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RESEARCH ARTICLE

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Construction of a molecular map for melon (*Cucumis melo* L.) based on SRAP

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Abstract A molecular map of melon (*Cucumis melo* L.) was constructed with SRAP (Sequence-Related Amplified Polymorphism) markers using a population consisting of 114 F_2 individuals derived from the cross of 4G21 (*C. melo* var. *chinensis*) and 3A832 (*C. melo* var. *saccherinus*). Twenty-nine primer pairs were used and 187 polymorphic loci were produced. The map consists of 12 linkage groups that include 152 genetic markers and cover 2077.1 cM with an average genetic distance of 13.67 cM. Every linkage group has 6–32 genetic markers with average genetic distance of 9.72–19.19 cM. The length of linkage group is 85.3–496.1 cM.

Keywords Melon (*Cucumis melo* L.), SRAP (Sequence-Related Amplified Polymorphism), molecular map

1 Introduction

Melon (*Cucumis melo* L.) is an important fruit that is a favorite of many people. Melon breeding depends on the innovation of breeding technique and germplasm resource. A high-density genetic linkage map is a base of quantitative trait loci (QTL), map-based cloning and molecular assisted selection (MAS). So far, there is no integrated classical genetic linkage map of melon. Pitrat (1991) analyzed 28 markers of systematic molecules and mapped 23 markers on 8 linkage groups. In the 1980s, with the rapid development of molecular marking, melon map construction experienced a rapid progress. Baudracco-Arnas and Pitrat (1996) finished the first map that comprised 107 markers on 14 linkage groups, and covered 1390 cM using F_2 population. Later, other 6 melon maps were constructed using BC (Backcross) population (Wang et al., 1997) and F_2 impermanent population (Liou et al., 1998; Danin et al., 2002; Silberstein et al., 2003; Oliver et al., 2001; Brotman et al., 2000, 2002). In recent years, permanent mapping populations have been applied step by step. Périn et al. (2002) and Gonzalo et al. (2004) have constructed melon maps using recombinant inbred lines (RIL) and doubled haploid (DH) populations (Danin-Poleg et al., 2001) respectively, merging and comparing different maps with co-dominant markers.

China is an important sub-origin area of western Asia thick-skinned melons and eastern Asia thin-skinned melons. However, there has been no report about constructing molecular maps of melon of Chinese germplasm resources in the development of melon genetic breeding in China, because of the weak base of melon research in genetics and breeding. SRAP is a novel molecular marker based on PCR which is simple and reliable (Li and Quiros, 2001). SRAP has been applied in map construction (Murry and Thompon, 1980), comparative genetics (Li et al., 2003) and genetic diversity (Ferriol et al., 2003). Our study used 4G21 (*C. melo* var. *chinensis*) and 3A832 (*C. melo* var. *saccherinus*) as parents and F_2 population to construct a molecular map of melon with SRAP markers. The aim was to establish a good foundation for melon molecular breeding.

2 Materials and methods

2.1 Plant materials and population generation

The mapping population was developed from the interspecific cross of 4G21 (*C. melo* var. *chinensis*) × 3A832

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(*C. melo* var. *saccherinus*) including 114 F_2 individuals derived from the self-population of a single F_1 plant.

2.2 DNA extraction

Genomic DNA was isolated from frozen leaves of F_2 individuals and parents via a CTAB procedure.

2.3 SRAP-PCR amplification

We designed 64 forward and reverse primers according to Li and Quiros (2001). For PCR amplification, each 10-μL PCR reaction mixture consisted of 50 ng genomic DNA, 50 ng primer, 100 $\mu mol \cdot L^{-1}$ dNTPs, 1 × Taq buffer and 0.5 unit Taq polymerase.

The procedure of PCR was conducted according to Li and Quirous, with some modifications as follows: initial denaturalization at 94°C for 3 min, followed by the first five cycles at 94°C for 30 s, 35°C for 45 s and 72°C for 1 min, for denaturing, annealing and extension, respectively. Then the annealing temperature was raised up to 50°C for another 35 cycles. The last was run at 72°C for 10 min.

2.4 SRAP analysis

The PCR products are separated using 6% denatured polyacrylamide gels (acrylamide:bisacrylamid=19:1). The gel was pre-run in 1 × TBE buffer at 2500 V and 80 W for 1 h; After loading the samples, the gel was electrophoresed at 2000 V of constant voltage for about 2 h until the xylene cyanol reached 2/3 of the gel surface towards the bottom and the temperature was kept under 50°C to avoid breaking the gel-support glass. After electrophoresis, the gel was stained using $AgNO_3$ solution (Bassam et al., 1991).

2.5 Data collection and analysis

For each SRAP locus, the genotype of 4G21 was marked as 'a' and that of 3A832 as 'b'. Accordingly, the genotype of individual F_2 plants was marked as 'a' or 'b', and the lost data were marked as '-'.

MAPMAKER/EXP3.0 was used to construct the linkage map of melon with the LOD $\geqslant$ 3.0 and the longest map distance $\leqslant$ 37.2 cM. Thereafter, markers were presequenced using the commands 'group' and 'order' and the sequence was confirmed using the 'ripple' and 'compare' commands. Markers that could not be confidently located were placed via the 'try' command. The recombination rate was converted into genetic distance using Kosambi function.

2.6 Marker coding

The loci were encoded by 'primer combination + locus number'. For example, a locus name 'me2em1-1' represents the first locus produced by the primer combination of me2 and em1.

3 Results and analysis

3.1 Marker distribution

Fifteen forward primers were randomly selected and combined with 64 reverse primers into a total of 960 primer pairs. Among them, 29 primer pairs with better polymorphisms and clearer bands were picked out to amplify and analyze the F_2 population. A single primer combination could generate 1–15 polymorphic bands. One hundred and eighty-seven polymorphic bands were generated in all with an average of 6.45 per primer pair (Table 1).

Table 1 Distribution of polymorphic loci in mapping parents generated by 29 SRAP primer pairs

primer pair	polymorphism distribution		number of polymorphism	primer pair	polymorphism distribution		number of polymorphism
	4G21	3A832			4G21	3A832	
me1em18	7	4	11	me17em62	3	3	6
me2em1	6	4	10	me21em2	1	1	2
me3em24	2	2	4	me21em7	1	1	2
me3em42	2	2	4	me21em20	1	1	2
me3em51	2	2	4	me21em21	3	0	3
me3em53	6	5	11	me45em1	2	2	4
me10em7	0	1	1	me45em2	5	5	10
me10em55	2	2	4	me45em7	2	0	2
me12em11	0	2	2	me45em9	5	7	12
me45em32	6	1	7	me45em35	3	3	6
me45em42	6	4	10	me45em45	4	4	8
me45em58	4	11	15	me46em6	7	1	8
me49em34	3	6	9	me49em56	3	3	6
me49em62	2	4	6				
average	3.31	3.14	6.45				
amounts	96	91	187				

The frequency of the 4G21 and 3A832 alleles of all the loci studied was 48.7% (96) and 51.3% (91) respectively, fitting a 1:1 ratio ($P = 0.796$). Skewed markers did not show systemic bias towards either parent.

3.2 Distortion analysis

Chi-square test indicated that 179 (95.92%) of the 187 markers conformed to Mendelian expectation. Thus, only 8 (4.07%) of the markers exhibited distorted segregation; the P values computed for these loci indicated that 2 (1.07%) of them showed a lower level of distortion ($P < 0.05$) while 6 (3.2%) showed a highly skewed distortion ($P < 0.01$). Then all the markers were used to construct a linkage map with Mapmaker 3.0. It was found that 35 (18.7%) markers remained unlinked, and 1 marker was distorted at a ratio of 3:1.

3.3 SRAP map construction in melon

Among the 187 bands scored, 152 markers were grouped at a LOD $\geqslant$ 3.0 in 12 linkage groups (Fig. 1; Table 2). From Fig. 1 and Table 2 we can see that: (i) 152 markers were arranged in 12 linkage groups, which was the same number as the basic chromosome number of the species. (ii) The distribution of loci was even. The map spanned

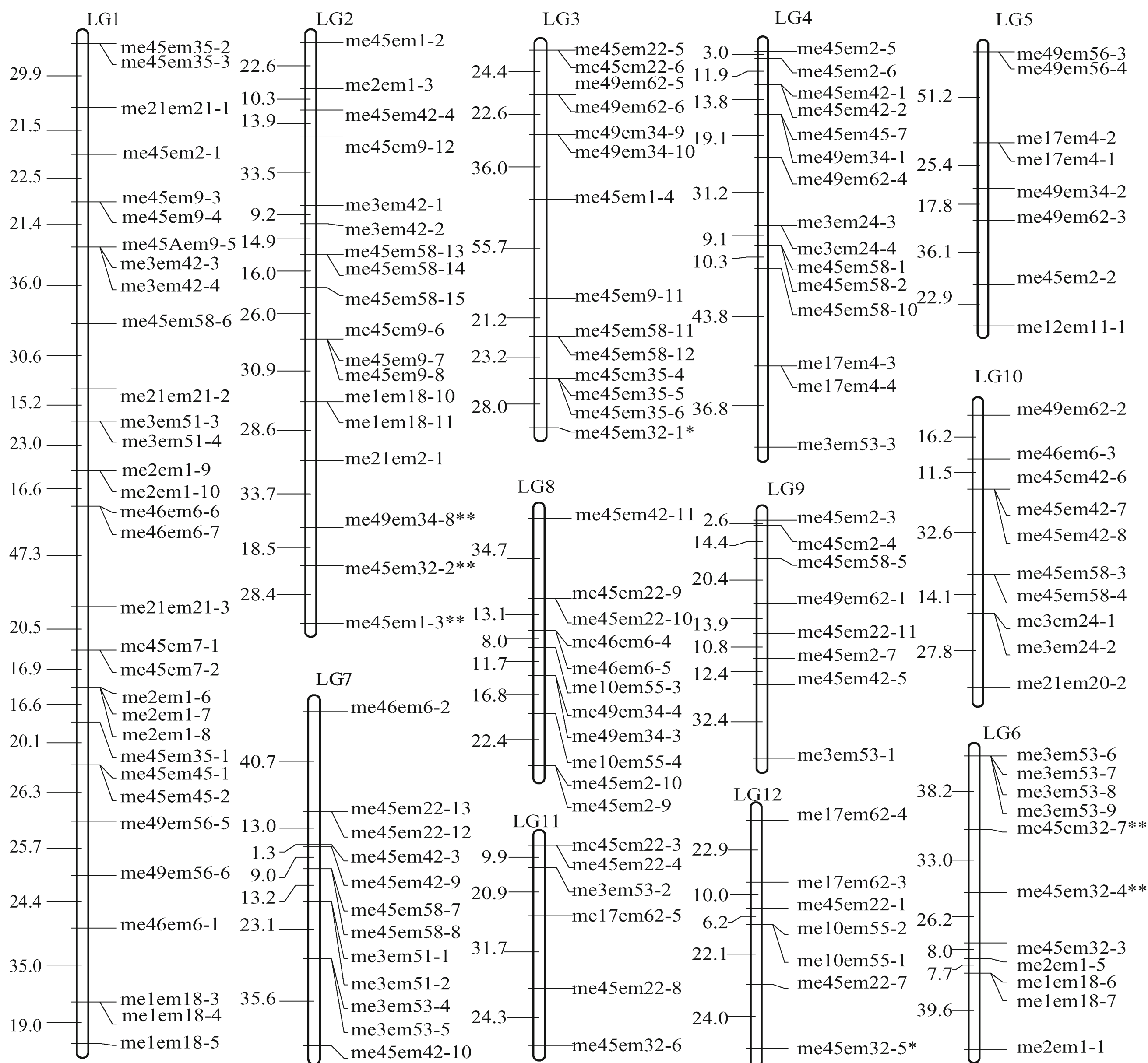

Fig. 1 Molecular genetic map of melon (*Cucumis melo* L.)

Note: Absolute distance values in cM are indicated on the left side of linkage groups and locus names on the right. * and ** indicate $P < 0.05$ and $P < 0.01$, respectively.

Table 2 Distribution of 152 molecular markers on the map

linkage group	length/cM	number of markers	number of distorted markers	maximum distance/cM	average distance/cM
LG1	469.1	32	0	43.4	14.7
LG2	286.9	18	3($P < 0.01$)	33.5	15.94
LG3	211.4	14	1($p < 0.05$)	55.7	15.1
LG4	179.2	15	0	43.8	11.95
LG5	153.5	8	0	51.2	19.19
LG6	153.0	11	2($P < 0.01$)	39.6	13.67
LG7	135.9	12	0	40.7	11.33
LG8	106.9	11	0	34.7	9.72
LG9	106.8	8	0	32.4	13.35
LG10	102.3	10	0	32.6	10.23
LG11	86.8	6	0	31.7	14.47
LG12	85.3	7	1($P < 0.05$)	24.0	12.19
amounts	2077.1	152	7	38.6	13.67

2077.1 cM, with an average marker interval of 13.67 cM. The density of markers among the linkage groups ranged from 9.72 cM per marker in LG8 to 19.19 cM per marker in LG5. The number of markers among the linkage groups ranged from 6 in LG11 to 32 in LG1. The longest linkage group spanned 469.1 cM, and the shortest one spanned 85.3 cM. The maximum marker interval was 55.7 cM in LG 3. A total of 7 (4.6%) distorted markers distributed in 4 linkage groups. LG2 and LG6 contained 3 (em45me1–3, em45me32–2, em49me34–8) and 2 (em45me32–4, em45me32–7) distorted markers respectively. They all highly deviated from the expected ratio ($P < 0.01$) and clustered in a small area.

4 Discussion

So far, several molecular maps of melon have been constructed by RFLP, RAPD, AFLP, SSR, ISSR and systematic molecular. The study used a population consisting of 114 F_2 individuals derived from the cross of 4G21 (*C. melo* var. *chinensis*) and 3A832 (*C. melo* var. *saccherinus*) to construct the first molecular map of melon (*Cucumis melo* L.) with SRAP markers in China. From the whole process, we can draw the following conclusions:

4.1 SRAP marker has a high polymorphism to mapping parents of melon

Twenty-nine SRAP primer pairs were used to detect the polymorphisms between 4G21 (*C. melo* var. *chinensis*) and 3A832 (*C. melo* var. *saccherinus*) and generated 187 polymorphic bands with an average of 6.45 polymorphic bands per primer pair. The primer pairs showing polymorphism produced 1–15 polymorphic bands. Baudracco-Arnas and Pitrat (1996) used 13 RAPD polymorphic primers and generated 16 polymorphic bands with an average of 1.23 per primer. Liou et al. (1998) found that in RAPD analysis, restriction enzymes were used to digest template DNA before PCR was very helpful to detect new polymorphisms. They used 60 RAPD primers and produced 125 polymorphic bands with an average of 3.1 per primer. Though our study had different parents from the above mentioned study, relatively, SRAP marker had a high polymorphism to mapping parents of melon. The result showed that SRAP marker has a high polymorphism to mapping parents of melon.

4.2 SRAP marker has a low distortion

Distortion is a universal phenomenon in molecular map construction of plants including melon (*Cucumis melo* L.). In Baudracco–Arnas and Pitrat's (1996) study in melon, RAPD was used, the percentage of skewed markers was 12%; Wang et al. (1997) found that the percentage of skewed markers was 14% with AFLP. Liou et al. (1998) used restriction enzymes to digest template DNA and 19.4% markers distorted expected ratio with RAPD. Oliver et al. (2001) drew a conclusion that the percentage of skewed markers was 5.8% by RAPD and AFLP. Périn et al. (2002) found that 19.8% markers skewed away expected ratio by IMA and AFLP. In our study, the percentage of skewed markers was 4.6%, lower than that previously reported in melon. The result showed that SRAP marker is fit for constructing the melon map.

At present, there are 3 kinds of hypothesis to explain the distortion. First, markers tightly linked to the gene affecting the distortion are highly distorted (Xu et al., 1997). Second, gametophyte's selection leads to distortion and distorted markers are segregated with the ratio of 0:2:1 (parent 1 : progeny : parent 2). Finally, effect of the pollen selection results in distortion and distorted markers are segregated with the ratio of 0:1:1 (parent 1 : progeny : parent 2) (Haanatra et al., 1999). In our study, 8 markers were distorted and there was no obvious deflection to either parent. The distortion may be related to the first reason, but cannot exclude the effect of artificialness and surroundings in the procedure of established F_2 population.

4.3 The distribution of SRAP loci is even

An ideal molecular map has short intervals and has no clusters on a linkage group. In our study, the map consists of 12 linkage groups, which include 152 genetic markers, and cover 2077.1 cM with an average genetic distance of 13.67 cM. One hundred and fifty-two loci were not clustered on the linkage groups. However, the number of loci was small and the interval between two markers was long. The maximum marker interval was 55.7 cM in LG3. The genome size of melon had been estimated between 2276–3250 cM. When a map is applied to quantitative trait loci (QTL) or map-based cloning, the mean interval between two markers of the intended area should be less than 1 cM. However, the existing melon map cannot meet the requirement. The maps constructed by Oliver et al. (2001) and Périn et al. (2002) had small mean intervals of 3.0 cM and 2.5 cM, respectively, but the loci had clusters on the chromosome. In order to fill the gap and diminish the mean interval of the map, we need to: (i) establish a population derived from different crossing, and (ii) to apply the characteristics of different molecular techniques to construct a map or merge the maps to improve the density and the usefulness of the maps.

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