Quantitative Trait Locus Analysis of Plant Height– Related Traits in Cucumber Using $F_{2:3}$ Population

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Summary

In present study, C18 (determinate) and C19 (indeterminate) cucumber lines were crossed and the hybrid plants were self-pollinated to produce the F2:3 generation. Thirteen ISSR (Inter Simple Sequence Repeat) primers, two retrotransposons and two IRAP (Inter-Retrotransposon Amplified Polymorphism) combination primers were screened for QTLs (Quantitative Trait Loci) controlling plant height in cucumber. Five QTLs using SIM (Single Interval Mapping) were identified but only two major of them were observed using CIM (Composite Interval Mapping) method for plant height in F2:3 generation. The distance of one of the identified QTLs to nearest marker was very low and 0.01 CentiMurgan (cM), which showed a high linked marker to this QTL.

Key words

ISSR, IRAP, ERAP (Exon-Retrotransposon Amplified Polymorphism), QTL, Retrotransposon

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Introduction

Cucumber (*Cucumis sativus* L. 2n = 2x = 14) is a cross-pollinated crop and selection is difficult due to unsuitable pollen. Furthermore, plant height is a quantitative trait controlled with many genes (Whittaker *et al*., 1996). These traits are difficult to study due to the complex nature of their inheritance. So, in selection programs, breeders have to use traits that have high and positive correlation with this trait.

Quantitative trait loci (QTL) mapping includes: phenotypic evaluation of a relatively large number of plants from a segregating population; genotyping the population for polymorphic genetic markers, and statistical analyses to identify loci affecting quantitative traits (Haley and Knott, 1992). Application of molecular markers had enabled the identification of the QTLs information involved in important agronomic traits (Qin *et al*., 2008). The methods to detect quantitative trait loci include single marker analysis; interval mapping; composite interval mapping, and mixed linear models (Whittaker *et al*., 1996; Haley and Knott, 1992). Inter-simple sequences repeat (ISSR) PCR is a fast and inexpensive genotyping technique based on variation in regions between microsatellites. This method can be used for characterization of genetic relatedness among populations; genetic fingerprinting; gene tagging; detection of clonal variation; identification of cultivars; phylogenetic analysis; detection of genomic instability, and to assess hybridization (Tanyolac, 2003; Fernandez *et al*., 2002).

Retrotransposons (also called transposons via RNA intermediates) are genetic elements that can amplify themselves in a genome and are ubiquitous components of the DNA of many eukaryotic organisms. They are 1 of 2 subclasses of transposon, the other is DNA transposon, which does not involve an RNA intermediate. They are particularly abundant in plants, where they are often a principal component of nuclear DNA (SanMiguel and Bennetzen, 1998; Li *et al*., 2004). Inter-retrotransposon amplified polymorphism (IRAP), a marker system based on transposable elements in barley, was developed by Kalendar *et al*. (1999). In contrast to other markers, IRAP aims to genomic polymorphism between two nearby retrotransposons in the genome (Kalendar and Schulman, 2006).

A population of 113 cucumber recombinant inbred lines (RILs) derived from a cross between Europe 8 and Qiupeng was used as materials in Chen *et al*. (2014) study and a total of 58 QTLs were detected in their study. Among them, 1 QTL conferring single plant yield (SPY) was located on the linkage group LG4; 2 QTLs conferring the number of fruit setting (NFS) were located on LG2 and LG4. The above-mentioned QTLs were detected in only one cropping season. There were 5 QTLs conferring female flower number (FFN) on LG2, of which, ffn2a and ffn2b, were detected in both cropping seasons with the same positive genetic effects.

The F2:F3 RAPD analysis and mapping of yield components in cucumber by Serquen *et al*. (1997) resulted in the identification of QTLs describing main stem height, days to anthesis, sex expression, multiple lateral branching, fruit number and weight, and height/diameter ratio. However, the presence and strength of QTLs were underestimated by these RAPD marker loci (Serquen *et al*., 1997).

The recent development of ISSR and retrotransposone (Tanyolac, 2003; Fernandez *et al*., 2002; SanMiguel and Bennetzen, 1998; Li *et al*., 2004; Kalendar *et al*., 1999; Kalendar and Schulman, 2006), should allow for increased map saturation and more precise QTL estimation in cucumber. Therefore, a study was designed to employ these markers in the analysis of a F3 progeny to characterize plant height associated QTLs in cucumber.

Materials and methods

The experiment was conducted at the University of Guilan from 2014 to 2015. The lines C18 (determinate) and C19 (indeterminate), released from the cucumber breeding program at the University of Guilan, were crossed and hybrid plants were self-pollinated as parent lines (P1 and P2, respectively) to produce F2:3 generation. The sandy loam soil was prepared by plowing and disking and formed into raised beds by plough and harrow prior to plant establishment. Rows were on 1 m centers and plants were about 50 cm apart in the row. Prior to planting 150 kg·ha^{-1} of nitrogen from urea, 100 kg·ha^{-1} of phosphorous from triple superphosphate, and 80 kg·ha^{-1} of potassium sulfate were applied. Side dressing with the same amount of nitrogen and phosphorus occurred at 50% flowering stage. Irrigation begun at plant first flowering with 250 m^3·ha^{-1} three times weekly. During April of 2014 and 2015, seed of the generations (parent and F3 during 2015) were planted with 10 plants in a row for each parent. The F2 population contained 86 plants and F3 contained 10 progenies from each selfed F2 plants. A randomized complete block design with three replications was applied. Pests and weeds were controlled with the bio-methods. Yellow and blue cards for trips, white fly and aphid's absorption and no pesticides were used. Weeds were controlled by hand without use of herbicides. Plant height at harvesting time was measured.

The DNA was extracted from fresh young leaves using the procedure of Guo (2003). The inter simple sequence repeat (ISSR) protocol employed was similar to that of Fernandez *et al.* (2002) and the retrotransposon protocol employed was similar to that of SanMiguel and Bennetzen (1998). Thirteen ISSR primers, two retrotransposon and two combination primers (IRAP) (Table 1) exhibiting polymorphism between parents C18 and C19 were further screened for QTLs loci controlling number of fruit per node. The PCR analysis was performed in 15 µl of substrate containing 40 mg DNA, 2.5 mM primer, 10 mM dNTPs, 1.50 µl 10× assay buffer, 0.20 µl Taq polymerase, and 8.10 mL sterile distilled water. Amplification was carried out in a thermal cycler with initial denaturation for 5 min at 94°C, 40 cycles of denaturing for 1 min at 94°C, annealing for 2 min at 50°C, extension for 2 min at 72°C, and final extension for 5 min at 72°C. Amplified products were separated by electrophoresis on 1.2% (w/v) agarose gel and visualized by staining with ethidium bromide.

Genotype data were used to perform linkage analyses using Win QTL Cartographer (Basten *et al*., 2001). A minimum LOD of 2.5 was set as a threshold to relegate marker loci into linkage groups, to order markers, and to estimate interval distances (Kosambi function). A graphic representation of the linkage groups was created using MapManager QTXb17 (Manly and Olson, 1999). Trait distributions were examined for fit to normality using the SAS.

Composite interval mapping (CIM) was performed using Model 6 of QTL Cartographer (version 1.21) with a walking speed of 0.5 cM, a window size parameter of 3 cM, and the inclusion of 15 maximum background marker loci in a stepwise forward regression procedure. The inclusion of background marker loci allowed for greater sensitivity in QTL detection and a conservative estimation of their effects (R2). The significance of each QTL interval was tested by a likelihood-ratio statistic (LOD). The LOD threshold for significance at p = 0.05 was determined using 1,000 permutations.

Table 1. List of ISSR and retrotransposon primers used in this study

Primer name	Primer cod	Annealing temperature (˚C)	Primer sequence	Number of alleles	Number of polymorphic alleles
UBC811	*P1*	43	*$(GA)_8C$*	7	7
UBC812	*P2*	42	*$(GA)_8A$*	7	7
UBC813	*P3*	42	*$(CT)_8T$*	6	6
UBC814	*P4*	41	*$(CT)_8A$*	10	10
UBC808	*808*	45	*$(AG)_8C$*	10	10
UBC815	*P5*	42	*$(CT)_8G$*	9	9
UBC816	*P6*	47	*$(CA)_8T$*	10	10
UBC817	*P7*	47	*$(CA)_8A$*	7	7
UBC823	*P8*	44	*$(TC)_8C$*	10	10
UBC824	*P9*	45	*$(TC)_8G$*	10	10
UBC825	*P10*	48	*$(AC)_8T$*	9	9
UBC826	*P11*	49	*$(AC)_8C$*	7	7
UBC827	*P12*	43	*$(GACA)_4$*	9	9
TOS1	*TOS1*	51	*TGTTGGGAATAGTCCCACA*	1	1
TOS2	*TOS2*	45	*TGTTGAATAGTTCCACATT*	4	4
UBC811+TOS2	*P1(2)*	44	*TGTTGAATAGTTCCACATT+$(GA)_8C$*	11	11
UBC812+TOS2	*P2(2)*	43.5	*TGTTGAATAGTTCCACATT+$(GA)_8A$*	6	6

Results

Plant height distributions were fitted to normality in the F2:3 generation (Figure 1). Phenotypic values of plant height in P1 and P2 parents and F2:3 populations showed that two parents had a significant difference using t-student test (Table 2). The highest polymorph allele number was obtained in IRAP when UBC811 (an ISSR marker) and TOS2 (a retrotransposon marker) were applied simultaneously (Table 3). Furthermore, as presented in Table 3, all of the used primers showed a 100 percent polymorphism.

Five QTLs were detected for plant height in cucumber using SIM method, but two of them that showed the highest LOD value were identified by CIM (Table 3 and Figure 2). These QTLs that identified using both SIM and CIM, were located on chromosomes 3 and 4. The additive and dominant effects for the QTL linked to UBC876-2 and UBC823-7 showed that alleles inherited from these positions had a decreasing effect on plant height (Table 3).

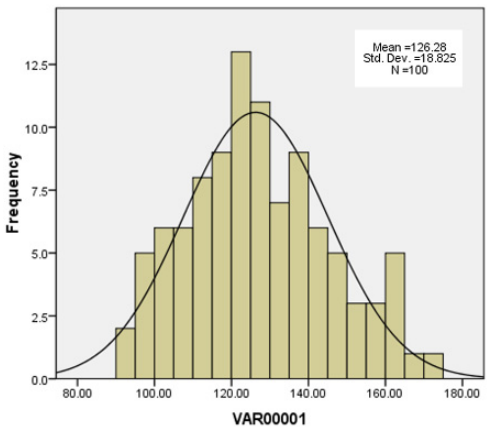

Figure 1. Frequency distribution of the means of the F2:3 population for plant height

Table 2. Phenotypic values of plant height in parents (C118 and C119) and F2:3 populations

C118 (m±SD)	C119 (m±SD)	t-value (P1-P2)	F2:3 (m±SD)	F2:3 Kurtosis	F2:3 Skewness
110.18±5.5	179.19±7.6	-8.481**	126.8±18	-0.49	0.29

Table 3. Information on QTL identified from F2:3 population through the simple interval mapping (SIM) and composite interval mapping (CIM) for plant height

Mapping method	QTLs	Flanking markers	Distance to nearest marker	Chromosomal location	LOD score	QTL Peak position (cM)	Additive effect	Dominant effect	QTL effect (%)
SIM	qPL1	p9-5-p2(2)-1	34.04	1	4.72	443.24	-7.63	-15.38	11
	qPL2	p12-4-tos1-3-tos1-4-tos2-3	17.22	2	4.83	618.52	1.22	30.20	0
	qPL3	808-7-p2(2)-3	9.01	3	6.82	9.01	-8.35	-20.46	17
	qPL4	p9-9-p10-2	0.01	4	6.66	0.01	-13.88	37.62	26
	qPL6	p4-7-p8-4	6.63	6	2.66	44.47	7.07	-7.58	12
CIM	qPL3	808-7-p2(2)-3	9.01	3	4.54	9.01	-5.85	-15.86	7
	qPL4	p9-9-p10-2	0.01	4	3.59	0.01	-9.03	-14.87	9

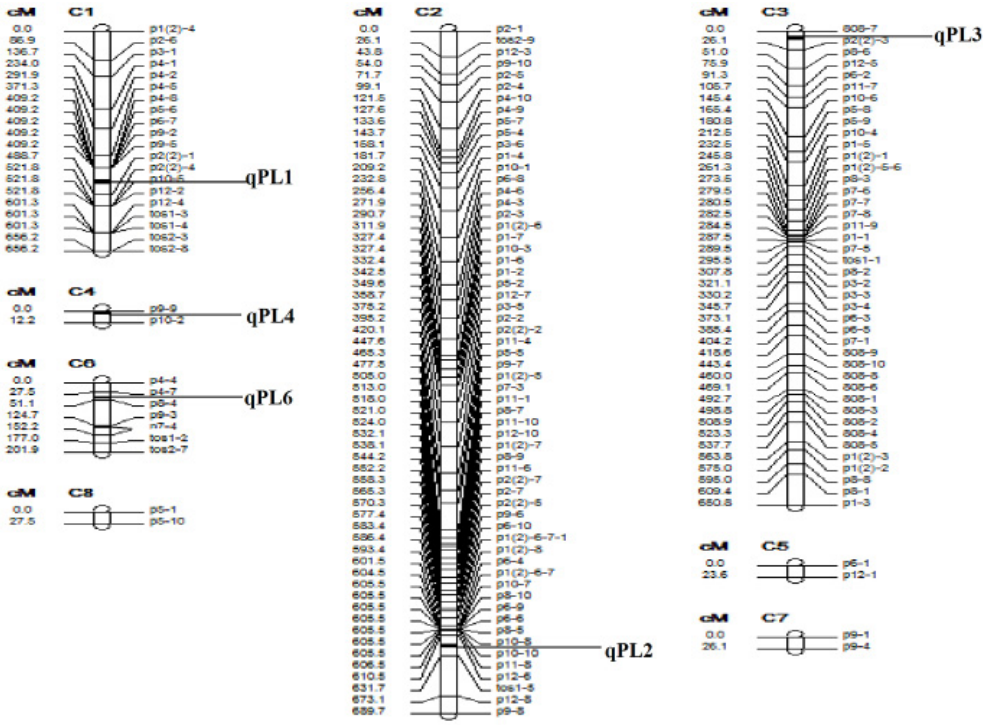

Figure 2. The chromosomal map and QTLs controlling plant height mapped in the F2:3 population

Discussion

Molecular markers used in the present study have enough polymorphism that is important for cucumber because many molecular markers cannot produce enough polymorphism for breeding program based on molecular markers such as marker assisted selection (Witkowicz et al., 2003).

It is known that vegetative growth related traits in cucumber can be affected by environments, but QTLs with relative high and stable phenotypic variation for vegetative traits were detected in this study. We speculate that this region might contain a few genes related to the hormonal biosynthesis and signal transduction pathways that lead to the vegetative growth. Previous researchers have reported that plant height is genetically controlled by quantitative trait loci (QTL) (Marza *et al*., 2006; Huang *et al*., 2003). In the present study, the location of two highly significant QTLs of plant height were detected in chromosome 3 and 4 and were consistent with the expected location of (UBC808)-7 + (UBC812+TOS2)-3 and (UBC824)-9 + (UBC825)-2 flanking markers.

Using the molecular markers tightly linked to targeted agronomic traits is an efficient breeding approach for crops improvement, especially for the improvement of some complex traits (having complex environmental relationships like plant height) (Yu et al. 2000; Fan et al. 2006; Zhu and Sun 2006; Lecomte et al. 2004; Yuan et al., 2008).

Application of MAS for the agronomic traits in cucumber has been proved to be very effective (Fan et al., 2006). Application of this sequence specific marker information on the stable QTLs with high phenotypic variation to the fresh-eating cucumber breeding with MAS is our further project for improving precocity and fruit shape in cucumber production.

Conclusion

In general, although a different QTL numbers were detected for plant height by different QTL mapping methods, all identified QTLs by using CIM mapping method could find those identified by SIM. Therefore, focusing on the markers linked to the QTLs that were found by both methods, can be used for improvement of plant height in cucumber breeding programs. In this study, (UBC808)-7 + (UBC812+TOS2)-3 and (UBC824)-9 + (UBC825)-2 flanking markers were found to be closely linked with the QTLs for plant length.

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