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SRAP MARKERS FOR FRUIT SHAPE IN CUCUMBER

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Abstract

Fruit shape is one of the most important quality factors in many domesticated plants. In this study, we used bulked segregate analysis (BSA) to identify two sequence-related amplified polymorphism (SRAP) markers linked to fruit shape in cucumber (*Cucumis sativus* L.). A segregating F_2 population of 130 plants from a cross (B-2-2×Y-3) of B-2-2 with long fruit shape trait and Y-3 with round fruit shape trait was used. Correlation and regression analysis showed that markers ME21/EM18M600 and ME9OD3M190 were linked closely to the allele of round at the fruit shape locus. Marker ME21/EM18M600 accounted for 49.5% of the phenotypic variation in fruit shape, and ME21/EM18M600 accounted for 33.1% of the phenotypic variation.

Introduction

Cucumber (*Cucumis sativus* L., 2n = 2x = 14), belongs to the genus *Cucumis* of the family Cucurbitaceae. It is grown in all over the world and is one of members of the economically important Cucurbitaceae family (Jeffrey, 1980; Kirkbride, 1993; Li *et al*., 2008). There are many types of cucumber fruit, different in size, shape, spine and color (Esteras, 2008). Fruit shape is an important quality factor for many domesticated plants (Grandillo *et al*., 1996) and current cultivars are classified into different cultivar-groups based on fruit shape. At present, consumer choice is driven by a range of individual preferences: quality, size, shape and so on. According to statistics of the Food and Agriculture Organization of the United Nations, 20% cucumber produce was sold as fresh cucumber (van Eck *et al*., 1998), with the improvement of the living standards, some consumers are not satisfied with the common cucumbers and begin to look for newer, more unique and better quality cucumbers in market. So for cucumbers and many other crops, size and shape of fruits are important quality criteria. Plant breeders try to develop new varieties with improved size and shape features (van Eck *et al*., 1998). However, cucumber is a crop with narrow genetic base (Kupper & Staub, 1988; Fan, 2006), and it will take a long time to breed some characteristic varieties if the traditional breeding methods are used. Marker Assisted Selection (MAS) is an efficient way to breed a new variety (Gubbuk *et al*., 2004; Hu *et al*., 2012). It may shorten the breeding time the breeders spend compared to the traditional breeding method. Sequence related amplied polymorphism (SRAP) is a PCR- based marker system as described by Li & Quiros (2001). The SRAPs is a simple and efficient marker system that can be adapted for a variety of crops, such as cucurbits (Ferriol *et al*., 2003; Ferriol *et al*., 2004; Qian *et al*., 2006).

In order to speed up the breeding of the variety characteristics, the SRAP molecular marker of round shape was studied in our research. A F_2 population with 130 individuals, which was derived from the cross of B-2-2×Y-3, was used as the plant material to identify the fruit shape of cucumber at the commercial fruit stage and to carry out SRAP molecular marker analysis. The objective of this study was to find the molecular markers linked with the round shape trait of cucumber fruit which was useful for breeders to identify the fruit shape of cucumber breeding materials at seedling stage, to investigate the genetic patterns of the gene linked with this trait and to clone some related gene.

Materials and Methods

Plant material: The plant population consisted of 130 F_2 individuals derived from a cross made by the cucumber research group of Northwest A & F University between B-2-2 with long fruit and Y-3 with round fruit. Y-3 comes from a local cultivar which is an inbred line and B-2-2 which has a distant relationship with Y-3 is another inbred line with the long fruit shape. The F_1 was hybridized in spring of 2006, and the F_1 individual was inbred and seeds were reserved separately. The 15 individuals of P_1 and P_2 were planted separately and 130 individuals of F_2 were planted in the Horticulture Research Station of College of Horticulture in the spring 2007. Similar management practices to commercial cucumber production were applied to the experimental plots.

SRAP primers: The primers were obtained following the literature on cucumbers (Li *et al*., 2001, 2003; Ferriol *et al*., 2003, 2004). In total, 19 forward primers and 20 reverse primers were selected. The primers were synthesized by the SanboYuanzhi Science and Technology Company, and the primers selected were listed in the Table 1.

Phenotypic analysis: The fruit shape of F_2 individuals were evaluated at the commercial ripe stage. The fruit length and fruit diameter at least ten fruits per plant were measured with a hand-held caliper and the fruit shape index (L/D) was calculated with the ratio of fruit length to the diameter.

DNA isolation and PCR reaction: Genome DNA was extracted from fresh leaves using the procedure of CTAB (Chen *et al*., 2006). PCR amplification was carried out as follows: initial de-moisturized for 5 min at 94°C; 8 cycles for: 30 s at 94°C, 45 s at 36°C, 90 s at 72°C; then 38 cycles for: 30 s at 94°C, 45 s at 50°C, 90 s at 72°C; and final extension for 7 min.

Table 1. List of SRAP primers.

Forward primers	Sequence of primers (5'-3')	Reverse primers	Sequence of primers (5'-3')
ME1	TGAGTCCAAACCGGATA	EM1	GACTGCGTACGAATTAAT
ME2	TGAGTCCAAACCGGAGC	EM2	GACTGCGTACGAATTTGC
ME3	TGAGTCCAAACCGGAAT	EM3	GACTGCGTACGAATTGAC
ME4	TGAGTCCAAACCGGACC	EM4	GACTGCGTACGAATTTGA
ME5	TGAGTCCAAACCGGAAG	EM6	GACTGCGTACGAATTGCA
ME6	TGAGTCCTTTCCGGTAA	EM8	GACTGCGTACGAATTCTG
ME7	TGAGTCCTTTCCGGTCC	EM9	GACTGCGTACGAATTGAT
ME8	TGAGTCCTTTCCGGTGC	EM14	GACTGCGTACGAATTCAG
ME9	TGAGTCCAAACCGGAGG	EM18	GACTGCGTACGAATTCCT
ME10	TGAGTCCAAACCGGAAA	EM20	GACTGCGTACGAAATTCTT
ME11	TGAGTCCAAACCGGAAC	OD3	CCAAAACCTAAAACCAGGA
ME12	TGAGTCCAAACCGGTAG	OD15	GCGAGGATGCTACTGGTT
ME13	TGAGTCCAAACCGGCAT	OD17	GTTAGTATCAAGGTTAGAGTT
ME14	TGAGTCCAAACCGGTCT	SA4	TTCTTCTTCCTGGACACAAA
ME21	TGAGTCGTATCCGGTCT	SA7	CGCAAGACCCACCACAA
ME22	TGAGTCGTATCCGGAGT	SA14	TTACCTTGGTCATACAACATT
ME23	TGAGTCGTCTACGGTAG	SA17	ATAAGAATCAGCAGACGCAT
DC1	TAAACAATGGCTACTCAAG	SA18	ACGAGTTGCGGAAGTGG
PM8	CTGGTGAATGCCGCTCT	GA2	TTGAACTGGCAGAAAGGGT
		GA33	GTTATGGGAAATTAGGTGAG

Bulked segregant analysis: DNA from 10 round and 10 long fruit plants were pooled to prepare the round fruit and the long fruit bulks, respectively. 380 primer combination SRAP markers were chosen to screen the polymorphism between the round fruit and long fruit bulks, as well as the 2 parental lines. The polymorphic markers were used in the 130 plants of the segregating population. The PCR products were separated using the poly acrylamide gel electrophoresis. The polymorphic bands were observed after silver staining of the gel, the primer combinations which could produce polymorphic bands were selected.

The test of SRAP marker in F_2 populations: The DNA of F_2 individuals was amplified using the polymorphic primer combinations. The separation of the polymorphic bands in the F_2 individuals was observed and recorded.

Correlation analysis of the SRAP molecular markers: The correlation coefficient was the covariance of 2 sets data (fruit-shaped traits and molecular markers) divided by the square root of the product variance.

Results and Discussion

Fruit shape variation: Fruit shape exhibited continuous variation in the individual of F_2 population, typical of quantitative traits. In Y-3, the average fruit shape index was nearly 1.0 as determined by the ratio of polar to equatorial diameters and in B-2-2 the fruit shape is long and the fruit shape index is nearly 4.5, so distinct segregation for this trait was observed in the F_2 population. The fruit shape index of the F_2 population was between 0.8 and 4.8, with only 2 individuals in 4.7, and the mean of the population was 1.8. The fruit shape index in this population fits the normal distribution in statistics (Fig. 1).

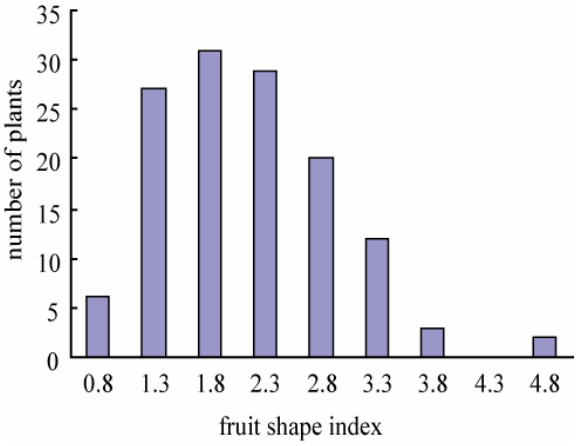

Fig. 1. Frequency distribution for fruit shape index in F_2 population.

The SRAP molecular marker linked to the round fruit shape of cucumber: A total of 380 SRAP primer combinations were screened by using the DNA of two parents as the template, respectively, out of which 184 showed polymorphism with a rate of 48.4%. Each primer combination produced 20-30 clear and stable bands, and these bands were distributed between 100-800 bp in size. The strong bands constituted 80% of those present (Fig. 2). The 184 primers combinations screened in the round and long shape DNA pools as the templates revealed 34 primer combinations that produced clear and stable bands with a polymorphism rate of 18.5%. The primer pairs ME6/OD3, ME8/EM14, ME9/OD3, ME21/EM8 and ME21/EM18 showed correlation in the bulk for round fruit shape. To confirm whether the marker was linked to this trait, it was essential to conduct further tests on the F_2 population and using other cucumber materials.

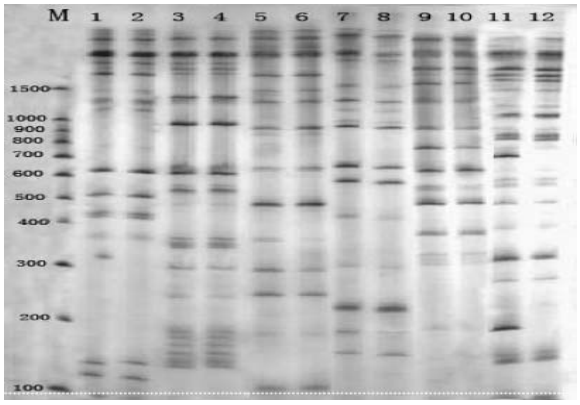

Fig. 2 Primers screening in"Y-3" and"B-2-2"
M: 100bp ladder maker, 1,2, ME13/EM3; 3,4, ME13/EM4; 5,6, ME13/EM8; 7,8,.ME13/EM18; 9,10, ME13/EM20; 11,12,ME13/GA2; 1,3,5,7,9,11: template is"B-2-2"; 2,4,6,8,10: template is"Y-3"

Confirmation of polymorphism primer combinations in the F_2 population: Using the polymorphic marker combinations ME6/OD3, ME8/EM14, ME9/OD3, ME21/EM8 and ME21/EM18 to screen the 130 F_2 individuals, we found that ME8/EM14, ME9/OD3 and ME21/EM18 were polymorphic (Figs. 3 and 4). The correlation coefficient between the ME21/EM18 M600 marker and the phenotype of F_2 population was significant ($|r|=|-0.7038| > r_{0.01,129}= 0.2252 > r_{0.05,129}= r=0.1723$), which revealed that the coefficients between ME21/EM18 M600 marker and the round fruit shape trait was significant at 1% level, and it also reflected that the presence of ME21/EM18 M600 marker was closely linked to the round fruit trait. Regression analysis showed that ME21/EM18 M600 accounted for 49.5% of the phenotypic variation, the correlation coefficient between ME9/OD3 M190 marker and phenotype of F_2 population was significant ($|r|=|-0.5732| > r_{0.01,129}= 0.2252 > r_{0.05,129}= r=0.1723$), and the coefficient between ME9/OD3 M190 marker and phenotype of F_2 population was significant at the 1% probability level, showing that the ME9/OD3 M190 marker was linked closely to the round shape fruit trait, and ME21/EM18 M600 accounted for the 33.1% of the phenotypic variation.

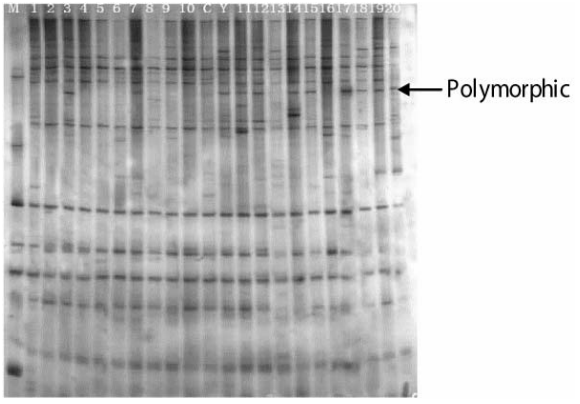

Fig. 3 Primers ME21/EM18 screening in individuals of two pools
M: 100bp ladder maker, 1~10: 10 individuals of long pool; 11~20: 10 individuals of circle pool; C: mixed long pool;Y: mixed circle pool; the arrow is different band

Phenotype and marker polymorphic: Cucumber has a narrow genetic base (Kupper & Staub, 1988; Fan, 2006; Zhou, 2009). In this study, Y-3, a self-bred line with round shape fruit is a local cultivar. B-2-2, another self-bred line with the long shape fruit is much different with Y-3**.** In their F_2 population, there were a variety of fruit shapes ranging from long shape to round fruit shape, mature fruit shape ranged from 1 to 4.7 approximately in fruit shape index. SRAP marker is characterized to be highly sensitive in detecting DNA structural differentiation in plant genome. So the large number of bands and the high polymorphism rate revealed by SRAP molecular marker between two parents and two DNA pools. It showed that this strategy was effective and efficient to found the molecular marker which liked with the trait of round fruit shape of cucumber.

The marker of round fruit shape and it's future use in cucumber: Fruit shape is a very important marketing quality trait for horticulture crops, not only in plant variety rights or cultivar registers, but also in the evaluation of consumer preference (Currie *et al*., 2000; White *et al*., 2000). Therefore, plant breeders always try to develop new varieties with improved fruit shape features (van Eck *et al*., 1998). The selection of fruit shape features is time-consuming in plant breeding process, and the shape features of product are often visually scored into a limited number of classes. However, the molecular marker linked to this trait is of great value for the identification and selection of plant genotypes with the desire characters long before the trait.

According to the history about this knowledge, this is the first report identifying molecular marker of round shape trait in cucumber. The marker linked with fruit shape has been analyzed in the other plants such as tomato, pepper, sponge gourd and eggplant. In tomato, several major genes controlling fruit shape were identified (Ku *et al*., 2000; Van der Knaap & Tanksley, 2001; 2003; Van der Knaap *et al*., 2004).

In this study, two SRAP markers linked closely with the round shape fruit of cucumber were developed using the strategies of BSA and the correlation analysis. Furthermore, the recombinant inbred lines will be used to develop the molecular mapping and the QTL analysis about the fruit related traits, which will be helpful to the breeding of unique cultivar in cucumber.

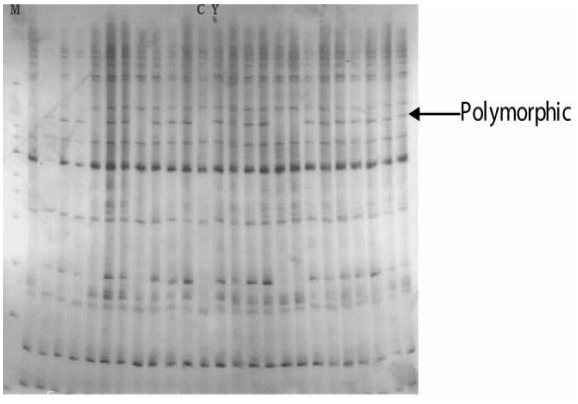

Fig. 4 Primers ME8/EM14 screening in "Y-3"、"B-2-2" and part of F_2
M: 100bp ladder maker,C: template is"B-2-2"; Y: template is "Y-3"; the other is F_2 individuals;

Conclusion

By using 130 seedlings of the F_2 segregating population of the cross combination B-2-2×Y-3, the closely-linked markers with round shape fruit in cucumber were selected. Correlation and regression analysis showed that the markers ME21/EM18M600 and ME9OD3M190 were linked closely to the allele of round at the fruit shape locus. Marker ME21/EM18M600 accounted for 49.5% of the phenotypic variation in fruit shape, and ME21/EM18M600 accounted for 33.1% of the phenotypic variation.

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