2500	121	2090.3	19.98	38.97	Resistant to SCN	20.00	R	24.76	MR	58.60	R	66.64	MR	40.80	R	29.231 ± 0.64
Fengyuan 3	Ha 94-9656 × An 9656	Semi-determinate	83	Round	White	Yellow	Light brown	21.0	2450	121	2156.5	21.73	38.23	Resistant to SCN	13.33	R	34.81	MR	18.10	MR	58.83	MR	34.40	R	22.405 ± 1.08
Nenfeng 20	Developed by hybridization and pedigree selection using Hefeng 25 as the female parent, and An 7811-277 as the male parent	Semi-determinate	88	Round	White	Yellow	Light brown	21.7	2500	118	2207.4	19.82	41.72	Resistant to SCN	25.00	MR	23.00	MR	30.05	MR	46.12	MR	46.00	MR	29.100 ± 1.71
Kangxian 2	Derived from the cross between Hefeng 9 (the female parent), and F_2 generation of Nengfeng 10 × Frenklin (the male parent)	Indeterminate	95	Round	White	Yellow	Brown	18.0	2530	122	2334.3	20.54	38.00	Highly resistant to SCN race 3, resistant to salt, drought and poor fertility; not resistant to leaf spot	25.38	MR	38.00	MR	8.45	R	37.60	MR	49.60	MR	29.513 ± 0.90

Table 3

Cultivar	Parents	Stem termination	Plant height /cm	Leaf shape	Flower color	Seed coat color	Hilum color	100-seed weight /g	Accumulated temperature /℃	Days of growth period /d	Yield /(kg·ha^{-1})	Oil content /%	Protein content /%	Characteristics	SMV1 disease index/%	Resistance	SMV3 disease index/%	Resistance	SCN disease index/%	Resistance	Mortality rate caused by root rot	Resistance	Average value of resistance to leaf spot	Resistance	*Pn*
Kangxian 6	Generated through the introduction of exogenous DNA via pollen tube pathway, with the genomic DNA of a Hainan soybean cultivar as the donor, and Kangxian 2 as the receptor	Indeterminate	85	Round	White	Yellow	Brown	20	2500	121	2053.6	22.06	38.17	Resistant to SCN	20.00	R	18.40	R	14.90	MR	67.33	MR	61.00	S	29.174 ± 0.50
Kangxian 4	Developed by hybridization and pedigree selection using 8105-5 as the female parent, and Jiufeng 1 as the male parent	Semi-determinate	70	Round	White	Light yellow	Brown	20 – 22	2350	113	2302.2	20.77	38.20	Resistant to SCN race 3, salt and drought	25.71	MR	43.48	MS	4.65	R	45.74	MR	53.00	MR	27.886 ± 0.03
Nenfeng 14	Derived from 70-417	Semi-determinate	60 – 80	Round	Purple	Yellow	Light brown	20 – 23	2500	115	1839.3	19.70	43.90	Resistant to drought salt and SCN	20.00	R	58.95	MS	68.95	S	60.08	MR	60.80	MR	29.597 ± 0.83
Kangxian 11	Developed by hybridization and pedigree selection using Dongnong 434 as the female parent, and F_1 generation of An 01-1767 × An 87-7163 as the male parent	Indeterminate	85	Round	White	Yellow	Black	21	2550	123	2402.3	21.50	39.41	Resistant to SCN	21.67	MR	24.55	MR	5.45	R	79.83	S	40.80	R	27.917 ± 0.26
Fengdou 3	Developed by hybridization and pedigree selection using Kangxian 4 as the female parent, and Suinong 14 as the male parent	Semi-determinate	80	Round	White	Yellow	Brown	22	2400	120	2065.1	21.22	39.61	Resistant to SCN race 3	25.60	MR	56.15	S	29.10	MR	24.96	R	35.20	R	29.156 ± 0.70
Kangxian 5	Derived from the cross between Hefeng 25 (the female parent) and An 8804-33 (8201-205 Kangxian 2 × 8314-1222) (the male parent)	Semi-determinate	80	Long	Purple	Light yellow	Brown	20	2550	120	1792.8	19.75	41.18	Resistant to SCN race 3, drought and salt	20.00	R	34.17	MR	2.70	R	53.82	MR	33.20	R	23.814 ± 0.15

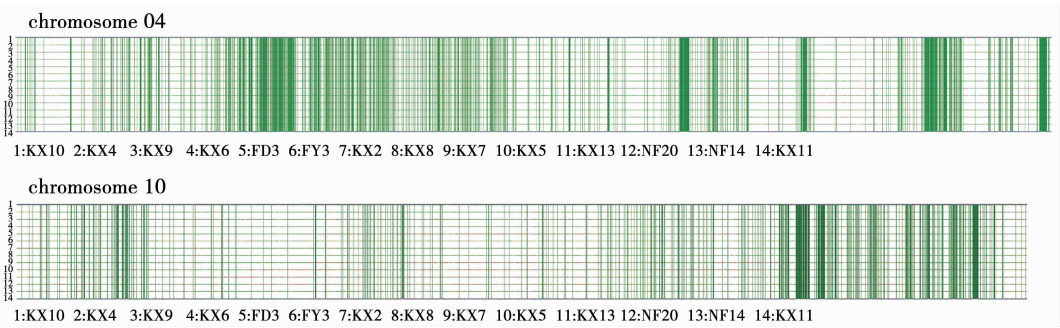

Fig. 3 Identical allelic variation on Gm04 and Gm10 chromosomes between the SCN resistant cultivars from Heilongjiang province

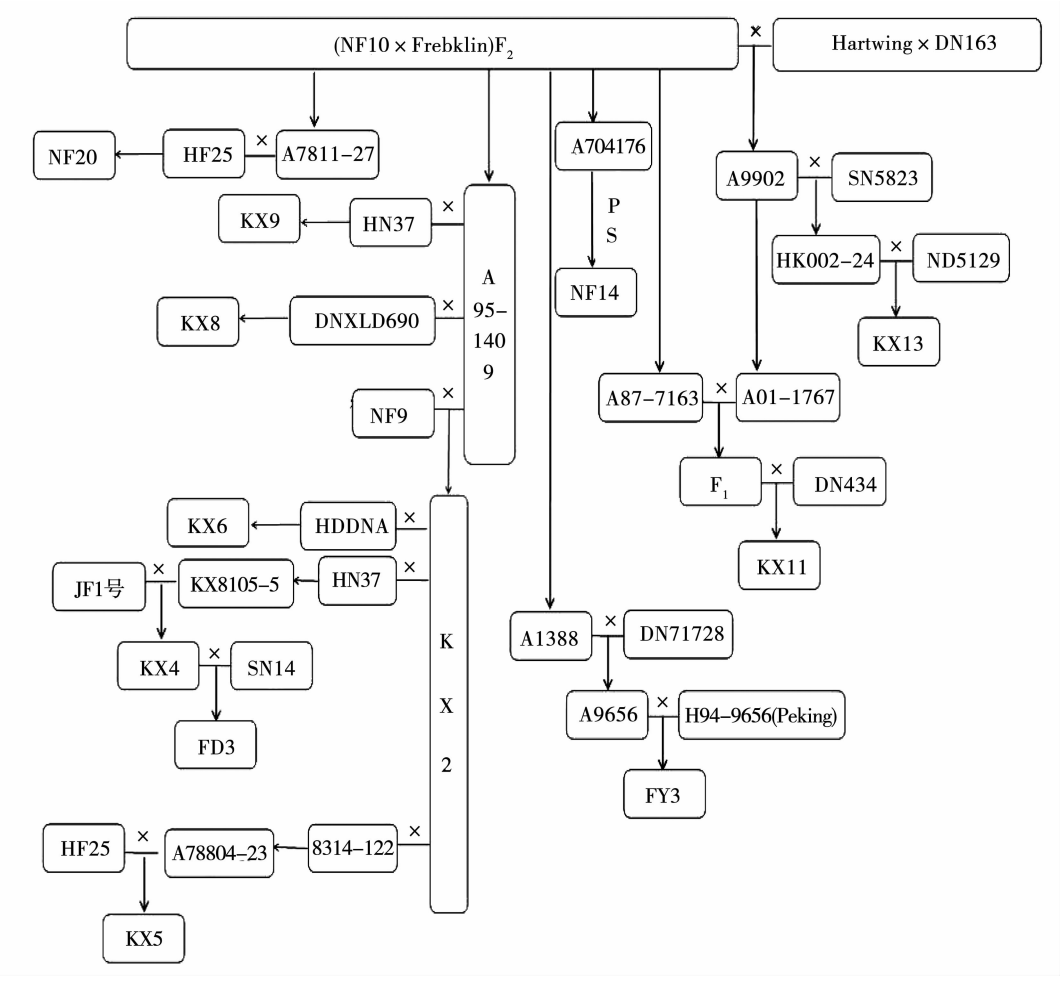

Fig. 4 Genetic relationship of the SCN resistant cultivars from Heilongjiang province

2.4 Important loci and candidate genes responsible for the resistance to SCN race 3

Genome-wide association mapping for SCN resistance of the 326 soybean cultivars were performed using a mixed model and a linear model. By genome-wide association mapping, six SNP loci significantly related to the incidence rate of SCN race 3 diseases were detected on chromosome 11 and 19 (Fig. 5 and Fig. 6), among which, the SNP locus Gm11: 37323330 showed the most significant association, followed by Gm11: 37419001 with their contribution rates were 6.03% and 5.63%, respectively. They were detected in 88% and 95% of the lines resistant to SCN race 3 and by the two models. Moreover, SNP loci related to the incidence rate of SCN race 3 diseases were also found on chromosomes 1 and 8.

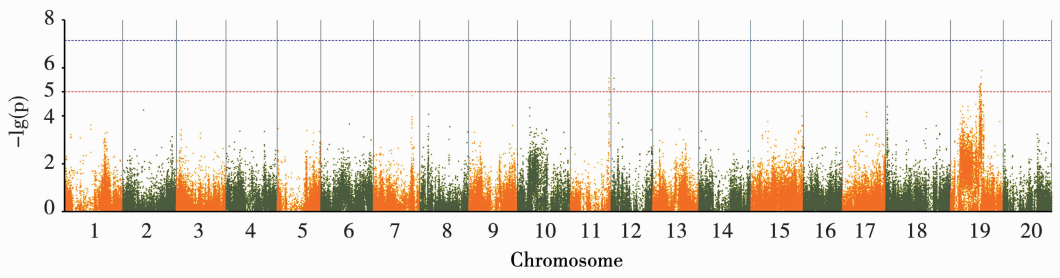

Fig. 5　The genome-wide association of SCN_3

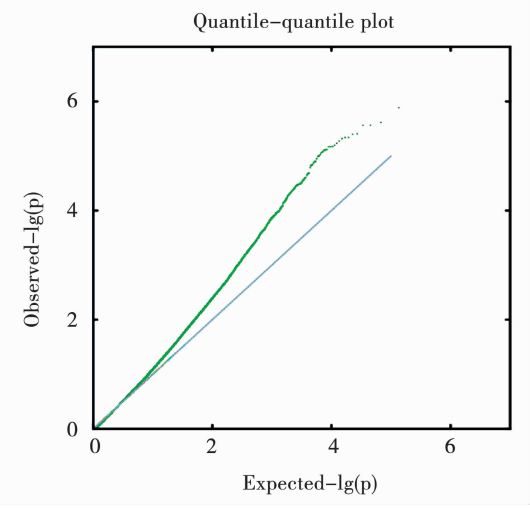

Fig. 6　SCN3 of Expected-lg(P)

A total of 15 loci significantly correlated with the disease index of SCN race 3 were found on chromosomes 8, 11 and 18, among which the locus Gm18: 1581688 showed the most significant association with the disease index of SCN race 3, followed by Gm18: 1554392, and their *P* values were $8.90E^{-12}$ and $3.61E^{-09}$, respectively.

Four genes that were possibly associated with SCN resistance were found (Table 4 and Fig. 7): Glyma11g35700.1, which encodes a laccase, is a member of laccase gene family. It is involved in the synthesis of lignin and highly expressed in lignified tissues. When soybean plant is infected by pathogens, Glyma11g35700.1 is largely expressed, which promotes the lignification of the infected part of roots to resist further infection of the pathogens. In addition, it possibly participates in ABA treatment, stress response and lignin catabolism[23]. Glyma11g35820.1, encodes alpha-soluble NSF attachment protein 2 (α-SNAP2) and mediates membrane fusion. In detail, the adaptor protein of N-ethylmaleimide sensitive fusion proteins recognizes and binds to the receptors V-SNARE (soluble NSF attachment protein receptor residing on the vesicle) on vesicle and t-SNARE on target membranes, initiates the assembly of the fusion complex, which catalyzes the fusion of vesicle with the target membrane. Glyma11g35640.2 encodes DEAD-box ATP-dependent RNA helicase 18, which is involved in the unwinding of RNA duplexes. Glyma11g35651.1 which encodes an autophagy related protein is involved in autophagy of plant cells, and maintains the growth balance *in vivo* by degrading damaged intracellular organelles, long-lived proteins and macromolecular aggregates.

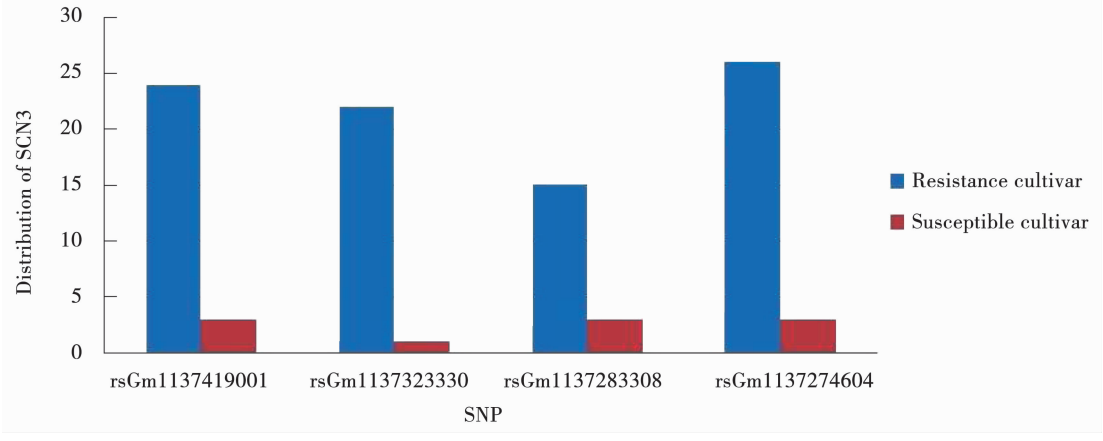

Fig. 7　Distribution of SCN3 related SNP marks in material

Table 4 SCP loci and candidate genes significantly associated with SCN resistance of soybean

SNP	Position	Gene	Start	Stop	Distanceto SNP/bp	Beneficial Allele	*P* value	*R*/%	Anotation
rsGm1137274604	Gm11:37274604	Glyma11g35640.2	37272846	37278083	5237	C	7.89E-06	5.548	RNA helicase
rsGm1137283308	Gm11:37283308	Glyma11g35651.1	37280268	37287704	7436	C	3.90E-06	5.548	Autophegy protein
rsGm1137323330	Gm11:37323330	Glyma11g35700.1	37321654	37326536	4882	A	2.73E-06	6.028	Enzymes and Ligui metabolism
rsGm1137419001	Gm11:37419001	Glyma11g35820.1	37416391	37421254	4863	A	6.67E-06	5.624	*a* soluble NSF attachment protein

3 Discussion

3.1 Target loci under ecological selection

The percentage of identical allelic variants on both chromosomes 4 and 10 was more than 91% between Kangxian 2 and its derived lines: Kangxian 4 and Kangxian 6. Especially, the percentage of identical allelic variants on chromosome 4 was more than 95%. The results proved the genetic transmission between Kangxian 2 and its derived lines. In contrast, the percentage of allelic variants on chromosome 17 between Kangxian 2 and Kangxian 4, Kangxian 6, was only 66.8% and 65.33%, indicating that more genes on chromosome 17 were replaced by new ones. Numerous loci related to important traits of soybean such as SCN resistance, 100-seed weight, root morphology, growth period, lodging resistance and hilum color were detected on chromosome 4, and these traits are inherited by later generations, which are also the results of artificial selection of target loci.

The percentage of identical allelic variants on chromosome 10 was more than 90% between Kangxian 4, Kangxian 6 and Kangxian 2. According to the association analysis on markers and phenotypic data, we found that the resistances to SCN, phytophthora root rot, drought, root weight, photosynthetic rate, SMV1 resistance and other dominant traits were preferentially retained and passed on to later generations under ecological selection in arid areas. In addition, on chromosomes 4 and 10 there are some traits those have high ability to transmit and are preferentially selected as the target loci by breeder under ecological conditions. So, we speculated that there may be particular genome sections on chromosomes 4 and 10 related to the main agronomic traits such as SCN resistance, drought resistance, high water use efficiency, root rot resistance, root weight and virus resistance, which are the main traits selected under the ecological conditions in western Heilongjiang, and also the genetic basis of the SCN resistant cultivars.

3.2 Identification of SNP markers associated with resistance to soybean cyst nematode

The QTLs associated with the resistance of soybean cyst nematode were identified by QTL mapping. A total of 60 QTLs associated with resistance to soybean cyst nematode have been reported by Concibido, et al[9]. By mining candidate genes for resistance to soybean cyst nematode based on meta-analysis and domains annotations, Chang, et al[24] found that Satt315-Sat400 was a QTL associated to the resistance to SCN race 3, with a genetic contribution rate of 26.2%. In the study of Zhang, et al[25], ESSR197 on Gm08 was proved to be a marker related to SCN, and Satt197 on Gm11, St193 on Gm16, and satt723 on Gm19 were QTL domains and loci associated with resistance to SCN race 3.

In the present study, 21 SNP loci associated with SCN race 3 were found in natural populations of soybean from Northeast China, and they were mapped on Gm11, Gm19, Gm8 and Gm18. Association analysis on the incidence rate and disease index of SCN race 3 revealed that the loci that showed extremely significant association were all on Gm11.

The SNP markers: rsGn11 37419001, rsGm11 37323330 and rsGm11 37274604 detected in the present study can be used for molecular marker assisted selection.

Among the four genes related to stress response we detected, Glyma11g35700.1 encodes a laccase and is involved in the synthesis of lignin, and helps to resist infection of pathogens by promoting the lignification of the infected parts. Cell wall is the natural barrier of plants against the invasion of pathogens. The lignification and lignin accumulation of cell walls play a major

role in resisting the invasion of pathogens. Laccase is a key enzyme during lignin polymerization, and is involved in the stress response to pathogens. When the plants are infected by pathogens, lignin is induced by a complex metabolic pathway to form a new cell wall to resist the mechanical stress caused by pathogen invasion and prevent the degradation of the cell wall by the pathogens, blocking the exchange of materials between the pathogens and the host plants. Glyma11g35651.1 gene probably encodes an autophagy-related protein which is involved in the autophagy of plant cells. Autophagy is a highly conserved pathway of cellular degradation present in all eukaryotes, and maintains the growth balance *in vivo* by degrading damaged intracellular organelles, long-lived proteins and macromolecular aggregates. In recent years, many studies have confirmed that it plays a vital role in plant defense response and resisting pathogen infection. In addition to the four genes mentioned above, some other genes interacting with pathogens were detected on Gm11, such as Glyma11g35880.1, which may encode a subunit of NADH: Ubiquinone oxidoreductase, respiratory chain complex I, involved in respiration of plants. Some genes related to chlorophyll synthesis and light migration were also mapped on chromosome 18 by association analysis, suggesting that the resistance of soybean to cyst nematode is controlled by multiple genes.

4 Conclusions

By identifying the resistance of a natural population consisting of 326 soybean cultivars to SCN race 3 from Northeast China, a total of 105 563 SNP loci were amplified from Kangxian 2 and its derived lines, among which 4 352 loci were genetically conserved, accounting for 4.12% of the total. The percentage of identical allelic variants ranged from 56% to 96.3% among the tested soybean cultivars, which differed between cultivars and chromosomes. Kangxian 2 was genetically close to Kangxian 6 and Kangxian 10, and genetically distant from Fengdou 3. The percentage of identical allelic variants on chromosomes 4 and 10 was more than 90% between the SCN resistant soybean cultivars. In addition, four loci significantly associated to the resistance to SCN race 3 were detected on chromosome 11, and among them rsGm11 37323330 has the greatest synergistic effect, and can be used for molecular marker assisted selection of soybean.

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