Construction of a cucumber genetic linkage map with SRAP markers and location of the genes for lateral branch traits

WANG Gang*, PAN Junsong*, LI Xiaozun, HE Huanle, WU Aizhong & CAI Run

Agriculture and Biology School of Shanghai Jiaotong University, Shanghai 201101, China
Correspondence should be addressed to Cai Run (email: cairun@sjtu.edu.cn)

Received July 28, 2004

Abstract Using SRAP (sequence-related amplified polymorphism) markers a genetic linkage map of cucumber was constructed with a population consisting of 138 F_2 individuals derived from a cross of the two cucumber lines, S06 and S52. In the survey of parental polymorphisms with 182 primer combinations, 64 polymorphism-revealing primer pairs were screened out, which generated totally 108 polymorphic bands with an average of 1.7 bands per primer pair and at most 6 bands from one primer pair. The constructed molecular linkage map included 92 loci, distributed in seven linkage groups and spanning 1164.2 cM in length with an average genetic distance of 12.6 cM between two neighboring loci. Based on this linkage map, the quantitative trait loci (QTL) for the lateral branch number (lbn) and the lateral branch average length (lbl) in cucumber were identified by QTLMapper1.6. A major QTL lbn1 located between ME11SA4B and ME5EM5 in LG2 could explain 10.63% of the total variation with its positively effecting allele from S06. A major QTL lbl1 located between DC1OD3 and DC1EM14 in LG2 could account for 10.38% of the total variation with its positively effecting allele from S_{06}.

Keywords: cucumber, SRAP (sequence-related amplified polymorphism), molecular linkage map, lateral branch gene, quantitative trait loci (QTL).

DOI: 10.1360/062004-11

Cucumber (*Cucumis sativus* L. $2n = 2x = 14$), that belongs to Cucurbitaceae family, is one of major vegetables with a planting area second to that of tomato in the world[1]. Due to its economical importance plant breeders and geneticists have paid much attention to the genetic study on this important vegetable crop, but the research progress in cucumber is much less than that in tomato. In 1990, Pierce[2] reviewed all the reported genes of cucumber that had been genetically analyzed since the 1930s and integrated them into a linkage map. Later Kennard[3] constructed two cucumber linkage groups with isozyme markers alone and isozyme plus morphological trait markers, respectively. But DNA molecular markers, such as RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphism DNAs), had still not been applied to cucumber until 1995, when Lee[4] developed the first molecular linkage map with 28 RAPD markers only. So far four molecular linkage maps in cucumber have been reported, of which two

* These authors contributed equally to this work

were constructed by Kennard with RFLP, RAPD and isozymes for a narrow (58 loci) and a wide cross (70 loci), respectively, while the third one was constructed with 80 RAPD loci by Serquen[5]. As compared with the above three molecular maps, the fourth is a much saturated linkage map including 131 loci in seven linkage groups with a total coverage of 706 cM, that was reported in 2003[6].

It has been known that a high-density genetic linkage map is of great importance to molecular assisted selection (MAS), quantitative trait loci (QTLs) mapping, map-based cloning, and comparative genomic studies. But, in the reported cucumber linkage maps, both of the marker densities and marker types are far less than enough to meet the requirement for the above applications. To saturate the cucumber molecular linkage map more markers should be explored and added. Nevertheless, the polymorphisms between cucumber varieties revealed by the general DNA markers, such as RAPD, RFLP, and SSRs (simple sequence repeats), have been proved too poor to make a saturated map, that may be attributed to the narrow genetic basis of cucumber[7−10]. So it is required to apply new type of markers to the cucumber linkage map construction. Recently, a new marker system, sequence-related amplified polymorphism (SRAP), was developed by Li and Quiros[11], which is aimed for the amplification of open reading frames (ORFs). The forward primer is 17 bp 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 18 bp 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 with promoters, which tend to be rich in A and T. The polymorphism revealed by SRAP fundamentally originates from the variation in the length of the introns, promoters, and spacers among individuals and among species. SRAP combines simplicity, reliability, moderate through-put ratio and facile sequencing of selected bands, which has been applied in genetic linkage map construction, transcriptome map construction[12], comparative genetics and genetic diversity in a number of crops, including cauliflower, Chinese cabbage, potato and cotton[13].

Lateral branch growing ability is an important agronomic trait in cucumber because the strong lateral growing ability may affect the main stem growth and have some advantages or disadvantages depending on the mode of production management. In China, cucumber lines with weak lateral branch growing ability are much preferred than the strong ones, whereas, in the United States and other western countries, the cucumber lines with strong lateral growing ability are popular due to their suitability to autonomous robots harvesting. In this study, to genetically analyze and map the gene for the lateral branch trait in cucumber, we constructed a molecular marker linkage map with an F_2 population from a cross between the cucumber line S52 and S06 by using SRAP markers.

1 Materials and methods

1.1 Segregating population

The two cucumber parental lines, S06 and S52, were used to develop an F_2 mapping population including 138 F_2 individuals, which were selected randomly from a total of 200 F_2 individuals planted in the green house. The S06 line (from Isareal) has a multiple and strong lateral branching habit, but the S52 (from Dabie mountain of China) dose not possess this habit.

1.2 Field evaluation and characteristics examined

The lateral branch numbers, the lateral branch average length and node numbers of lateral branch were evaluated approximately 50 days after cucumber seedling was planted. In order to control environmental effects during the cucumber growing a series of standard cultivation measures, such as cultivation-in-bag, drop watering and fertilization were taken.

1.3 DNA extraction

Genomic DNA was extracted from freeze-dried leaf tissue of S06, S52 and F_2 individuals according to the CTAB method[14].

1.4 PCR amplification and product analysis

The SRAP primer sequences used in this study are listed in table 1. The sequences of ME1-ME5, DC1, PM8, EM1-EM6, OD3, SA4, and GA18 were provided by Li and Quiros[11,12]; the sequences of ME7 and ME8 were by Ferriol et al.[15]; and those of EM14 and EM15 (that is represented by EM8 in Table 1) by Lin et al.[13]. The other primer sequences were designed by us. A total of 182 different combinations with 13 forward and 14 reverse primers were employed (Table 1). All the primers were synthesized by Sangon Biological Engineering Technology and Service Co. Ltd., Shanghai.

Reactions were performed in 20 μL volume in 0.2 μL polypropylene tubes containing 60 ng of DNA, 30 ng of primer, 200 μmol/L dNTPs, 1.5 mmol/L $MgCl_2$, 1x*Taq* buffer and 0.5 unit of *Taq* polymerase (Progmega). The procedure of PCR was basically according to Li and Quiros[11], and modified as follows: 94℃ 3 min; 94℃ 30 s, 37℃ 30 s, 72℃ 90 s, 8 cycles; 94℃ 30 s, 48℃ 30 s, 72℃ 90 s, 32 cycles; 72℃ 7 min. The products were segregated on 4% denatured polyacrylamide gels. The gel was run at 70 W constant power for 1.5－2 h until xylene cyanol reached the 2/3 of the gel towards the bottom and the temperature was kept under 50℃ after loading sample. After electrophoresis, the gel was stained by $AgNO_3$ solution[16].

1.5 Data collection and markers named

For each of SRAP loci the genotype of S06 was recorded as 1 and that of S52 as 2. In accordance, the genotypes of the individual F_2 plants were scored as 1 or 2, and the lost data were scored as 0. The located markers were named after primer combination. For instance, ME9EM2 represents the combination of ME9 and EM2, and the followed A,B, and C represent the first, second and third band (locus) that derived from the same combination, respectively, from top to bottom in the gel.

1.6 Genetic linkage map analysis

MAPMAKER/EXP3.0[17] was used to construct the genetic linkage map of cucumber with the LOD≥3.0 and the longest distance≤37.2 cM. The recombination rate was converted into genetic distance using Kosambi mapping function.

1.7 Genetic analysis of lateral branch traits

QTLMapper1.6[18] was used to map QTLs for the lateral branch number (lbn) and lateral branch mean length (lbl) with a log-likelihood (LOD) score threshold of 2.5.

2 Results

2.1 Screening polymorphisms between parents

Totally 182 SRAP primer combinations were used to survey the polymorphisms between the two

Table 1 SRAP primers and their sequences

Type	Name	Sequence (5′-3′)	Type	Name	Sequence (5′-3′)
Forward primers	ME1	TGAGTCCAAACCGGATA	Reverse primers	EM1	GACTGCGTACGAATTAAT
	ME2	TGAGTCCAAACCGGAGC		EM2	GACTGCGTACGAATTTGC
	ME3	TGAGTCCAAACCGGAAT		EM3	GACTGCGTACGAATTGAC
	ME4	TGAGTCCAAACCGGACC		EM4	GACTGCGTACGAATTTGA
	ME5	TGAGTCCAAACCGGAAG		EM5	GACTGCGTACGAATTAAC
	ME6	TGAGTCCTTTCCGGTAA		EM6	GACTGCGTACGAATTGCA
	ME7	TGAGTCCTTTCCGGTCC		EM7	GACTGCGTACGAATTCAA
	ME8	TGAGTCCTTTCCGGTGC		EM8	GACTGCGTACGAATTCTG
	ME9	TGAGTCCAAACCGGTAG		EM9	GACTGCGTACGAATTGAT
	ME10	TGAGTCCAAACCGGCAT		EM14	GACTGCGTACGAATTCAG
	ME11	TGAGTCCAAACCGGTCT		EM18	GACTGCGTACGAATTCCT
	DC1	TAAACAATGGCTACTCAAG		OD3	CCAAAACCTAAAACCAGGA
	PM8	CTGGTGAATGCCGCTCT		SA4	CCAAAACCTAAAACCAGGT
				GA18	GGCTTGAACGAGTGACTGA

parents, S06 and S52. Each primer pair could produce 20—30 clear bands separated from 100 bp to 800 bp in the gel, of which approximately 80% were strong bands. It was found that 64 primer pairs could reveal parental polymorphisms. From these selected primers 108 polymorphic bands were generated, that could be used as molecular makers in the F_2 population analysis.

2.2 Map construction

The segregation data of the 108 SRAP markers were used to construct a linkage map with MAPMAKER/EXP3.0, and 92 loci were mapped into seven linkage groups at a LOD≥3.0, which spanned 1164.2 cM in length with an average genetic distance of 12.6 cM between two loci while the greatest distance between two neighboring loci was 25.0 cM and the shortest 3.2 cM. The linkage groups had 7—22 markers, with the largest one covering 281.0 cM and the shortest covering 86.6 cM. All markers were distributed evenly in the whole linkage groups without clustering of loci in some linkage region.

2.3 The lateral branch phenotypes among the parental lines and their F_2 population

The significant difference in the lateral branch number between the two parental lines was observed 50 days after planting. At that time, S06 and S52 had 14 and 6 lateral branches in average, respectively, whereas in the F_2 population the lateral branch numbers showed a continuous distribution, in which 31 plants had the lateral branch numbers less than the average number of S52, four plants had the numbers more than the average number of S06, and the other 88 plants had the numbers between the two averages. As shown in Fig. 1, the lateral branch numbers in the F_2 population showed a typical normal distribution.

The lateral branch node numbers were also evaluated approximately 50 days after planting. At that time the lateral branches of the S52 plants were all shorter than the five nodes and those of the S06 plants were longer than the five nodes. With the lateral branch node number of five as a criterion to investigate the lateral branch node numbers distribution in the F_2 population, the individual plants could be divided into two groups. In the first group each plant had at least one lateral branch with five or more nodes while in the second group the plants had lateral branches all with less than five nodes, resulting in 98 individuals in the first group and 40 in the second. A *chi*-square test indicated that this segregation ratio was not significantly different from the expected 3:1, so the lateral branch trait, when judged with the criterion of the node number of five, could be genetically analyzed as a qualitative trait, termed lb.

In addition, we measured the lateral branch average lengths of the two parents and the F_2 individuals in the mapping population. The lateral branch average length of S06 and S52 were 29 and 8 cm, respectively, whereas those of the F_2 individual plants ranged from 1 to 43 cm with a peak at 16 cm, showing a normal distribution (Fig. 2).

2.4 Mapping of the genes for lateral branch traits

When the segregation data for the 108 SRAP loci and the lateral branch trait (lb) were integrated together with MAPMAKER/EXP3.0, the lb was located

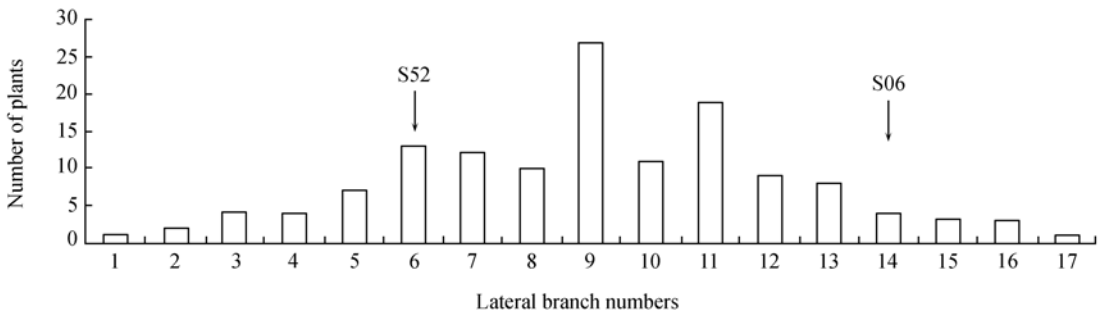

Fig. 1. Lateral branch number variation in S06, S52 and F_2 population (the arrows show the lbn of S52 and S06).

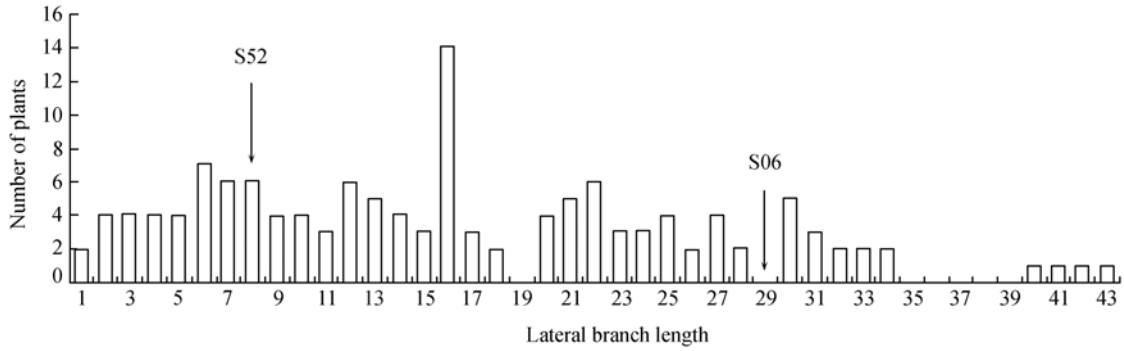

Fig. 2. Lateral branch length (lbl) variation in S06, S52 and F_2 population (the arrows show the lbl of S52 and S06).

between the marker DC1GA18 and ME11SA4B in linkage group 2 (LG2) with the genetic distances of 9.6 cM and 10.8 cM, respectively (Fig. 3).

Then QTLMapper1.6 was used to map the genes (QTLs) for the two quantitative traits of lateral branch, the lateral branch number (lbn) and the lateral branch average length (lbl) with a log-likelihood (LOD) score threshold of 2.5. For lbn one major QTL, lbn1, was detected between ME11SA4B and ME5EM5 on LG2, that could explain 10.63% of the variation, and its allele from S06 provided a positive effect on the lateral branch number (Table 2, Fig. 3). Other three QTLs with effect less than lbn1were detected too, which were located in LG1, LG2 and LG7, respectively (Table 2, Fig. 3).

For lbl four QTLs were identified (Table 2, Fig. 3). lbl1 was a major QTL located between DC1OD3A and DC1EM14 in LG2, that explained 10.38% of the total variation, and the allele from S06 had a positive effect on the lateral branch average length. Another QTL, lbl2, was also located in LG2 but between DC1EM2C and DC1GA18, that explained 6.22% of the total variation, and the allele from S06 had a positive effect too. The other two QTLs, lbl3 and lbl4, both with minor effect, were located in LG1 and LG7, respectively. The S06 allele of lbl3 had a positive effect on the trait but the S52 allele of lbl4 had a negative effect. It was noted that no epistatic effect between the QTLs of lbn and lbl was detected when they were analyzed in combinations.

3 Discussion

As a new marker system, SRAP has only been applied to a few crop plants[11−13]. In the study, we modified the SRAP procedure described by Li and Quiros[11], adapted it to our experiment with cucumber, and obtained satisfied results. In our laboratory, SRAP presented higher polymorphic primer ratio and generated more polymorphic bands as compared with RAPD (data not shown).

Of the 108 polymorphic bands, 96 markers showed a separation ratio of 3:1 in the F_2 population after a *chi*-square test for fit. Among the 12 markers unfitted for 3:1 separation ratio, eight leaned to S52 and four to S06. The skewed segregation may be attributed to the gamete selection. Notably, all skewed segregation markers could not be mapped into any linkage groups. We had constructed a genetic linkage map with only 93 individual plants from the same F_2 population, in which 74 loci had been mapped into eight linkage groups. In the present study, when the mapping population was expanded to 138 individuals, some previously unlinked makers were mapped into larger linkage groups, and the resulting linkage group numbers reduced to seven equal to the numbers of cucumber chromosome pairs. In the meantime, the average genetic distance (cM) between two adjacent markers shortened too. These results suggested that increasing the number of individuals in a mapping population is the most effective means to improve the linkage map quality.

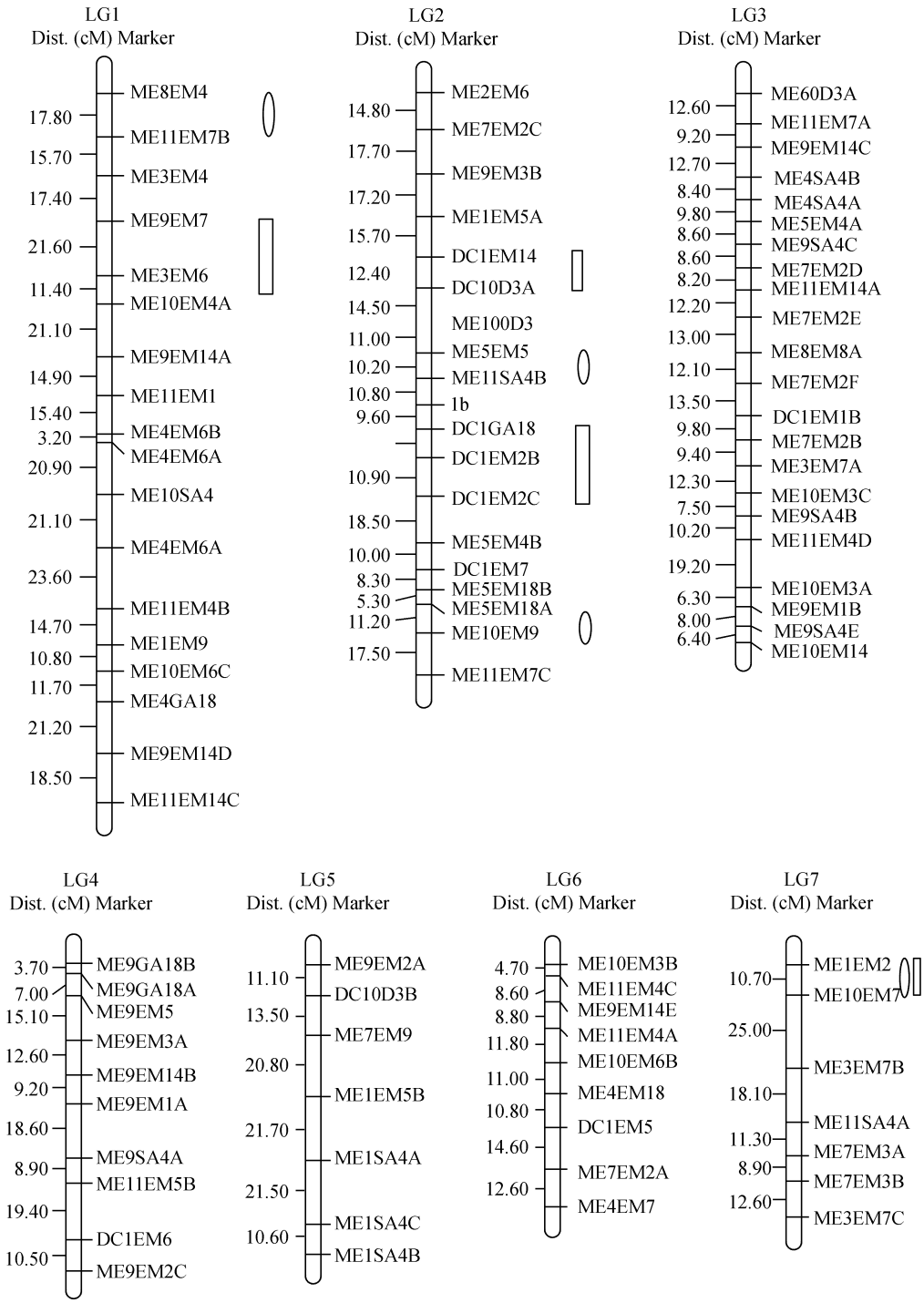

Fig. 3. Cucumber linkage map based on SRAP. The seven cucumber linkage groups are arranged as LG1—LG7. Dist. indicates the genetic distance (cM) between markers on the left of each linkage group. lb represents the gene for the lateral branch trait, ⬯ lbn represents the QTL for the lateral branch number, and ▯ lbl represents the QTL for lateral branch average length.

Table 2 QTLs for lateral branch number and lateral branch average length

QTL	Interval	Linkage group No.	LOD	Variance explained (%)	Effect
lbn1	ME11SA4B—ME5EM5	2	33.08	10.63%	0.9942
lbn2	ME10EM9—ME5EM18A	2	4.32	7.56%	0.6574
lbn3	ME11EM7B—ME8EM4	1	3.41	6.47%	0.7276
lbn4	ME10EM7—ME1EM2	7	4.44	9.33%	–0.8185
lbl1	DC1OD3—DC1EM14	2	3.52	10.38%	0.9886
lbl2	DC1EM2C—DC1GA18	2	23.52	6.22%	0.7577
lbl3	ME10EM4A—ME9EM7	1	2.64	7.65%	0.78168
lbl4	ME10EM7—ME1EM2	7	2.54	6.86%	–0.9857

SRAP has been used to constructed the linkage maps of cauliflower[11], Chinese cabbage[12] and cotton[13]. The SRAP linkage map of cauliflower includes nine linkage groups, and the greatest distance between any two loci is 34.6 cM. The SRAP map of cotton has 39 linkage groups, spanning 3030.7 cM with a genomic coverage of 65.4%. Its average genetic distance is 12.79 cM between adjacent markers with the biggest interval of 42.8 cM and the smallest one of 0.2 cM[13]. The SRAP linkage map we constructed consists of seven linkage groups spanning 1164.2 cM, in which the greatest genetic distance, shortest distance and the average distance between two adjacent loci are similar to those in the map of cauliflower and cotton. In addition, the resulting total genetic distance approaches the estimated total length of 800－1000 cM for cucumber[8]. These results demonstrated that SRAP is indeed a type of effective molecular marker, and can be easily applied to varieties of crops.

With the SRAP linkage map, we further analyzed three lateral branch traits, i.e., the lateral branch number (lbn), the lateral branch average length (lbl) and the lateral branch with over five nodes (lb), all of which are important breeding targets of cucumber, related to the growth and development of lateral branches. The node numbers of all lateral branches in S52 were less than five but the counterpart numbers in S06 were more than five when they were investigated 50 days after planting. With the criteria of five nodes, the segregation of the lateral branch trait (lb) in the F_2 population fitted for the 3:1 ratio. Previously, this trait had also been evaluated in other way, i.e., approximately 30 days after planting when the parent S52 still had no lateral branch or had the lateral branches shorter than 2 cm while the parent S06 and F_1 had their branches longer than 2 cm. In a smaller F_2 population from the same cross, with the lateral branch length of 2 cm as a criterion, we had observed the segregation ratio of the lateral branch trait fitting for 3:1 and mapped the gene into a region around the marker DC1EM2B in LG2 (data not shown), that is the same location where we mapped lb in the present study.

The lateral branch number (lbn) showed great difference between the two parental lines, S06 and S52, and a continuous distribution in the F_2 population, indicating that lbn was a quantitative trait, for which a total of four QTLs were detected. A major QTL lbn1 was located between ME11SA4B and ME5EM5 in LG2 and its allele from S06 had a positive effect on the lateral branch number. Another quantitative trait, the lateral branch average length (lbl) is related to the growth strength of lateral branches in cucumber, for which four QTLs were detected. A major QTL lbl1 was located between DC1OD3A and DC1EM14 in LG2, and its allele from S06 had a positive effect on the lateral branch length. When the QTLs for lbn and QTLs for lbl were compared in their linkage positions, we found lbn4 and lbl4 both were located in the same region between ME10EM7 and ME1EM2 in LG7, and their negative alleles were both from S52.

It was reported that SRAP is a kind of dominant marker but with some being co-dominant[11]. However, in this study, we did not find any co-dominant SRAP markers. Using dominant marker is unable to distinguish homozygote and heterozygote at a particular

marker locus among F_2 individual plants, therefore it is impossible to distinguish the dominant effect and additive effect of any QTL mapped between dominant markers. In addition, F_2 population is a kind of temporary segregation population, therefore, with an F_2 population, it is unable to replicate the QTL mapping experiments. Considering the weakness in our mapping population, we analyzed three lateral branch traits in the meantime, which are all related to the growth and development of lateral branches in cucumber. It is noticed that the mapped gene and QTLs were distributed in LG1, LG2 and LG7 but with an obvious concentration in LG2. Two QTLs (lbn1 and lbl2) and one gene (lb) are located in the same region between DC1EM2C and ME5EM5, indicating that a gene related to lateral branch growth and development may exist in this region.

In addition, we observed transgressive segregation of the two quantitative traits, lateral branch number and the lateral branch average length, in the F_2 population (Figs. 1 and 2). For example, some F_2 plants had lateral branches less and shorter than S52, as can be explained by the separation of the related QTLs. This result indicates that it is possible to breed new cucumber cultivars with less lateral branch numbers and weaker strength of lateral branch development by marker-assisted selection of the related QTLs.

Acknowledgements This work was supported by the Shanghai Municipal Scientific & Technological Commission (Grant Nos. 033107019) and Shanghai Municipal Agricultural Commission (Grant Nos. 2003-2-1-2).

References

1. Li, H. Z., The status and the development trends in cucumber planting of China, Vegetables, 2003, 8: 3－4.
2. Pierce, L. K., Whener, T. C., Review of genes and linkage groups in cucumber, HortScience. 1990, 26: 605－615.
3. Kennard, W. C., Poetter, K., Dijkhuizen, A. et al., Linkages among RFLP, RAPD, isozyme disease resistance and morphological markers in narrow and wide crosses of cucumber, Theor. Appl. Genet., 1994, 89: 42－48.
4. Lee, Y. H., Use of random amplified polymorphic DNAs for linkage group analysis in interspecific hybrid F2 generation of cucurbica, Journal of the Korean Society Horticultural Science, 1995, 36(3): 323－330.
5. Serquen, F. C., Bacher, J., Staub, J. E., Mapping and QTL analysis of horticultural traits in a narrow cross in cucumber (*Cucumis sativas* L.) using random amplified polymorphic DNA makers, Molecular Breeding, 1997, 3(4): 257－268.
6. Fazio, G., Staub, J. E., Genetic mapping and QTL analysis of horticultural traits in cucumber (*Cucumis sativus* L.) using recombinant inbred lines, Theor. Appl. Genet., 2003, 107: 864－874.
7. Zhang, H. Y., Wang, Y. J., Xu, Y. et al., RAPD used in analysis of genetic relationships of cucumber (*Cucumis sativus* L.) germplasm, Acta Horticulturae Sinica, 1998, 25(4): 345－349.
8. Staub, J. E., Serquen, F. C., Gupta, M., Genetic makers, map construction, and their application in plant breeding, HortScience, 1996a, 31: 729－741.
9. Dijkuizen, A., Kennard, W. C., RFLP variability and genetic relationships in cultivated cucumber, Euphytica, 1996, 90: 79－87.
10. Knerr, L. D., Staub, J. E., Genetic diversity in *Cucumis sativus* L. assessed by variation at 18 allozyme coding loci, Theor. Appl. Genet., 1989, 78: 119－128.
11. Li, G., Quiros, C. F., Sequence-related amplified polymorphism (SRAP), a new maker system based on a simple RCR reaction: Its application to mapping and gene tagging in *Brassica*, Theor. Appl. Genet., 2001, 103: 455－461.
12. Li, G., Gao, M., Yang, B., Quiros, C. F., Gene for gene alignment between the *Brassica* and *Arabidopsis* genomes by direct transcriptome mapping, Theor. Appl. Genet., 2003, 107: 168－180.
13. Lin, Z. X., Zhang, X. L., Nie, Y. C. et al., Construction of a genetic linkage map for cotton based on SRAP, Chinese Science Bulletin, 2003, 48(15): 1676－1679.
14. Clark, M. S., Plant Molecular Biology——A Laboratory Mannual, Heidelberg: Springer Verlag, Berlin, 1998.
15. Ferriol, M., Pico, B., Nuez, F., Genetic diversity of a germplasm collection of *Cucubita pepo* using SRAP and AFLP markers, Theor. Appl. Genet., 2003, 107: 271－282.
16. Zhu, Z. G., Jia, J. Z., Sun, Z. X., Improvement of AFLP protocal for silver-staining in rice, Chinese J. Rice Sci., 2002, 16(1): 71－73.
17. Lander, E. S., Green, P., Abrahamson, J. et al., MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations, Genomics, 1987, 1: 174－181.
18. Wang, D. L., Zhu, J., Li, Z. K. et al., User Manual for QTKMapper Version1.6, 1999.