Localization of genes for lateral branch and female sex expression and construction of a molecular linkage map in cucumber (*Cucumis sativus* L.) with RAPD markers*

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Abstract A cucumber (*Cucumis sativus* L.) molecular linkage map, including 79 random-amplified polymorphic DNAs (RAPD) and two genes, *lb* for lateral branch and *f* for female sex expression, is constructed from a cross between a line, S52, with weak lateral growing ability and staminate from Dabieshan Mountains area in China and another line, S06, with strong lateral growing ability and gynoecious from Europe. The map contains nine linkage groups and spans 1110.0 cM with an average distance of 13.7 cM between loci. The *lb* locus is located in a longer linkage group LG-2 and flanked by two markers, OP-Q5-1 and OP-M-2-2, at 9.3 cM and 15.9 cM, respectively. In the meantime, the RAPD loci, OP-Q5-2 and BC151, in a short linkage group were found to flank *f* at 13.7 cM and 13.4 cM, respectively. The construction of RAPD map has paved a way for further study of the genes for lateral branch, female sex expression and other agronomic traits in cucumber.

Keywords: cucumber (*Cucumis sativus* L.), lateral branch, gynoecy, RAPD, genetic mapping.

Cucumber (*Cucumis sativus* L.), with the obvious diversities in many agronomic traits, such as sex expression and lateral branch growing ability, is one typical species of the Cucurbitaceae family, which includes several economically important vegetable crops, and has been drawing much attention from breeders and geneticists[1—5]. Lateral growing ability is an important agronomic trait in cucumber because strong lateral growing ability may affect the main stem growth and cause much trouble to 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[6,7]. Sex expression is a unique trait in cucumber. Farmers prefer gynoecious cucumber with its advantage of having female flowers on every node and high output. Therefore, mapping the genes for lateral branch and gynoecy and developing their molecular markers are of great importance.

Molecular marker-assisted selection (MAS) is a new and efficient approach in crop breeding because it works through the molecular markers linked to targeted trait genes on the genome level and is immune to environment impacts and disturbances of allele's dominance and recessiveness. In addition MAS can be performed in the early time of plant growth to shorten breeding periods and to raise efficiency in breeding[8]. In 1987, Fanourakis et al.[9] constructed the first cucumber linkage group based on morphological trait markers. In 1990, Pierce et al.[4] reviewed all the reported genes that had been genetically analyzed since the 1930s and integrated a linkage map consisting of 43 morphological traits according to the relative genetic data. Two years later Vakalounakis discovered a recessive leaf shape marker and analyzed its linkageship with disease resistance and some other trait genes[10]. In 1996 Knerrd et al.[11] and Meglic et al.[12] constructed cucumber linkage groups using isozymes alone and isozymes plus mophorlogical traits, respectively. Along with the appearance of molecular markers such as restriction fragment length polymorphism (RFLP) and random amplified polymorphism DNAs (RAPD), construction of cucumber molecular genetic map became possible. Till now, in addition to a merged map presented by Bradeen et al.[5], three molecular linkage maps in cucumber each consisting of not less than 58 markers

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loci have been reported, of which two maps were constructed by Kennard et al.[13] with RFLP, RAPD and isozymes for a narrow (58 loci) and a wide cross (70 loci), respectively, and the other one was constructed by Serquen et al.[6] with 80 RAPD loci. Although the gene for gynoecy was located in the three maps, the results yet cannot be applied to molecular-assisted breeding in China due to the lack of comparability of the randomly amplified markers among different varieties. Serquen et al. also used the molecular linkage map to analyze quantitative traits loci (QTL) of lateral branch. It is noted that, because the chromosomes of cucumber are too small and not easily to be viewed, by now the established molecular linkage groups have not been associated with cucumber chromosomes.

In this study, by using RAPD markers, an F_2 population from a cross between the line S52, showing weak lateral growing ability and bearing more male flowers, from Dabieshan Mountains area, and the gynoecious line S06, showing strong lateral growing ability, from Europe was used to construct a molecular marker linkage map, in which both lateral branch gene (*lb*) and gynoecious expression gene (*f*) were mapped.

1 Materials and methods

1.1 Materials

Inbred-line S06, a greenhouse type from Europe with strong lateral growing ability and gynoecious, and S52, a variety from Dabieshan Mountains area in China, with weak lateral growing ability and bearing more male flowers, both were developed by Cucumber Breeding Laboratory, Shanghai Jiaotong University.

Taq DNA polymerase and dNTPs were bought from Promega, Shanghai. The RAPD primers were synthesized by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. according to the sequences of QIAGEN Operon RAPD 10-mer Sets and NAPS Unit standard primers (University of British Columbia, Canada).

1.2 Methods

1.2.1 Population development The F_1 between S06×S52 was obtained in the autumn of 2002 and subsequently self-pollinated to produce F_2 progeny in the summer of 2003.

1.2.2 Field evaluation and characters examined A total of 200 F_2 individuals, their parental plants, and F_1 hybrid progeny were planted in a greenhouse of Agriculture and Biology School, Shanghai Jiaotong University. Of the F_2 population 93 individuals were selected randomly to construct the linkage map. 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.

The data of lateral branch growing ability of individual plants were collected about 30 days after they were planted and at the time when the parent, S52, still had no lateral branches while the parent, S06, had branches with the mean length of 2 cm. We took the length of 2 cm as a criterion to judge the lateral branch growing ability of the F_2 individuals, which were then divided into two classes, one had the lateral branches and the other had no lateral branches.

Investigation of sex expression began from the first node and the rate of female flowers within all the 40 nodes of each individual was recorded. Those whose female flower rate between 90%—100% were designated as gynoecious while those that had none female flowers were designated as staminate. Others were designated as the middle phenotype.

1.2.3 Genomic DNA extraction When a young plant fully extended the first two leaves, its cotyledons were picked off for DNA extraction with CTAB phenol/chloroform extraction procedure[14].

1.2.4 RAPD analysis The optimized PCR amplification system (20 μL in total volume) contained 12.5 pmol of RAPD primer, 2.0 mmol/L of $MgCl_2$, 2 μL of 10× buffer, 40 ng of genomic DNA and 0.6 U of *Taq* DNA polymerase. PCR was conducted with a thermocycler using the following cycling profile: 94 ℃/3 min; 40 cycles of 94 ℃/30 sec, 37 ℃/30 sec; 72 ℃/1.5 min ramp to 72 ℃/7 min and indefinite soak at 4 ℃. After completion of PCR, the samples were electrophoresed in 1.5%—2.5% agarose gels.

1.2.5 Data collection and analysis Polymorphic markers were named according to their corresponding primer codes. Putative multiple loci revealed by one primer were designated by a hyphen after the primer array designation (e.g. two loci at OP-H6-1 and OP-

H-6-2). The genotype polymorphism of S06 was recorded as 1 and S52 as 2. In accordance, the genotypes of F_2 plants were scored as 1 or 2, and 0 represents that failed to amplify. The linkage map was constructed using MAPMAKER/Exp. Version 3.0 with the smallest LOD score of 4.5 and the biggest interval of 37.2 cM.

2 Results and analysis

2.1 Screening of parental polymorphic primers

A total of 640 primers with GC content 60%—70% (Opreron primers, 400; NAPS Unit primers, 240) were screened and 120 primers (18.8%) could produce polymorphic bands, from which 109 polymorphic primers were applied to F_2 population due to their good repeatability and stability. Totally 140 polymorphic loci were identified from the 109 primers (i.e. approximately 1.3 marker bands per primer), including 15 primers each revealing two polymorphic loci, five primers (OP-H-6, OP-M-6, OP-D-7, OP-T-14 and OP-S-13) each revealing three polymorphic loci, and one primer (OP-E-1) four polymorphic loci. Of the 140 polymorphic loci, 45.2% of the bands were from S06 and 54.8% from S52.

2.2 Genetic analysis of lateral branch and gynoecy

Survey of lateral branch was conducted about 30 days after planting when the parent S52 still had no lateral branch and the parent S06 and F_1 had branches with the mean length of 2 cm of the first branches. With the mean length of 2 cm of the lateral branches as the criterion the F_2 population were investigated and grouped into two classes, one was considered having lateral branch and the other having no lateral branch. The data showed that, in the F_2 population, 87 individuals belonged to the type of having branch and 26 ones belonged to the type of having no branch. A *chi*-square test indicated that the observed segregation was not significantly different from the expected ratio of 3∶1, so the trait of lateral branch in cucumber could be analyzed as a qualitative trait.

As to the segregation of gynoecious character in the F_2 population, 28 individuals presented gynoecious, 20 presented staminate and 47 presented middle phenotype. A *chi*-square test for fit of 1∶2∶1 (single codominant gene) indicated that the segregation was not significantly different from the expectation.

2.3 Construction of the linkage map

Of the 140 polymorphic markers ranging from 100 bp to 3000 bp there were 132 markers fitting for 3∶1 separation ratio after a *chi*-square test for fit. As a result 132 RAPD markers plus two morphic markers (lateral branch and gynoecy) were used to construct a linkage map.

A molecular linkage map of 79 RAPD markers and two genes (*f* for gynoecy and *lb* for lateral branch), distributed in nine linkage groups (designated LG1 to LG9) spanning 1100.0 cM with average distance between loci of 13.7 cM, was constructed (Fig. 1, Table 1). Among the nine linkage groups four were longer ones and five were shorter. The longest linkage group (LG-1) spanned 293.6 cM consisting of 17 loci with the average distance between loci of 17.3 cM. The linkage group with the most markers contained 18 markers spanning 229.9 cM with the average distance between loci of 12.8 cM (LG-2). The linkage group with the shortest average distance between loci of 9.6 cM had 16 markers spanning 153.1 cM. The four shorter linkage groups (LG-5, 6, 7, 8 and 9) all contained 4 markers spanning 43.6 cM to 68.4 cM with the average distance between loci of 11.1 cM to 17.1 cM.

2.4 Genetic mapping of the genes for lateral branch and gynoecy

The gene *lb* for lateral branch trait was mapped to a region flanked by RAPD marker OP-Q5-1 at 9.3 cM and OP-M2-2 at 15.9 cM in a longer linkage group (Fig. 1, LG-2). The gene *f* for gynoecy was mapped to a shorter linkage group and flanked by OP-Q5-2 and BC151 at 13.8 cM and 13.6 cM, respectively (Fig. 1, LG-8).

3 Discussion

3.1 RAPD polymorphism

RAPD markers are relatively simple, inexpensive and amenable to the high throughput analysis and have been successful in genetic mapping of many crops. The polymorphisms revealed by RAPD mainly depend on the genetic relationship of experimental materials.

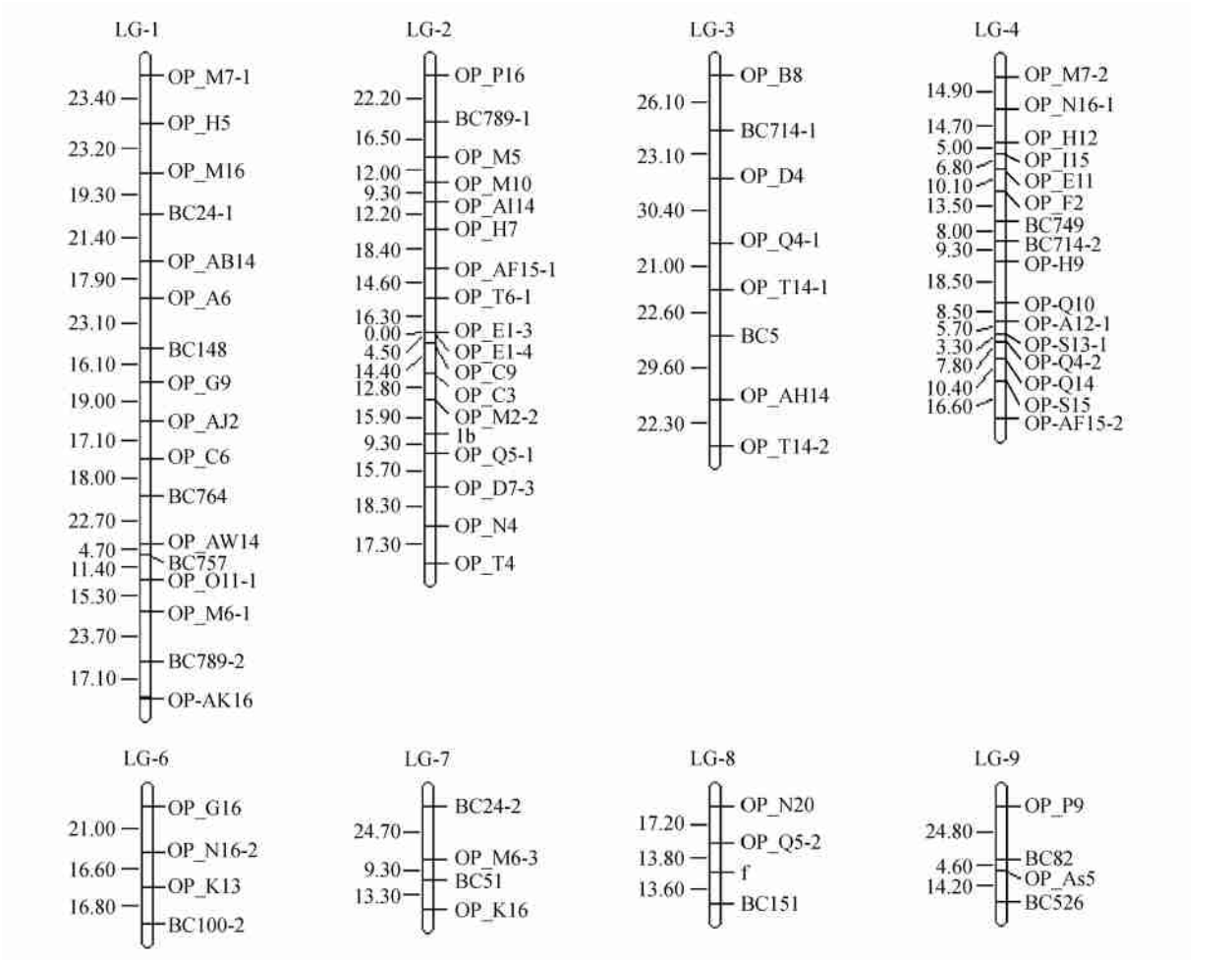

Fig. 1. Genetic linkage map of cucumber (*Cucumis sativus* L.) based on RAPD. The symbols on the right are RAPD markers and genes, the numbers on the left are the intervals between flanking markers.

Table 1. Basic parameters of the cucumber RAPD linkage groups

Linkage group	Number of markers	Length of LG (cM)	Max interval (cM)	Min interval (cM)	Mean interval (cM)
LG-1	17	293.6	23.7	4.7	17.3
LG-2	18	229.9	22.2	0.0	12.8
LG-3	8	175.2	30.4	21.0	21.9
LG-4	16	153.1	18.5	3.3	9.6
LG-5	4	68.4	27.7	15.3	17.1
LG-6	4	54.4	21.0	16.6	13.6
LG-7	4	47.3	24.7	9.3	11.8
LG-8	4	44.4	17.4	13.4	11.1
LG-9	4	43.6	24.8	4.6	14.5
Total	81	1110.0	—	—	13.7

Additionally, optimizing PCR reaction condition and agarose concentration for electrophoresis can also improve the polymorphism-revealing ability of RAPD analysis. Screening of parental stocks with 640 RAPD primers resulted in detection of 120 (18.8%) polymorphic primers, furthermore linkage analyses resulted in 70 primers finally applied to the map with a rate of 10.9%, which was much higher than 5.5% in a narrow cross (GY-14×PI432860) by Kennard et al.[13] and 4.8% in a narrow cross (GY-14×PI432860) by Serquen et al.[6]. The difference may be attributed to the difference in plant materials used in the experiments. One parental material in this study was a variety from China Dabieshan Mountains area and the other belonged to Europe greenhouse type, so they were distant in genetic relationship. In this study, by optimization of the PCR reaction condition, most of PCR products appeared clear bands ranging from 100 bp to 3000 bp in electrophoresis when the agarose concentration (1.5%—2.5%) was appropriately regulated according to the banding array. These measures were sure to benefit the polymorphism-revealing ability of RAPD.

3.2 RAPD molecular map

The constructed map consisted of nine linkage groups distributed by 79 RAPD markers and two gene

loci (*lb* and *f*), spanning 1110.0 cM. The resulting total genetic distance was longer than those of Kennard et al.[13] and Sequen et al.[6] (480 cM, 766 cM and 599.6 cM respectively) and similar to the length 800—1000 cM estimated by Beckman and Soller[15]. Although the mean interval distance of 13.7 cM in the map was longer than that of Serquen et al.[6], even distribution of all the markers in our map was one remarkable advantage of the map by Serquen et al.[6].

Cucumber should have seven linkage groups corresponding to its seven pairs of chromosomes, but our constructed map still contained nine linkage groups similar to that of Serquen et al. This result suggested a low saturation of the existing maps. With the addition of new markers and the enlargement of mapping population, the mergence of linkages will happen and as a result the number of linkage groups will be equal to the number of the chromosomes. This can be deduced from our data processing course. For example, when we performed linkage analysis, two linkage groups LG-1 and lg-4 would be merged into a longer group if the score of LOD was set at a lower value.

3.3 Genetic mapping of lateral branch and gynoecy

Both lateral growing ability and sex expression are important agronomic traits of cucumber and have been attached much importance by cucumber geneticists and breeders. Previously, there were several studies on *f* gene and some researchers had roughly mapped the genes related to female expression by means of traditional genetics[16, 4]. In the present study we used molecular markers to tag these two genes. The gene *f* for gynoecy was mapped to a shorter linkage group and flanked by OP-Q5-2 and BC151 at 13.8 cM and 13.6 cM, respectively. Serquen et al. mapped this gene in the same way, but the two flanking markers were 31.2 cM and 43.9 cM away from the gene *f* locus[6]. The results suggest that our experimental materials are more suitable to the study on the gene *f*. It should be noted that, in our field test, due to the impact of high temperature in the cucumber growing period, the parent S52 plants and part of F_2 individuals did not bear female flowers, which might result in some segregation data different from those generated in normal weather. The gene *lb* for lateral branch trait was mapped in linkage group LG-2 and flanked by RAPD marker OP-Q5-1 at 9.3 cM and OP-M2-2 at 15.9 cM.

As we know, lateral branch has never been studied as a qualitative trait previously. Indeed, most of agronomic traits in cucumber as well as other crops present quantitative traits, but under certain conditions some quantitative traits can also be studied as qualitative ones depending on certain criteria taken to score the related traits by researchers[17]. Take lateral branch for example, in the early growth period of cucumber, according to our criteria, it could be regarded as a qualitative trait for genetic analysis and gene mapping, but after this period the previously examined branches extended and then presented as a quantitative trait. The similar situations have been suggested in mapping genes for other quantitative traits in other plants.

MAS might improve breeding efficiency and has been studied relatively deeply in tomato and other vegetable crops. Fazio et al. tried MAS and proved its feasibility in cucumber breeding[18]. Due to the larger distance of *lb* and *f* to their respective flanking markers developed in this study, selection of *lb* and *f* genes with the linked single markers is still less efficient. However, if for each of the two genes its two flanking markers are selected in combination, the right rates of selecting lateral branch and gynoecy are 98.5% and 97.4%, respectively. Therefore, the linked markers identified in this study can be applied to cucumber breeding practice.

The construction of the cucumber molecular linkage map has paved the way for the further study on genetic bases of lateral branch and gynoecy. In order to precisely locate the genes (including QTL) relative to lateral branch and gynoecy, the next step is to establish an F3 family, to introduce new types of molecular markers and to improve map saturation. Moreover, because RAPD markers are a kind of random marker, it is necessary to transform the target-linked RAPD markers into the sequence characterized amplified region (SCAR) markers for application of MAS to cucumber breeding.

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