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GENETIC LINKAGE MAPS OF PEAR BASED ON SRAP MARKERS

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Abstract

Genetic maps for two pear cultivars 'Red Bartlett' (*Pyrus communis* L.) and 'Nanguo pear' (*Pyrus ussuriensis* Maxim.) were constructed using SRAP molecular markers. The mapping population consists of 74 F1 individuals derived from the cross of a cultivar 'Red Bartlett' with good fruit quality as the female parent and a cultivar 'Nanguopear' with good cold tolerance as the male parent. Linkage maps for both parents were built with Joinmap3.0. 103 markers have been mapped to 20 linkage groups covering 602.2cM with an average distance between markers of 4.89cM on maternal map, while 105 markers have been mapped to 20 linkage groups covering 650cM with an average distance between markers of 5.20cM on the paternal map. The map could lay a foundation for high density molecular genetic map construction for pear, and provide technique support for cold hardiness QTL detection and MAS (marker assisted selection) in the future.

Introduction

High density genetic linkage map is the prerequisite for gene mapping and MAS (marker assisted selection). It's difficult to create an ideal mapping population in a short time due to its high heterozygosity, large plant, self incompatibility and long life cycle. These limited the development of genetic mapping studies. Since Hemmat *et al*., (1994) proposed the "double pseudo test cross" and applied it to the molecular genetics map construction for fruit tree, the fruit tree genetic mapping research has experienced a rapid developing period. So far, genetic maps have been built in most of the fruits, such as peach (Rajapakse *et al*., 1995; Dirlewanger *et al*., 1998; Blenda *et al*., 2007), apple (Hemmat *et al*., 1994; Kenis *et al*., 2005), pear (Iketani *et al*., 2001; Yamamoto *et al*., 2007; Pierantoni *et al*., 2007; Terakami *et al*., 2009), grape (Welter *et al*., 2007; Xu *et al*., 2008), citrus (Chen *et al*., 2008), olive (La *et al*., 2003), sour cherry (Wang *et al*., 1998), kiwifruit (Testolin *et al*., 2001), apricot (Vilanova *et al*., 2003), papaya (Ma *et al*., 2004) and lychee (Zhao *et al*., 2011).

Pyrus ussuriensis Maxim., which is native to China, is one of the most cold hardy species in *Pyrus*, and is widely cultivated in Northeast China. It is a good material for cold hardiness breeding. Previous genetic linkage maps mainly focused on the research of *Pyrus communis* L. and Japanese pear (Dondini *et al*., 2004; Yamamoto *et al*., 2007; Pierantoni *et al*., 2007; Iketani *et al*., 2001; Yamamoto *et al*., 2002) , however there is no report about *Pyrus ussuriensis* Maxim.

Sequence-related amplified polymorphism (SRAP) was developed by Li *et al*., (2001), which aimed for the amplification of open reading frames (ORFs). The forward primer is 17bp long containing a fixed sequence of 14 nucleotides rich in C and G, and three selective bases at the 3' end. This primer preferentially amplifies exonic regions, which tend to be rich in C and G. The reverse primer is 18bp long, containing a sequence of 15 nucleotides, rich in A and T, and three selective bases at the 3' end. This primer preferentially amplifies intronic regions and regions within promoters, which tend to be rich in A and T. The polymorphism was fundamentally originated from the variation of the length of these introns, promoters and spacers, both among individuals and among species. SRAPs were easily amplified in crops such as potato, rice and lettuce, showing that SRAP combines simplicity, reliability, moderate through-put ratio and facile sequencing of selected bands (Li *et al*., 2001).

Here we report a linkage map based on SRAP molecular analysis of the F1 population derived from the cross between 'Red Bartlett' (*Pyrus communis* L.) and 'Nanguo pear' (*Pyrus ussuriensis* Maxim.) so as to provide support for QTL (quantitative trait locus) mapping and MAS in the future.

Materials and Methods

Plant materials: Experiments were carried out in molecular biology lab, Shenyang Agricultural University. Seventy four F1 individuals obtained from an interspecific cross between the European pear cultivar (*Pyrus communis* L.) 'Red Bartlett' and the Chinese pear (*Pyrus ussuriensis* Maxim) cultivar 'Nanguo pear', were used in this study. The double pseudo-testcross strategy was adopted to construct genetic linkage maps. All the plant materials were obtained from Pomology institute, Liaoning Agricultural Institute.

DNA extraction: Genomic DNA of all the materials was extracted from young leaves according to the modified CTAB method described in Murray *et al*., (1980) and Dellaporta *et al*., (1983).

SRAP reaction system and amplification protocol: The reaction system was 20μL, including: 1×PCR buffer, $MgCl_2$ 2.0 mmol·L^{-1}, dNTPs 0.1 mmol·L^{-1}, primer 0.5 μmol·L^{-1}, template DNA15 ng, Taq DNA polymerase 1.5U. The protocol for PCR amplification was: initial denaturation (5 min at 94°C); denaturation (60s at 94°C),annealing (60s at 35°C), extension (90s at 72°C), for 5 cycles; denaturation (60s at 94°C), annealing (60s at 50°C), extension (90s at 72°C), for 35 cycles; final extension (10min at 72°C). The amplification products were separated by electrophoresis on 6% polyacrylamide gels.

Data analysis: According to the results of electrophoresis, if there was an amplified band (band present) it was scored as 1, otherwise (band absent) scored as 0. 'CP' model was applied to construct the genetic map (Van Ooijen & Voorrips, 2001). A LOD score of 4.0 was used to define LGs, and map distances

were calculated according to Kosambi's mapping function (Kosambi, 1943). Genetic linkage maps were drawn using MapChart 2.2 software (Voorrips, 2002).

Results

Primer screening and separated markers screening: Six randomly chosen F1 progenies, along with 2 parents were used as a small population. Using this small population, we screened 240 SRAP primer pairs, among which 48 generated stable, clear and polymorphic bands. These 48 primer pairs were used to amplified the large population (74 individuals), and then the segregation situation was scored according to electrophoresis results (Fig. 1, Table 1).

Three hundred and twenty five polymorphic markers were obtained between parents, among which 102 markers are peculiar to female parent (female loci), 95 to male parent (male loci) and 128 common to both parents (meaning that markers present in both parents but separate in the population, the ratio being 3:1 theoretically). X^2 test revealed that, among the markers peculiar to male parent, 19 (20.00%) showed distorted separation; among the markers peculiar to female parent, 16 (15.69%) showed distorted separation; among common markers, 20 (15.63%) showed distorted separation.

Table 1. Forward and reverse primer sequences used in SRAP analysis.

Forward primers	Reverse primers
me1 5'-TGAGTCCAAACCGGATA-3'	Em1 5'-GACTGCGTACGAATTAAT-3'
me2 5'-TGAGTCCAAACCGGAGC-3'	em2 5'-GACTGCGTACGAATTTGC-3'
me3 5'-TGAGTCCAAACCGGAAT-3'	em3 5'-GACTGCGTACGAATTGAC-3'
me4 5'-TGAGTCCAAACCGGACC-3'	em4 5'-GACTGCGTACGAATTTGA-3'
me5 5'-TGAGTCCAAACCGGAAG-3'	em5 5'-GACTGCGTACGAATTAAC-3'
me6 5'-TGAGTCCAAACCGGTAG-3'	em6 5'-GACTGCGTACGAATTGCA-3'
me7 5'-TGAGTCCAAACCGGTTG-3'	em7 5'-GACTGCGTACGAATTATG-3'
me8 5'-TGAGTCCAAACCGGTGT-3'	em8 5'-GACTGCGTACGAATTAGC-3'
me9 5'-TGAGTCCAAACCGGTCA-3'	em9 5'-GACTGCGTACGAATTACG-3'
me10 5'-TGAGTCCAAACCGGAGG-3'	em10 5'-GACTGCGTACGAATTTAG-3'
me11 5'-TGAGTCCAAACCGGAGA-3'	em11 5'-GACTGCGTACGAATTTCG-3'
me12 5'-TGAGTCCAAACCGGAAA-3'	em12 5'-GACTGCGTACGAATTGTC-3'
me13 5'-TGAGTCCAAACCGGAAC-3'	em13 5'-GACTGCGTACGAATTGGT-3'
me14 5'-TGAGTCCAAACCGGACA-3'	em14 5'-GACTGCGTACGAATTCAG-3'
me15 5'-TGAGTCCAAACCGGACG-3'	em15 5'-GACTGCGTACGAATTCTG-3'
me16 5'-TGAGTCCAAACCGGACT-3'	em16 5'-GACTGCGTACGAATTCGG-3'
me17 5'-TGAGTCCAAACCGGCAT-3'	em17 5'-GACTGCGTACGAATTCCA-3'
me18 5'-TGAGTCCAAACCGGGAC-3'	em18 5'-GACTGCGTACGAATTGAT-3'
me19 5'-TGAGTCCAAACCGGGTA-3'	em19 5'-GACTGCGTACGAATTCAA-3'
me20 5'-TGAGTCCAAACCGGGGT-3'	em20 5'-GACTGCGTACGAATTCAT-3'
me21 5'-TGAGTCCAAACCGGCAG-3'	em21 5'-GACTGCGTACGAATTCTA-3'
me22 5'-TGAGTCCAAACCGGCTA-3'	em22 5'-GACTGCGTACGAATTCTC-3'

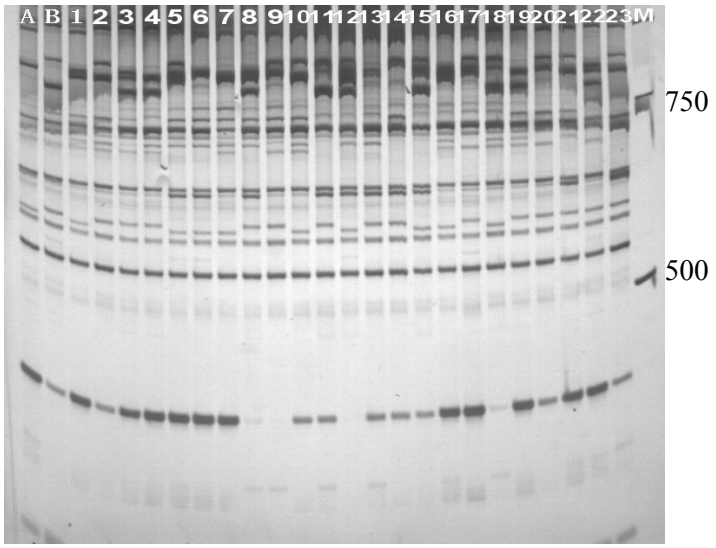

Fig. 1. A representative gel showing amplification result of parents and F_1 of Pear individuals using primer combination Me2/Em6 A. Red Bartlett ; B. Nanguopear; M. DNA maker; NO. 1□23 indicate F1 progenies of Red Bartlett × Nanguopear

Map construction: Using Joinmap 3.0 (LOD=3.0, maximum recombination value=0.4), 'CP' model was applied to construct the genetic map. The recombination rate was converted into map distance, cM. Maps were then drawn using Mapchart 2.2.

One hundred and three markers were mapped to 20 linkage groups in maternal map (Fig. 2), which covers 602.2cM with an average distance between markers of 4.89cM. The average linkage group length was 30.11cM. There were 5.15 markers per linkage group. The largest linkage group (RB1) contained 35 markers, and the total length was 97.1cM. 8 linkage groups contained only 2 markers.

One hundred and five markers were mapped to 20 linkage groups in paternal map (Fig. 3), which covers 650.05cM with an average distance between markers of 5.20cM. The average linkage group length was 32.5cM. There were 5.25 markers per linkage group. The largest linkage group (NG1) contained 40 markers, and the total length was 87.1cM. 10 linkage groups contained only 2 markers (Table 2).

Discussion

High-density linkage maps would also help to locate genes of interest for MAS and to identify quantitative trait loci (QTL). A framework map calls for an average distance between markers of no more than 20cM on the chromosome. QTL mapping requires an average distance between markers of less than 10cM. Gene clone requires an average distance between markers of less than 1cM.

With the development of molecular biology technique, woody fruit crop genetic mapping research has gained great progress in recent years even if it started late. Satisfactory genetic maps of ideal marker number and density have been constructed in apple, citrus and peach etc. and gene mapping has been done for fruit quality and disease resistance etc. (Kenis *et al*., 2007; Welter *et al*., 2007; Xu *et al*., 2008; Chen *et al*., 2008; Blenda *et al*., 2007).

The first pear map was constructed by Iketani *et al*., (2001) based on RAPD markers where 81 progeny plants from a cross 'Kinchaku' x 'Kosui' were used as the mapping population. For maternal map 'Kinchaku', 120 markers have been mapped into 18 linkage groups covering 768cM. For paternal map 'Kosui', 78 markers have been mapped into 22 linkage groups covering 508cM. Afterwards, Yamamoto *et al*., (2002) used 63 progenies derived from the cross between 'Bartlett' and 'Housui' as the mapping population and constructed the parental genetic maps by AFLP, SSR, isozymes and phenotypic marker. Terakami *et al*., (2009) increased the density of 'Housui' map, and compared with the apple map through co-dominant SSR markers. Dondini *et al*., (2004) used 99 progenies from the cross between 2 European pear cultivars ('Passe Crassane' x' Harrow Sweet') as the mapping population, and constructed the parental genetic maps. For maternal map 'Passe Crassane', 155 markers have been mapped into 18 linkage groups covering 912cM. For paternal map 'Harrow Sweet', 156markers have been mapped into19 linkage groups covering 930cM. Yamamoto *et al*., (2007) reported genetic linkage maps of the European pear cultivars 'Bartlett' and 'La France' based on simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers. These maps were composed of 17 linkage groups (LGs), including 447 and 414 markers and covering 1,000 cM and 1,156 cM of 'Bartlett' and 'La France', respectively. Pierantoni *et al*., (2007) used 95 progenies from a cross between two European pear cultivars ('Abbé Fètel'× 'Max Red Bartlett') as the mapping population and constructed the parental genetic maps by AFLP and SSR markers. 'Abbé Fètel' map contained 18 linkage groups covering 908.1cM with an average distance between markers of 7.4cM. 'Max Red Bartlett' map contained 19 linkage groups covering 879.8cM with an average distance between markers of 8.0cM.

In pear genetic map research, the materials are mostly *Pyrus communis* L. and *Pyrus*. *pyrifolia*, while *Pyrus ussuriensis* Maxim. map has not been reported. The molecular marker used are mostly AFLP, SSR and RAPD etc. For AFLP marker, the usage of radioactive isotope and DNA of high purity (Shi *et al*., 2012; Ji *et al*., 2012) and concentration is demanded, while for RAPD and SSR marker(Gomez *et al*., 2011; Hu *et al*., 2012) a large amount of manpower and material resources is needed for primer design and developing. However, SRAP marker avoids the disadvantages of these existing molecular markers and focuses on the amplification of ORFs, thus enhancing the relativity of application results and phenotype, Which the ability of SRAPs in saturating molecular linkage maps were observed (Liu *et al*., 2013), it can compensate SSR markers in genetic map construction study and also QTL mapping study. What's more, the obtained marker might be closer to genes.

Pyrus ussuriensis Maxim. is one of the hardiest species in *Pyrus*. It is of great value in cold area and is the best material for pear cold hardiness breeding and research. In this present work, we created an F1 mapping population derived from the cross between a less hardy cultivar Red Bartlett (*Pyrus communis* L.) and a cold hardy cultivar Nanguo pear (*Pyrus ussuriensis* Maxim.). And we constructed the first *Pyrus ussuriensis* Maxim genetic linkage map with SRAP makers. Even though there exists marker number limitations and gaps on the map, it yet lays a foundation for further research on pear, for example, cold hardiness QTL mapping.

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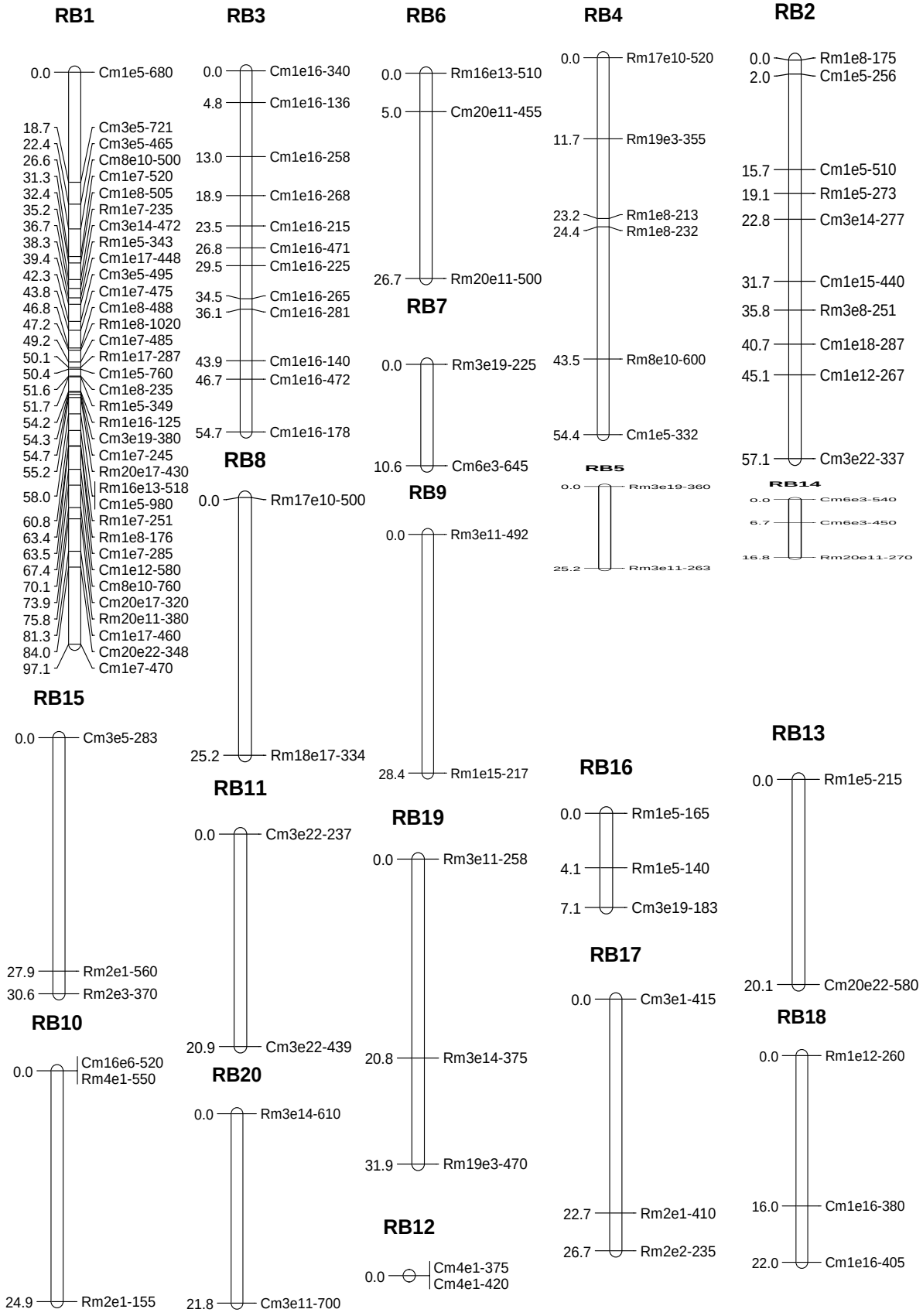

Fig. 2. Genetic linkage map of Red Bartlett.

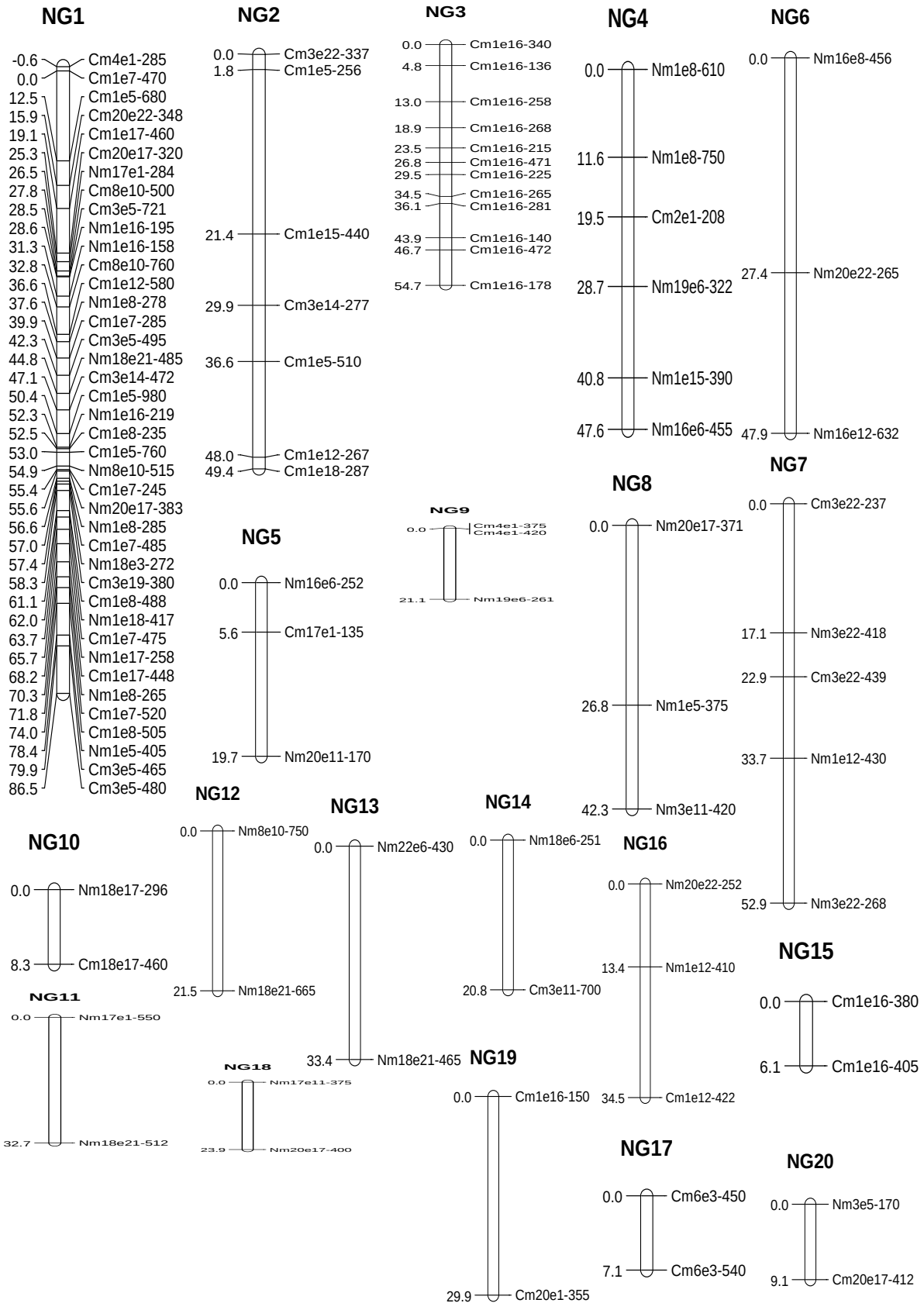

Fig. 3. Genetic linkage map of Nanguo pear.

Table 2. Main characteristics of the linkage groups.

Linkage group no.	Parent	Number of SRAP markers	Size (cM)	Gaps (>20 cM) number
1	Red Bartlett	35	97.1	0
	Nanguo pear	40	87.1	0
2	Red Bartlett	10	57.1	0
	Nanguo pear	7	49.4	0
3	Red Bartlett	12	54.7	0
	Nanguo pear	12	54.7	0
4	Red Bartlett	6	54.4	0
	Nanguo pear	6	47.6	0
5	Red Bartlett	2	25.2	1
	Nanguo pear	3	19.7	0
6	Red Bartlett	3	26.7	1
	Nanguo pear	3	47.9	2
7	Red Bartlett	2	10.6	0
	Nanguo pear	5	52.9	0
8	Red Bartlett	2	25.2	1
	Nanguo pear	3	42.3	1
9	Red Bartlett	2	28.4	1
	Nanguo pear	3	21.1	1
10	Red Bartlett	3	24.9	1
	Nanguo pear	2	8.3	0
11	Red Bartlett	2	20.9	1
	Nanguo pear	2	32.7	1
12	Red Bartlett	2	0	0
	Nanguo pear	2	21.5	1
13	Red Bartlett	2	20.1	1
	Nanguo pear	2	33.4	1
14	Red Bartlett	3	16.8	0
	Nanguo pear	2	20.8	1
15	Red Bartlett	3	30.6	1
	Nanguo pear	2	6.1	0
16	Red Bartlett	3	7.1	0
	Nanguo pear	3	34.5	0
17	Red Bartlett	3	26.7	1
	Nanguo pear	2	7.1	0
18	Red Bartlett	3	22.0	0
	Nanguo pear	2	23.9	1
19	Red Bartlett	3	31.9	1
	Nanguo pear	2	29.9	1
20	Red Bartlett	2	21.8	1
	Nanguo pear	2	9.1	0
Total	Red Bartlett	103	602.2	11
	Nanguo pear	105	650.0	10
Average	Red Bartlett	5.15	30.11	
	Nanguo pear	5.25	32.5	

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