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Mapping of fruit apex shape related QTLs across multi-genetic backgrounds in cucumber (*Cucumis sativus* L.)

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A B S T R A C T

The shape of fruit apex is critical to appearance quality in cucumber (*Cucumis sativus* L.), of which the genetic basis was poorly understood, and the use of marker-assisted breeding for fruit apex improvement is not available yet. In this study, the variation of fruit apex in different cucumber ecotypes was evaluated by fruit apex angle (variation coefficient from 7.1% to 15.7%) and fruit apex index (variation coefficient from 8.8% to 22.6%). Fruit apex associated QTLs were mapped by using 145 $F_{2:3}$ families and 155 $F_{2:6}$ population that were derived from the cross of different ecotype cucumbers. Phenotyping of the mapping populations were conducted in four experiments in 2 years. Four major-effect QTLs, *Bfal*4.1, Bfai4.1, *Bfad*6.1 and *Bfai*6.1 were consistently and reliably detected across two environments which could explain 11.6% - 33.6% phenotypic variations (R^2) in the $F_{2:3}$ families. Three major-effect QTLs, *Ofai*4.1 ($R^2 = 13.4\%–15.5\%$), *Ofal*4.1 ($R^2 = 10.7\%–12.8\%$), and *Ofad*6.1 ($R^2 = 11.6\%–12.4\%$) were stably detected in the $F_{2:6}$ population in two experiments. *Bfai*4.1, *Bfal*4.1, *Ofai*4.1 and *Ofal*4.1 were integrated to be consensus QTL *fa*4.1, within which 11 candidate genes were predicted. *Bfai*6.1 and *Bfad*6.1 were integrated to be consensus QTL *fa*6.1. QTL interaction analysis showed that *Bfai*6.1 has epistatic effect with *Bfai*4.1. This study revealed two reliable major-effect fruit apex related QTLs across multi-genetic backgrounds and environments in cucumber. The possible candidate genes regulating the shape of fruit apex, and the relationship between cell division and fruit apex morphogenesis were discussed.

Keywords: Cucumber; Fruit apex; Variation; QTL; Candidate gene

1. Introduction

Cucumber (*Cucumis sativus* L.) is a global economic important crop which is well-known for its rich diversity in fruit size and shape (Liu et al., 2020; Pan et al., 2020; Cheng et al., 2021). The abundant variation of fruit shape in cucumber was attributed to natural selection during domestication and artificial selection for fruit characteristic improvements. For example, the progenitor of cultivated cucumber *Cucumis sativus* var. *hardwickii* produces small and round fruits (Yang et al., 2012), while fruit shape of cultivated cucumbers varies dramatically among cultivars from round, oval round, short rodlike to long and extremely long. Beyond that, special structures in cucumber fruit such as warty, spines, ridge and stalk etc. further increased variance of cucum-

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ber fruit. Fruit shape is critical to the appearance quality and commodity value of crops, particularly in cucumber. To date, fruit shape improvement becomes to be a major objective for cucumber breeding, which is not only for adapting consumer preferences but also for reducing abnormal fruit rate when cultivating under unfavorable environmental conditions.

Fruit apex is an important part of fruit. Cucumber has a typical inferior ovary, thus the fruit apex develops from the fusion part between ovary and receptacle. This complex structure leads to a rich diversity of fruit apex in cucumber. For instance, North-China ecotype cucumber cultivar generally produce thin and tapering fruit apex, while the majority of South-China ecotype as well as a part of European greenhouse type cucumbers present round fruit apex. Xishuangbanna and Indian ecotype usually possess round fruit apex (Qi et al., 2013). In China, over half of cultivated cucumbers belong to North-China ecotype, because of their relatively high yield and resistance. But from the perspective of consumers, the fruit with sharp fruit apex is not as beautiful as round shape. Besides, sharp apex of fruit was more susceptible to mechanical damage in harvest or processing processes and injury by pathogen. In addition, cucumbers are usually consumed raw or processed into pickles, and tips in cucumber fruit, like fruit stalk, usually have a less desirable taste as compared with the rest of fruit, hence fruits with round apex have more edible proportion than the sharp apex fruits. A few studies showed that fruit apex was controlled by multiple QTLs in tomato, eggplant, and pepper. Brewer et al. (2007) detected four consensus QTLs (*dan7.1, dan7.2, dan8.1, dan12.1*) for fruit apex shape using fruit apex angle in tomato. Geng (2018) detected three QTLs associated with fruit tip shape in eggplant by a F_2 population derived from round fruit tip line '283′ and sharp fruit tip line '284′. Six QTLs (*fps1.1, fps1.2, fps2.1, fps*5.1, *fps8.1*, and *fps*9.1) controlling shape of fruit apex were detected in pepper (Zhang, 2014). However, the genetic basis of fruit apex morphogenesis in cucumber was poorly understood, and use of marker-assisted breeding for improvement of cucumber fruit apex is not available yet.

Many QTLs and genes related to fruit shape have been detected or cloned in different crops. The most intensive studies on genetic basis of fruit shape were performed in tomato. The variation of tomato fruit shape can be explained by four alleles including *OVATE, SUN, LOCULE NUMBER (lc)*, and *FASCIATED (fas)*. OVATE was detected on chromosome 2, in which the stop-gain SNV mutation results in a transition from round- to pear-shaped tomato fruit (Liu et al., 2002). *SUN* causes uniform elongation in both longitudinal directions of fruit (van der Knaap et al., 2014). The Rider retrotransposon-mediated duplication of fragment contained *SUN* causes an overexpression of *SUN*, inducing fruit elongation in several tomato cultivars (Xiao et al., 2008; Jiang et al., 2009). Locule number and flat shape were controlled by the *lc* and *fas* loci. *lc* is a mutation near *WUS* (*WUSCHEL*) of tomato, and is expected to cause increased expression by abolishing the binding site of its suppressor *AGAMOUS*. *Fas* encodes a transcription factor, and down-regulation off as is caused by a large insertion in the first intron results in fruits with high locule number (Cong et al., 2002; Xu et al., 2015; Li et al., 2016).

In cucurbits, QTL mapping was accelerated with the availability of draft genome of cucumber (Huang et al., 2009; Yang et al., 2012), melon (Garcia-Mas et al., 2012), watermelon (Guo et al., 2013) and so on. The genetic basis of fruit shape has been investigated in several studies. In melon, the andromonoecious (*a*) was cloned and confirmed to control the fruit shape and carpel numbers (Boualem et al., 2008). Forty-two QTLs (Diaz et al., 2011) and nine associations (Tomason et al., 2013) were correlated with fruit shape in melon. The fruit shape of watermelon was considered that controlled by an incompletely dominant gene, resulting in elongate, oval, and spherical fruits (Tanaka et al., 1995; Guner and Wehner, 2004). Recently, the major locus for watermelon fruit shape was identified that designated on watermelon chromosome 3 by GWAS profiles among 315 accessions (Dou et al., 2018). Among the cucurbits, bottle gourd and squash have the highest diversity in fruit shape, which may be spherical, oblate, obovoid, drum-shaped, pear-shaped, spindle-shaped, long and cylindrical, elongated, curved, and crooked-necked (Xu et al., 2014; Dhillon et al., 2016; Paris, 2016), however few studies have been conducted to investigate the genetic basis of fruit shape variation in these economically important crops.

Previous studies have been conducted to demonstrate genetic basis of fruit-shape relate traits in cucumber, including fruit length (Yuan et al., 2008; Wei et al., 2014, 2016; Weng et al., 2015; Gao et al., 2020), fruit diameter (Weng et al., 2015), ratio of fruit length and diameter (Miao et al., 2011; Bo et al., 2015; Pan et al., 2017), fruit stalk length (Miao et al., 2011; Zhang, 2014) and fruit bending (Wang et al., 2017). Several fruit shape related genes also have been cloned such as fruit length regulation gene *CsFUL1* (Zhao et al., 2019) and spherical fruit regulatory gene *CsSUN* (Pan et al., 2017). Although these detected QTLs or genes covered almost all components of fruit shape in cucumber, the underlying genes controlling shape of fruit apex have remained elusive.

In this study, the variation of cucumber fruit apex was investigated and evaluated by using 186 different ecotype cucumber lines. The heredity of fruit apex was analyzed based on 145 $F_{2:3}$ families derived from the cross of EC1 (round fruit apex, European greenhouse type) and 8419s-1 (sharp fruit apex, European greenhouse type) and 155 $F_{2:6}$ population derived from the cross of IL52 (round fruit apex, South-China ecotype) and CCMC (sharp fruit apex, North-China ecotype). In total, twenty-four QTLs were detected including two consensus major-effect QTL *fa4.1* and *fa6.1*. Eleven genes were predicted as the candidate genes of consensus QTL *fa4.1* based on whole genome resequencing of the parental lines. We also discussed the relationship between frequency and orientation of cell division to fruit apex morphogenesis.

2. Materials and methods

2.1. Plant materials

One-hundred and eighty-six cucumber inbred lines belonged to different ecotypes were used in this study, which were preserved in State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University. Four cucumber inbred lines were chosen to generate mapping populations including 'EC1' presented round fruit apex (RFA) derived from a European type 'Delta star', '8419s-1′ presented Sharp fruit apex (SFA) derived from European type 'Thaminbeit alpha', 'IL52' with RFA belonging to South-China ecotype (Zhang et al., 2018), and CCMC with SFA which is a typical North-China ecotype. One hundred and forty-five $F_{2:3}$ families were generated from the cross

between 'EC1' and '8419s-1′ (Wu et al., 2016). One hundred and fifty-five recombinant inbred lines ($F_{2:6}$ population) were derived from the cross of 'CCMC' and 'IL52' (Zhang et al., 2018). In addition, two new F_2 populations were constructed in present study, including 107 F_2 population of EC1 × 8419s-1 and 112 F_2 population of CCMC × IL52. All the plants were grown at green houses of Baima field (119.02 N, 31.65E) and Jiangpu (118.62 N, 32.05E) of Nanjing Agricultural University.

2.2. *Phenotyping of fruit apex*

Phenotypic data for fruit apex related traits, including fruit apex angle (FAA) and fruit apex index (FAI), were collected from the 10 day after pollination (DAP) fruits from 15 to 20th node. Number of enlarged fruits was limited to 2 per plant and lateral branches were cut off to reduce the effect of 'first-fruit inhibition' which could lead to abnormal fruit set or malformed fruit. Fruit shape index and fruit apex angel are used to describe fruit shape (Brewer et al., 2006, 2007; Weng et al., 2015; Geng, 2018). Hence, fruit apex angles (FAA = α) and fruit apex index (FAI = a/b), described in Fig. 1, B, were measured to evaluate the variation of fruit apex accurately in this study. As showed in Fig. 1, B, the angle between two tangents that draw from the top point of fruit apex was measured as FAA (α). The width alongside the horizontal line at the apex of mesocarp was measured as diameter of fruit apex (FAD, a), while the longitudinal line from apex of mesocarp to top point of fruit was measured as length of fruit apex (FAL, b). FAI was calculated by formula: FAL/FAD.

For the evaluation of fruit apex related traits, FAA and FAI of 186 cucumber inbred lines were investigated in three replicated experiments, including spring and fall of 2016 in Jiangpu (Nanjing, China), spring of 2018 in Baima (Nanjing, China). Phenotypic data of mapping population were collected in 4 environments in two years. Parent lines and their F_1 were included in all experiments. FAI data of 145 $F_{2:3}$ families were collected in two experiments, including spring of 2016 in Jiangpu (2016/Spring) and fall of 2016 in Baima (2016/Fall), respectively. While FAI data of 155 $F_{2:6}$ population were collected in two experiments, including spring of 2017 in Jiangpu (2017/Spring), fall of 2017 in Baima (2017/Fall), respectively. The FAI data of the new constructed populations i.e. 107 F_2 population of EC1 × 8419s-1 and 112 F_2 population of CCMC × IL52 were collected in spring of 2018 in Baima. Both experiments used the same randomized complete block design (RCBD) consisting of three replications with five plants per family per replication. Each cucumber was spaced 25 cm apart and placed 80 cm apart in rows. Local standard commercial production guidelines were followed for insect/weed control and fertilization by bees. In each family, over 15 fruits were harvested for phenotypic evaluation of fruit apex, and the family mean value were used in QTL analysis.

2.3. *Statistical analysis*

The software Statistical Analysis System (SAS, version 8.0) was used to analyze the phenotypic data of FAA and FAI. Violin and box plots depicted phenotypic data distribution of fruit apex related traits by using R package ggplot 2. Correlations between FAA and FAI of 186 cucumber inbred lines were analyzed by using PROCCORR function. Analysis of variance (ANOVA) of the FAI data of the segregation populations was conducted with GLM (general linear models) program to the estimate the effect of gene and environment with a model of $Y_{ijk} = mu + Genotype\ (G)_i + Environment\ (E)_j + G \times E_{ij} + Error_{ijk}$ in SAS. The broad sense heritability (h^2) was calculated based on variance components and estimated with model of $h^2 = \sigma^2{}_G$ / ($\sigma^2{}_G + \sigma^2{}_{GE}$ /$R_E + \sigma^2{}_E$/R_ERn). where $\sigma^2{}_G$ was the family variance, $\sigma^2{}_{GE}$ was the genotype × Environment interaction (G × E) variance, and $\sigma^2{}_E$ was the residual variance, respectively. R_E was the number of environment and Rn was the mode of individuals in each family. The segregation ratios in $F_{2:3}$ and $F_{2:6}$ populations were analyzed with a χ^2 goodness-of-fit test using FREQ (frequency) procedure in SAS.

2.4. *QTL analysis*

Two genetic linkage maps have been constructed in our previous studies. The genetic map of F_2 population from EC1 × 8419s-1 was constructed by Wu et al. (2016) (Table S2), including 133 SSR and 9 InDels markers. The genetic map of the $F_{2:6}$ population from CCMC × IL52 was constructed by Zhang et al. (2018) (Table S3), including 197 SSR and 19 Indel markers. Here, those genetic map of F_2 and $F_{2:6}$ population were used for QTL analysis with $F_{2:3}$ families and $F_{2:6}$ population fruit apex data, respectively.

The fruit apex related traits QTLs were detected by using WinQTLcart2.5 through the composite interval mapping (CIM) procedure based on phenotypic data of FAI, FAL and FAD mean value from the $F_{2:3}$ and $F_{2:6}$ populations. The presence of QTLs with a minimum LOD threshold of 2.5 were performed by using permutation test with 1 000 repetitions at 1.0 cM walk speed and threshold at $P \leq 0.05$. The intervals for the genetic map locations of significant QTL were calculated using a 1.5 LOD interval. The QTLs which can explain more than 10% observed phenotypic variations (R^2) and stable detected in multiple environments were considered as the major-effect QTLs.

QTLs respectively detected from $F_{2:3}$ and $F_{2:6}$ populations were integrated based on the physical location of flanking markers referencing Chinese long 9930 version 2.0 genome (http://cucurbitgenomics.org/organism/2). Based on the map integration, QTLs that were stably detected in multiple populations and environments and shared the same or had overlapped physical location were considered as consensus QTLs. Marker validation was conducted by Tukey-Kramer HSD test to determine the association of genotypes (markers) with fruit apex phenotypes (FAI) in the two F_2 populations and 186 cucumber inbred lines. Epistasis interactions of major-effect QTLs were performed by using the peak markers of the QTLs according to the methods of Yang et al. (2008) and Mackay (2014).

2.5. *Prediction and expression analysis of candidate genes within consensus QTL*

Candidate genes within consensus QTL *fa4.1* were predicted based on the published genome resequencing data of parental lines (Wu et al., 2016; Zhang et al., 2018). The common genes which have non-synonymous mutations or frameshift deletion/insertions between round fruit apex cucumber (EC1 and IL52) and sharp fruit apex cucumber (8419s-1 and CCMC) within CDS regions were considered as candidate genes. The specific

primers of the candidate genes were designed for Quantitative real time PCR by Primer Premier (Version 5.0). The sequences of these primers were listed in Table S9.

The apex part of ovary or fruit samples from the cucumber inbred lines 'EC1', '8419s-1', 'IL52' and 'CCMC' were harvested at different development stages (–3 DAP, 0 DAP, 3 DAP) with three biological repeats. Total RNA was extracted by using Trizol (Invitrogen, USA). The cDNA was then synthesized using a Prime Script™ RT Reagent Kit (TaKaRa, China). The qRT-PCR experiment was performed with a SYBR Premix Ex *Taq*™ Kit (TaKaRa, China) as described by Li et al. (2014). *CsActin3* (Csa6G484600) was used as a reference gene with constitutive expression in various tissues. To determine relative fold differences for each sample in each experiment, the Ct value for candidate genes was normalized to the Ct value for *CsActin3* and was calculated relative to a calibrator using the formula $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001). The experiment was repeated three times for each gene and sample.

2.6. *Paraffin section and microscope observation*

Ovaries of 'EC1', '8419s-1', 'IL52' and 'CCMC' at 0 DAP were collected from 15 to 20th node of plant. The apex part of the ovaries was isolated immediately and immersed in FAA solution (formalin: acetic acid: 75% ethanol = 5: 5: 90, v/v/v) for 24 h incubation as described as Li et al. (2014). Then, the samples were dehydrated through a graded ethanol/xylene series and embedded in paraffin. The paraffin blocks were cut into 5 μm thick sections along the vertical axis of ovaries and the sections stained with hematoxylin and eosin (HE) staining. For each sample, ten paraffin sections were prepared. All the paraffin sections were observed and photographed by using an Olympus BX53 microscope equipped with SPOT digital camera under the magnification of 40 × 10. Cell counting was conducted by ImageJ (Version 1.53a), for each replicate the numbers of the cell layers and cell density were measured 10 times.

3. Results

3.1. *Investigation and evaluation of fruit apex phenotypes among different cucumber ecotypes*

In this study, fruit apex phenotypes of 186 cucumber inbred lines were investigated. We divided these inbred lines into six ecotype groups including North-China ecotype (NC), South-China ecotype (SC), U.S. Processing ecotype (UP), European greenhouse type (EG), Xishuangbanna ecotype (XSBN) and the wild cucumber *Cucumis sativus* var. *hardwickii*. According to the appearance of fruit apex of commercial fruits, the cucumber lines can be simply classified into two categories such as round fruit apex (RFA) lines and sharp fruit apex (SFA) lines (Fig. 1, A; Table S1). Among the cucumber inbred lines, the fruit apex angle was in range of 99.4°–173.4°, and the fruit apex index was in range of 1.32–4.24 (Table 1). Tukey-Kramer HSD test ($P < 0.05$) suggested that the FAI and FAA were significantly correlated with fruit apex (Fig. 1, C, D). Correlation analysis suggested that the FAI was significantly positive-correlated with FAA (Fig. 1, E), however the effectiveness of FAI ($R^2 = 0.729$) was better than that of FAA ($R^2 = 0.711$). According to the data of FAI, the RFA and SFA categories could be well distinguished when the threshold was set to value 2.1 (Fig. 1, D). The variation of fruit apex in different cucumber ecotype populations was compared. The variation coefficient (CV) of

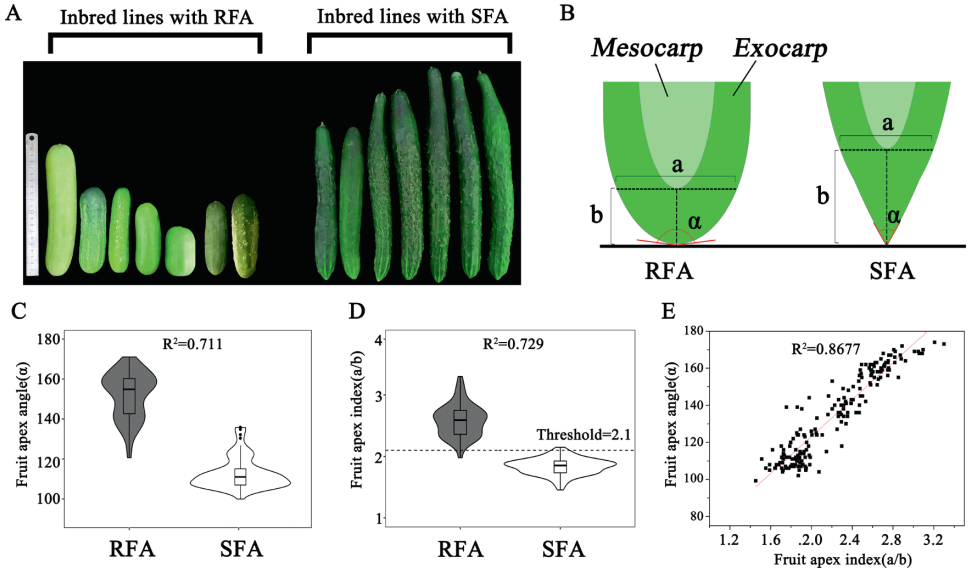

Fig. 1. Phenotypic evaluation of fruit apex shape among 186 cucumber inbred lines

(A) The commercial fruits at 10 day after pollination of different inbred lines that presented typical round fruit apex (RFA) and sharp fruit apex (SFA), respectively. (B) A diagram of cucumber fruit apex, 'α' means the angle of fruit apex, 'a' means FAD, 'b' means FAL; (C, D) The fruit apex angle (C) and fruit apex index (D) of RFA and SFA cucumber inbred lines were presented by violin and box plots. The effectiveness of FAA and FAI to distinguish RFA and SFA cucumber types were validated by Tukey-Kramer HSD test ($P < 0.05$). RFA and SFA cucumbers could be well distinguished by FAI data when threshold value was set to 2.1. (E) Correlation analysis of fruit apex angle and fruit apex index among the 186 cucumber inbred lines ($P < 0.05$).

Table 1 Variation of FAA and FAI in different ecotype cucumbers

	Population size	Means of FAA/°	Range of FAA/°	CV of FAA/%	Means of FAI	Range of FAI	CV of FAI/%
NC	63	115.3	99.4 - 157.6	12.3	1.89	1.32 - 2.74	12.9
SC	74	142.7	106.6 - 174.1	12.8	2.36	1.45 - 4.24	19.3
UP	23	155.7	110.4 - 173.4	9.5	2.63	1.90 - 4.19	17.2
EG	19	141.8	111.8 –170.4	15.7	2.32	1.51 - 3.34	22.6
XSBN	6	130.6	138.2 - 166.2	7.1	2.12	2.20 - 2.81	8.8
Hardwickii	1	166.8	160.8 - 170.3	–	3.23	3.21 - 3.31	–
Total	186	137.3	99.4 –173.4	14	2.27	1.32 - 4.24	18

Note: NC, North-China ecotype; SC, South-China ecotype; UP, U.S. Processing ecotype; EG, European greenhouse type; XSBN, Xishuangbanna ecotype; "-" represented that no data was detected.

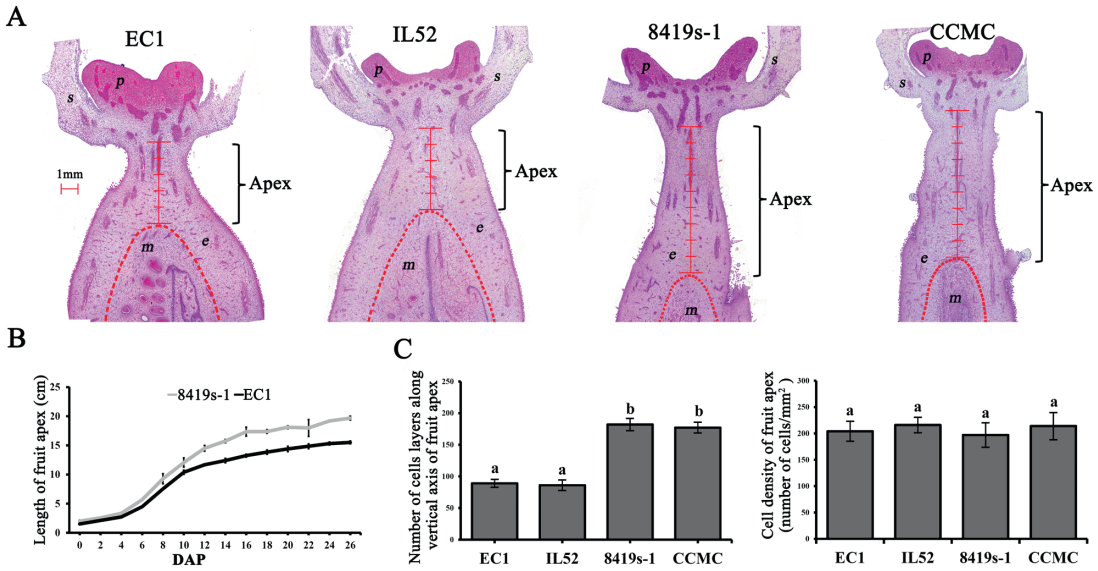

Fig. 2 Cytological observation of fruit apex in RFA and SFA cucumbers

(A) The apex of 0 DAP ovaries were harvested from 'EC1, 'IL52', '8419s-1' and 'CCMC'. Tissue boundary of exocarp (e) and mesocarp (m) was marked by red dotted lines. The central axis of fruit apex was marked by red scale lines. s, sepal; p, pistil. (B) The length of fruit apex was compared between 'EC1' and '8419s-1' from 0 DAP to 26 DAP. (C) Number of cell layers alongside the longitudinal axis of fruit apex and cell density (number of cells per mm^2) in fruit apex tissues were measured by ImageJ. Each value represents the mean ± SE of three replicates.

FAA and FAI showed similar tendency within each ecotype populations. However, among the different ecotypes the fruit apex variation of European greenhouse ecotype (EG) was the highest, while Xishuangbanna ecotype (XSBN) cucumbers showed the lowest variation (Table 1).

3.2. *Histological section analysis of round and sharp fruit apex in young fruits*

Many studies confirmed that cell division and expansion are critical to fruit morphogenesis in a variety of species such as tomato (Bohner and Bangerth, 1988; Gillaspy et al., 1993; Tanksley, 2004; Fanwoua et al., 2013), melon (Périn et al., 2002; Eduardo et al., 2007), pumpkin (Nakata et al., 2012), cucumber (Marcelis and Hofman–Eijer, 1993; Ando et al., 2012; Zhao et al., 2016; Colle et al., 2017). In this study, the histological structures of fruit apex in RFA (EC1 and IL52) and SFA (8419s-1 and CCMC) cucumbers were compared (Fig. 2). It was showed that SFA cucumbers have longer fruit apex than the RFA cucumbers throughout fruit development process (Fig. 2, A, B). Distribution of cells in fruit apex was measured. The number of cells alongside longitudinal axis of fruit apex was measured to determine the number of pericarp cell layers. The number of pericarp cell layers showed no significant difference within RFA cucumbers or within SFA cucumbers, however the SFA cucumber develops more cell lays along the vertical direction of fruit apex than the RFA cucumber (Fig. 2, C; Fig. S1). Interestingly, cell density of apex tissues showed no difference between the RFA and SFA cucumbers (Fig. 2, C), indicated that the frequency of cell division maybe has more impact on morphogenesis of fruit apex in cucumber.

3.3. *Inheritance analysis of fruit apex shape under different genetic backgrounds*

Shape of fruit apex, round in 'EC1' and 'IL52', and sharp in '8419s-1' and 'CCMC', can be distinguished easily on fruits after pollination (Fig. 3, A, C; Fig. S2). In most cucurbits such as cucumber, melon, watermelon, bitter gourd, and wax gourd, fruit length (FL) and fruit diameter (FD) are often used to describe fruit elongation and radial growth respectively, while fruit shape could be conveniently defined using fruit shape index which is the ratio of FL to FD. In present study, we used fruit apex index (FAI,

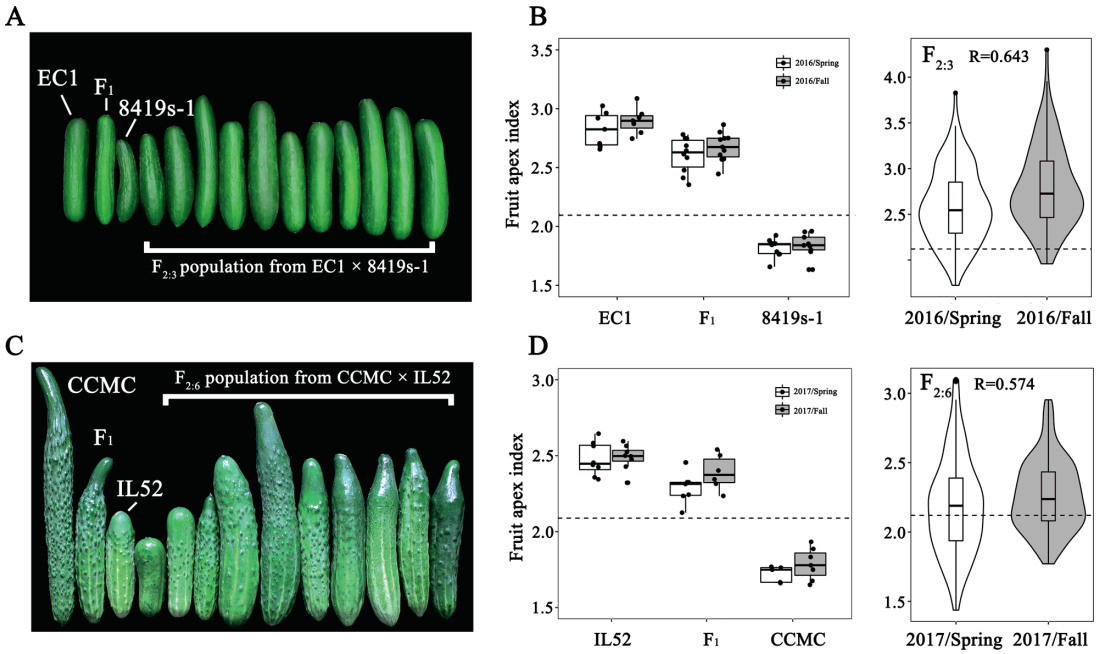

Fig. 3 The distributions of fruit apex index of parental lines, F_1 and segregation populations among different environments (A, C) $F_{2:3}$ families from EC1 × 8419s-1 (A) and $F_{2:6}$ population from CCMC × IL52 (C). (B) The FAI of 'EC1', '8419s-1', their F_1 and $F_{2:3}$ families were investigated in spring and fall of 2016. (D) The FAI of 'IL52', 'CCMC', their F_1 and $F_{2:6}$ population were investigated in spring and fall of 2017. The dotted line at value 2.1 is the threshold of FAI. 'R' mean the Spearman's rank correlation coefficient.

Table 2 Analyses of variance, variance component estimates and heritability for fruit apex index of $F_{2:3}$ and $F_{2:6}$ populations

Source of variations	$F_{2:3}$ families				$F_{2:6}$ population			
	df	Mean square	*F* value	*P* value	*df*	Mean square	*F* value	*P* value
Genotype(G)	144	1.51	10.56	< 0.0001	154	0.74	15.97	< 0.0001
Seasons(S)	1	6.01	42.06	< 0.0001	1	41.67	69.80	< 0.0001
G × S	144	0.60	4.17	< 0.0001	154	0.36	7.72	< 0.0001
Block	2	0.15	1.03	0.42	2	0.09	0.95	0.48
Residuals	288	0.14			308	0.05		
Heritability(h^2)	0.602				0.529			

FAD/FAL) to define the shape of fruit apex. Phenotypic data of the described parental lines, F_1 hybrids and segregation populations were collected in 2016 and 2017 by four experiments respectively. The FAI of commercial fruits at 10 DAP was measured. The FAI, FAL and FAD mean value ($n \geq 15$) of each individual plant were illustrated by box and violin plots (Fig. 3, B, D; Fig. S3). The frequency of FAL, FAD, and FAI mean value of both $F_{2:3}$ and $F_{2:6}$ population showed single-peak distributions in different seasons, suggested that fruit apex shape was controlled by multiple QTLs. Significant positive correlations were found in both FAI of $F_{2:3}$ ($R = 0.643$, $P < 0.001$) and $F_{2:6}$ ($R = 0.574$, $P < 0.001$) in different environments. ANOVA and variance component analysis of FAI showed that both genotypes, environments (seasons), and Genotype × Season (G × S) interactions significantly affected fruit apex shape in $F_{2:3}$ and $F_{2:6}$ population (Table 2). The broad sense heritability estimate (h^2) for fruit apex shape was 60.2% and 52.9% in $F_{2:3}$ and $F_{2:6}$ populations respectively.

3.4. *Detection of QTL associated with fruit apex index*

Mean phenotypic data of fruit tip related traits (FAI) was collected from $F_{2:3}$ and $F_{2:6}$ population in multiple environments. Six QTLs related to FAI were detected on chromosome 1, 2, 3 and 6 based on FAI means of the EC1 × 8419s-1 $F_{2:3}$ families in spring and fall of 2016 (Fig. 4; Table 3). QTLs on chromosomes 4 and 6 could be detected within two environments including *Bfai4.1* ($R^2 = 13.7\%$, 15.4% in 2016Spring and 2016Fall, respectively) and *Bfai6.1* ($R^2 = 21.0\%$, 25.9% in 2016Spring and 2016Fall, respectively). The additive effects of *Bfai4.1* and *Bfai6.1* were positive which indicated these alleles came from EC1 could increase fruit apex index. Four QTLs on chromosomes 3 and 4 were detected by the FAI data of the CCMC × IL52 $F_{2:6}$ population, of which only the *Ofai4.1* ($R^2 = 13.4\%$ and 15.5%) was repeatedly detected in spring and fall of 2017. The negative additive effect of *Ofai4.1* suggested that allele that comes from IL52 could increase fruit apex index. To verify whether the QTLs on chromosome 4 which were detected across different genetic background were the same alleles controlling FAI, the physical positions of flanked markers of *Bfai4.1* and *Ofai4.1* were compared. The result showed that the physical region of *Bfai4.1* (flanked by SSR05125–SSR21563, located at the interval of 13 529 693–17 666 521 bp on chromosome 4) and *Ofai4.1* (flanked by SSR15420-SSR29712, located at the interval of 11 388 783–15 473 324 bp on chromosome 4) was overlapped based on 9930 V2.0 reference genome, indicating *Bfai4.1*

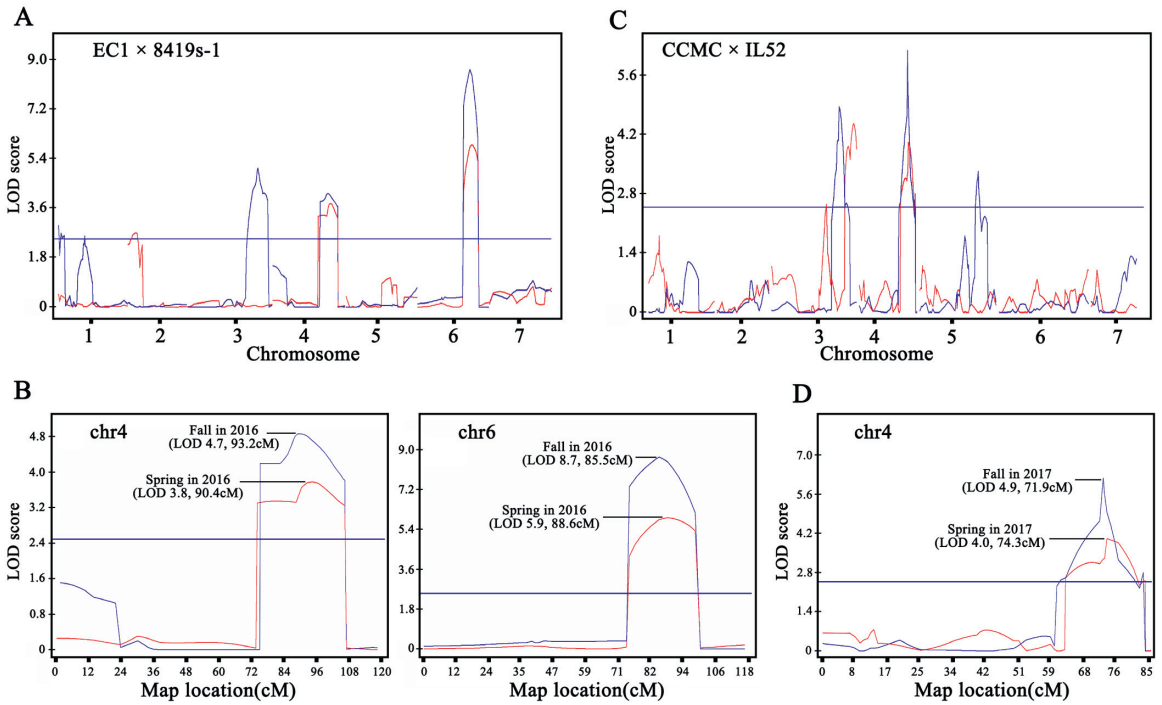

Fig. 4 Detection of fruit apex related QTLs

(A) All detected QTLs based on fruit apex index data from $F_{2:3}$ families of EC1 × 8419s-1 in spring and fall of 2016 and (C) $F_{2:6}$ population of CCMC × IL52 in spring and fall of 2017. (B) LOD curves of the major-effected QTL detected from $F_{2:3}$ families of EC1 × 8419s-1 on chromosome 4 and 6 and (D) $F_{2:6}$ population of CCMC × IL52 on chromosome 4. The peak LOD value and position(cM) were marked in brackets.LOD threshold = 2.5.

Table 3 QTLs related to fruit apex index (FAI) were detected in $F_{2:3}$ families of EC1 × 8419s-1 and $F_{2:6}$ population of CCMC × IL52 under different environments

QTL	Environments (Year/Season)	Mapping population	Chr.	Peak/cM	LOD value	R^2	Additive effect	1.5 LOD interval/cM		Marker interval
								Left	Right	
Bfai1.1	2016/Fall	EC1 × 8419s-1	1	0	3.0	10.6	0.166	0	2.1	SSR15108-SSR23757
Bfai1.2	2016/Fall	EC1 × 8419s-1	1	43.1	2.6	9.0	−0.157	31.3	56.2	SSR10134-SSR04805
Bfai2.1	2016/Spring	EC1 × 8419s-1	2	12.2	2.7	8.8	−0.123	0	19.2	SSR03070-SSR13532
Bfai3.1	2016/Fall	EC1 × 8419s-1	3	114.9	5.1	20.8	0.210	99.3	132.0	UW085395-SSR06791
Bfai4.1	2016/Spring	EC1 × 8419s-1	4	93.2	3.8	13.7	0.158	73.9	106.7	SSR05125- SSR21563
	2016/Fall	EC1 × 8419s-1	4	90.4	4.7	15.4	0.162	73.9	106.7	
Bfai6.1	2016/Spring	EC1 × 8419s-1	6	88.6	5.9	21.0	0.196	74.8	99.6	SSR19174-SSR12898
	2016/Fall	EC1 × 8419s-1	6	85.5	8.7	25.9	0.276	74.8	96.9	
Ofai3.1	2017/Spring	CCMC × IL52	3	74.8	2.5	7.0	−0.102	72.4	80.8	SSR04632-SSR20270
Ofai3.3	2017/Fall	CCMC × IL52	3	96.3	3.1	9.5	−0.078	92.3	99.9	UW085097-SSR05678
Ofai3.2	2017/Spring	CCMC × IL52	3	112.2	4.5	14.6	−0.133	107.2	115.2	SSR07131-SSR06791
Ofai4.1	2017/Spring	CCMC × IL52	4	74.3	4.0	13.4	−0.115	65.3	75.5	SSR15420-SSR29712
	2017/Fall	CCMC × IL52	4	71.9	4.9	15.5	−0.168	66.2	76.9	SSR15737- SSR29712

Note: '*Bfai*' means the QTLs were detected from $F_{2:3}$ families; '*Ofai*' means the QTLs were detected from $F_{2:6}$ population. The marker of $F_{2:3}$ families of EC1 × 8419s-1 from Wu et al. (2016) and $F_{2:6}$ population of CCMC × IL52 from Zhang et al. (2018).

and *Ofai4.1* can be integrated into a consensus QTL *fai4.1* (11 388 783–17 666 521 bp on chromosome 4).

Meanwhile, we also detected fruit apex related-QTL by fruit apex diameter and fruit apex length in $F_{2:3}$ families of EC1 × 8419s-1 and $F_{2:6}$ population of CCMC × IL52 (Table S4 and S5). A total of 7 fruit apex related-QTLs (*Bftd2.1*, *Bftd3.1*, *Bftd4.1*, *Bftd6.1*, *Oftd3.1*, *Oftd3.2*, *Oftd6.1*) were detected by fruit apex diameter from $F_{2:3}$ families and $F_{2:6}$ population (Table S4). Among those QTLs, the major-effect QTL *Bfad6.1* and *Ofad6.1* was consistently identified in two environments, which can explain 32.6%–33.6% and 11.6%–12.4% of the phenotypic variation in the $F_{2:3}$ families and $F_{2:6}$ population, respectively. A total of 7 fruit apex related-QTLs (*Bfal1.1*, *Bfal1.2*, *Bfal1.3*, *Bfal4.1*, *Oftl3.1*, *Oftl3.2* and *Oftl4.1*) were detected by fruit apex length from $F_{2:3}$ families and $F_{2:6}$ population, of which the major QTL *Bfal4.1* and *Ofal4.1* was consistently identified in two environments, explaining 11.6%–12.7% and 10.7%–12.8% of the phenotypic variation in the $F_{2:3}$ families and $F_{2:6}$ population, respectively (Table S4). These QTLs at the same or near physical interval probably belong to the same consensus QTL for the fruit apex shape. Hence, the fruit

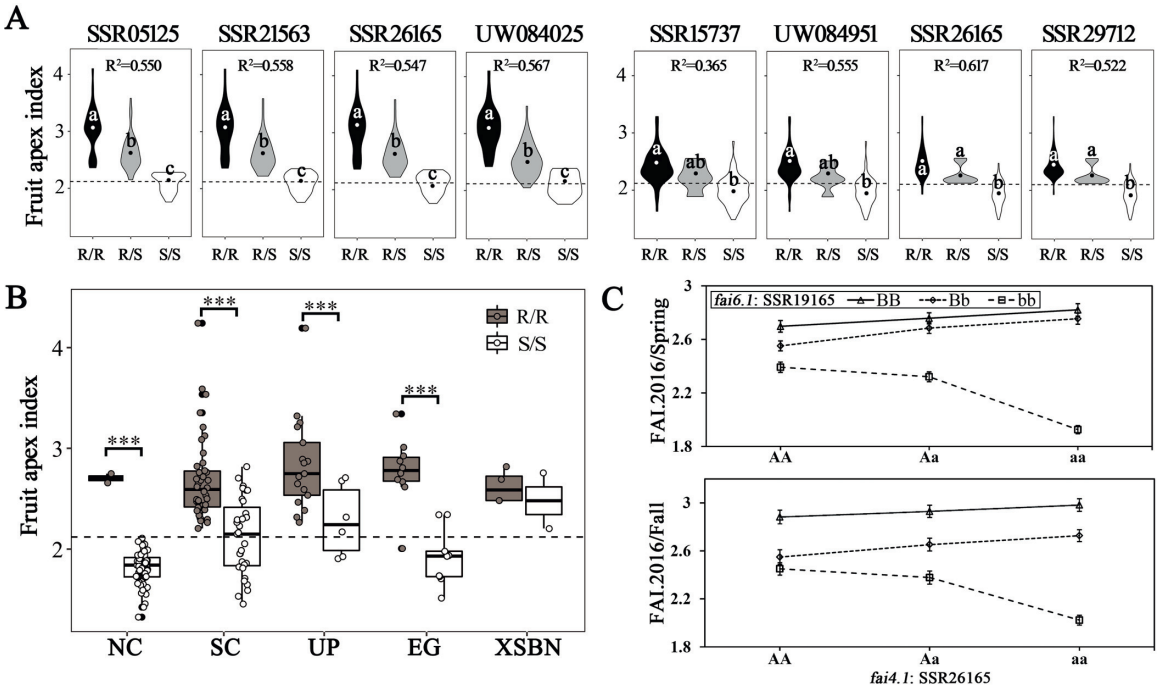

Fig. 5 Validation of the effectiveness of the markers linked to *Bfai*4.1 and *Ofai*4.1

(A) Effectiveness of makers SSR05125, SSR21563, SSR26165 and UW084025 linked to *Bfai*4.1 and SSR15737, UW084951, SSR26165 and SSR29712 linked to *Ofai*4.1 were validated in 107 F_2 population of EC1 x 8419s-1(A, left) and 112 F_2 population of CCMC x IL52 (A, right) respectively. R/R means homozygous allele of EC1 or IL52, S/S means homozygous allele of 8419s-1 or CCMC, R/S means the heterozygous alleles. Different letter indicates the significance between different genotypes at $P < 0.05$. R^2 values indicate effectiveness of the markers on FAI mean value (Tukey-Kramer HSD test, $P < 0.05$). (B) Effectiveness of the SSR26165 in 186 cucumber inbred lines was analyzed. * means significant difference between R/R and S/S genotypes among each ecotype ($P < 0.0001$). (C) Interaction plots of QTL pairs detected in $F_{2:3}$ families for FAI in spring and fall of 2016. 'EC1' and '8419s-1' carries A and B allele, respectively.**

apex length QTL *Bfal*4.1 and *Ofal*4.1 was co-localized with the fruit apex index QTL *fai*4.1 on chromosomes 4 as a consensus *fa*4.1 (Table S5). The fruit apex diameter QTL *Bfad*6.1 was co-localized with the fruit apex index QTL *fai*6.1 on chromosomes 6 as a consensus *fa*6.1 (Table S5).

3.5. *Validation of the effectiveness of the markers linked to fai*4.1

The markers SSR05125, SSR21563, SSR26165 and UW084025 linked to *Bfai*4.1 and SSR15737, UW084951, SSR26165 and SSR29712 linked to *Ofai*4.1 were used to genotype 107 F_2 population of EC1 × 8419s-1 and 112 F_2 population of CCMC × IL52, respectively (Fig. 5, A). The effects of the markers on FAI were tested with a Tukey-Kramer HSD ($P \leq 0.05$) procedure. We assigned the gene symbol *R* for round fruit apex in EC1 and IL52, and *S* for sharp fruit apex in 8419s-1 and CCMC. The FAI means of plants with homozygous EC1 alleles (*R*/*R*) at loci SSR05125, SSR21563, SSR26165 and UW084025 were 3.045 ± 0.417, 3.073 ± 0.400, 3.110 ± 0.392 and 3.205 ± 0.312, respectively, that were significantly higher than those homozygous *S*/*S* alleles (2.107 ± 0.161, 2.109 ± 0.168, 2.101 ± 0.162 and 2.102 ± 0.184). The FAI means of heterozygous genotype plant (*R*/*S*) at loci SSR05125, SSR21563, SSR26165 and UW084025 were significantly lower than the *R*/*R* plants but significantly higher than *S*/*S* plants. In the F_2 population of CCMC × IL52, FAI means of homozygous IL52 genotype plants (*R*/*R*) and heterozygous genotype plants (*R*/*S*) at loci SSR26165 and SSR29712 were significantly higher than the homozygous genotype plant (*S*/*S*), but there was no significant distinction between the two groups. FAI means of homozygous *R*/*R* at loci SSR15737 and UW084951 were significantly higher than the homozygous *S*/*S*, but there was no significant distinction between the heterozygous genotype *R*/*S* and *S*/*S*.

The marker SSR26165 was a common marker that was linked to both major-effect QTLs *Bfai*4.1 and *Ofai*4.1, which had significant effect on FAI means across two F_2 populations with R^2 values at 0.547 and 0.617 (Fig. 5, A). To further validate the effect of allele at loci SSR26165 on fruit apex shape, the relationship between genotypes of loci SSR26165 and FAI mean value in the 186 natural cucumber lines including 5 ecotype groups was analyzed (Fig. 5, B). The results showed that SSR26165 has significant correlation between genotype and phenotype of NC (North-China type cucumber), SC (South-China type cucumber), UP (U.S. Processing type cucumber) and EG (European greenhouse type) ecotype groups ($P < 0.0001$, Fig. 5, B). However, polymorphism of loci SSR26165 showed no association with fruit apex shape in XSBN (Xishuangbanna type cucumber) group which suggested that XSBN cucumber ecotype maybe harbored novel alleles to control fruit apex trait.

Table 4 Estimated epistatic effects (AA) interactions between *Bfai4.1* and *Bfai6.1* for FAI of $F_{2:3}$ families from EC1 x 8419s-1

QTL	Flanking marker	P	AA[a]	H^2 (AA,%) [b]
Bfai4.1	SSR05125 - SSR21563	$P < 10^{-4}$	1.81	12.87
Bfai6.1	SSR19174 - SSR12898			

Note: AA, Additive by additive interaction, positive value of AA indicates an increased value of the trait when parental genotypes are paired. Heritability (H^2) of QTL effect or percentage of variation in the total phenotypic variance that is explained by the component of the corresponding genetic source (AA).

3.6. *Interaction analysis between the QTLs* Bfai4.1 *and* Bfai6.1

We found that *Bfai6.1* was a stable major-effect QTL explained 21.0%−25.9% phenotypic variance of FAI, but it could be only detected in EC1 × 8419s-1 population (Fig. 4; Table 4). Interestingly, since both EC1 and IL52 were RFA inbred lines, both FAA and FAI of EC1 was significantly higher than that of IL52 (Fig. 3; Table S1). We speculated that *Bfai6.1* maybe has an additive effect on *Bfai4.1*. We investigated possible interactions among *Bfai4.1* and *Bfai6.1* by the methods of Yang et al. (2008) and Mackay et al. (2014). The marker SSR26165 with peak LOD score of *Bfai4.1* and SSR19165 with peak LOD score of *Bfai6.1* were chosen for locus interaction analysis in the 145 $F_{2:3}$ families from EC1 × 8419s-1. Effect plot showed that *Bfai6.1* had epistasis interaction with *Bfai4.1* (Fig. 5, C). In *Bfai4.1* background, FAI mean value of homozygous and heterozygous genotype plants (AA and Aa) at the loci SSR19165 were much higher than that of aa genotype. Additive by additive interaction (AA=1.81, $P < 0.001$) between *Bfai6.1* and *Bfai4.1* were analyzed by QTLNetwork 2.1(Yang et al., 2008), which accounted for 12.87% of the variation in the $F_{2:3}$ families (Table 4).

3.7. *Prediction of candidate genes of* fa4.1

Polymorphic SNP/Indels between EC1 and 8419s-1 as well as mutants between IL52 and CCMC were screened based on genome re-sequencing data of these parental lines. About 680 genes were located within the overlapped region of *fa4.1* (11 388 783 bp–17 666 521 bp on chromosome 4) referred to Chinese long 9930 version 2.0 reference genome (http://cucurbitgenomics.org/organism/2). The 69 and 152 genes were identified that have non-synonymous or frameshift deletion/insertion mutation sites between the two pairs of parental lines, respectively (Table S6 and S7). However, only 11 mutant genes were found to be commonly present in both RFA (EC1 and IL52) and SFA cucumbers (8419s-1 and CCMC) including 6 identical polymorphic sites within 4 mutation genes (Table S8).

The expression patterns of the 11 common mutant genes were analyzed during early developmental stages of apex part of ovary or fruit (−3 DAP, 0 DAP and 3 DAP) in EC1, 8419s-1, IL52 and CCMC. The 9 of the 11 genes showed changed expression during ovary/fruit development, only 5 genes performed different expression patterns between RFA and SFA cucumbers such as *Csa4G312230, Csa4G325540, Csa4G385800, Csa4G418540* and *Csa4G418560* (Fig. 6). *Csa4G312230* encodes a proliferation-associated protein. The homologous genes of *Csa4G312230* can regulate organ size through cell growth and proliferation in plants (Horváth et al., 2006; Wang et al., 2016). *Csa4G325540* is a regulatory associated-protein of TOR (Target of Rapamycin) which maybe plays a role in the stimulation of cell growth and metabolism in response to nutrients (Deprost et al., 2005; Pu et al., 2017). *Csa4G385800* is a leucine-rich repeat protein kinase family protein that may be involved in protein phosphorylation and regulation of pollen tube growth (Duckney et al., 2017). *Csa4G418540* belongs to heat shock protein 70 family which is involved in protein import into chloroplasts during plant early developmental stages (Su and Li, 2008). *Csa4G418560* encodes a KRI1-like protein (KRR1 interacting protein 1) which is responsible for ribosome biosynthesis. Among above five differentially expressed genes, *Csa4G385800* contains a common 3 bp deletion mutation at 2nd exon in SFA cucumbers by comparing to RFA cucumbers, while *Csa4G418560* contains 3 common non-synonymous SNPs in two mapping populations (Table S8).

4. Discussion

4.1. *Evaluation of fruit apex in different cucumber ecotypes*

Fruit represent an important part of the human diet and show extensive variation in size and shape within cultivated species (Monforte et al., 2014; Zhang et al., 2021). To explain and ultimately employ this variation towards crop improvement, numerous studies were conducted to investigate and evaluate morphological variability in many species. Cucumber is one of the most important cultivated cucurbit crops, which also presents a rich diversity in fruit shape and size. The genetic basis of fruit shape-related traits were well studied especially the fruit length, diameter and the fruit shape index (length/diameter ratio) (Wei et al., 2014; Bo et al., 2015; Weng et al., 2015). However, the fruit apex of cucumber was usually considered to be a low variance trait which has not been studied yet. In practice, shape of cumber fruit apex was simply divided into round and sharp types. In this study, the fruit apex angles and fruit apex index were employed as accurate parameters to describe feature of fruit apex. Interestingly, we found although cucumber cultivars can be easily classified into RFA and SFA groups with fruit apex index when threshold is set to 2.1, the FAI data showed typical normal distributions either in the natural population or in the $F_{2:3}$ and $F_{2:6}$ segregation populations. Besides, the cucumber inbred lines could not be simply clustered into two groups by fruit apex angle, suggesting that diversity of fruit apex in cucumber may be more complex than we imaged.

The diversity of fruit apex were significant different among different ecotype groups. Many studies confirmed that significant variations of fruit shape in crops owed to different domestication and improving pathways (Frary et al., 2000; Cong et al., 2002; Karimi et al., 2005). We speculated that different distribution of fruit apex variation between different ecotypes maybe owed to different selection and domestication strategy. For example, a part of breeders were interested in selecting gynoecious cucumber cultivars with limited fruit size to increase fruit load and single plant yield e.g. some European greenhouse type cultivars possess round fruit apex (141°–173°) that is similar to Cucumis sativus var. hardwickii. In China, breeders used to improve diameter and length of fruit to increase fruit weight. For instance, majority of South-China and Xishuangbanna ecotype cucumbers present relatively larger fruit diameter hence produce thick and round fruit apex (138°–166.2°), whereas most of North-China eco-

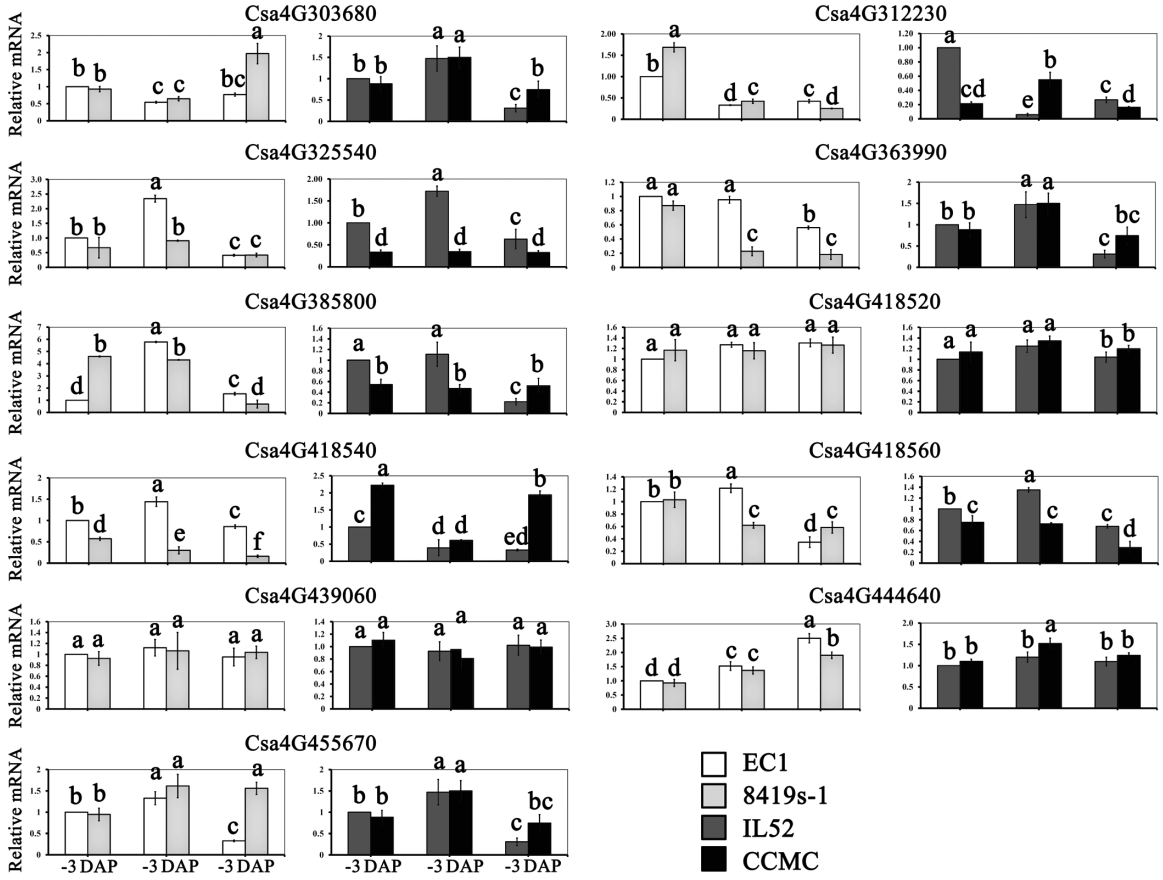

Fig. 6 Expression patterns of 11 candidate genes within consensus QTL *fa*4.1 during early stages of fruit development by qRT-PCR Data are expressed as relative values, based on the values of the −3 DAP ovaries of EC1 and IL52 respectively. Each value represents the mean ± *SE* of three replicates. Letters indicate the least significant differences (t-test, $P < 0.05$) between EC1 and 8419s-1 as well as between IL52 and CCMC.

type cucumber cultivars produce very long fruit with an obvious fruit stalk as well as a thin and tapering fruit apex (99.4°–139°).

4.2. *QTLs associated with shape of fruit apex*

Fruit apex is a critical part of fruits that associate with the appearance quality of fruit crops. A few studies demonstrated that traits of fruit shape were controlled by multiple QTLs. Six QTLs controlling shape of fruit apex were detected in pepper (Zhang et al., 2014). Three QTLs associated with fruit apex shape were identified in eggplant (Geng, 2018). In this study, phenotype data of fruit apex suggested that shape of fruit apex was controlled by multiple QTLs in cucumber. Fourteen fruit apex related-QTLs on chromosomes 1, 2, 3, 4 and 6 were detected by FAD, FAL, FAI in the $F_{2:3}$ families that derived from European greenhouse ecotype cucumbers, while ten fruit apex related-QTLs on chromosomes 3 and 4, 6 were detected by FAD, FAL, FAI in the $F_{2:6}$ population from cross of North-China ecotype and South-China ecotype. It seems that shape of fruit apex in different ecotype was controlled by different alleles. For example, *Bfai6.1* was a stable QTL that detected in multiple experiments ($R^2 > 10\%$), but could not be detected in IL52 (South-China ecotype). QTL *fa4.1* which was detected across different genetic background was considered as a stable major-effect QTL in cucumber, however, genotypes of loci SSR26165 (an effective marker associated with *fa4.1*) were not consistent with phenotypes of fruit apex shape in Xishuangbanna type cucumbers. It suggested that Xishuangbanna cucumber ecotype maybe harbored novel alleles to control fruit apex trait.

It was widely recognized that epistasis plays a significant role in the genetic regulation of quantitative traits (Causse et al., 2007; Phillips, 2008; Mackay, 2014). We found EC1 possess more round fruit apex than IL52. Besides the phenotype data of 186 cucumber inbred lines also suggested that the FAI mean value of European greenhouse type cucumbers were higher than that of North-China cucumbers. It seems that the fruit apex-associated QTLs maybe have interactions with each other. In present study, epistasis interaction analysis showed that *fai4.1* and *fai6.1* accounted for 12.87% of the phenotypic variance. Phenotype (fruit apex index) and genotype at alleles *fai4.1* and *fai6.1* were investigated in 107 F_2 plants from EC1 × 8419s-1. We found that both segregation ratios of genotype and phenotype in the F_2 population were significantly consistent with the expected ratios: 12 (B_, blunt round fruit apex): 3 (A_bb, oval round fruit apex): 1 (aabb, sharp fruit apex) (data not shown), implied that *fai4.1* and *fai6.1* maybe follow a two-gene model involved in inheritance of fruit apex. Further work of this study will focus on the effects of the two single loci (*fai4.1* and *fai6.1*).

4.3. *Prediction of candidate genes within fa4.1*

In present study, consensus QTL *fa4.1* was a reliable major-effect QTL controlling the shape of fruit apex. Within the chromosome region of *fa4.1*, mutant genes between EC1 and 8419s-1 as well as between IL52 and CCMC were screened respectively by comparing genome sequence of parental lines. Although the 69 and 152 mutant genes were respectively identified from the EC1/8419s-1 and IL52/CCMC parental line pairs, only 11 genes between the two pairs of parental lines may be promising candidate genes involved in cucumber fruit apex morphogenesis. It is widely recognized that phytohormones play critical roles during fruit development, but gene annotation suggested that none of the 11 genes was closely related to hormones related pathways. Six common mutant sites were identified in four genes including *Csa4G303680* (Glutathione S-transferase), *Csa4G363990* (JMS09K11.8 protein), *Csa4G385800* (Receptor protein kinase) and *Csa4G418560* (KRI1-like protein) which were the most possible candidate genes for QTL fai4.1. Besides, qRT-PCR showed that the 5 of the 11 polymorphism genes presented distinguished expression patterns between RFA and SFA cucumbers, suggesting these candidate genes should not be excluded.

4.4. *Variation of fruit shape owed to variation of cell division*

Coordinated cell division and expansion patterns regulate the shape and size of fruit. Specifically, the rate, duration and orientation of cell division, as well as isotropic and anisotropic cell enlargement contribute greatly to final morphology of fruit (van der Knaap and Østergaard, 2018). In tomato, variation of fruit shape can be explained by *SUN, OVATE, FAS* and *LC* (Rodríguez et al., 2011; Rodríguez and Kim, 2013). Distribution of *SUN*, *OVATE, LC*, and *FAS* in the tomato germplasm lead to variation of cell division patterns in ovary development to alter final fruit shape. For example, phenotypic analysis of *SUN* near-isogenic lines has demonstrated that fruit elongation was caused by increasing cell numbers in the longitudinal direction of the fruit while reducing them in the transverse direction. The relative increases in the proximal region cells and number of cell layer in *Slelf1* and *Slelf3* mutants resulted in elongated fruit (Rodríguez and Kim, 2013; Chusreeaeom and Ariizumi, 2014a,b; Wu et al., 2018).

Fruit development of cucumber generally follows the Gillaspy et al. (1993) model. The initial stage is marked by increases in cell division, followed by cell expansion. Fruit length is a key component of fruit shape in cucumber which displays tremendous variation, from 5 to 60 cm in length (Sebastian et al., 2010; Yang et al., 2012). A revealed *CsFUL1-CsSUP* model suggested that *CsFUL1* and *CsSUP* may regulate fruit elongation via modulating cell division and cell expansion (Zhao et al., 2019). The genetic basis of fruit stalk was also well studied in cucumber (Miao et al., 2011; Weng et al., 2015; Zhao et al., 2016). Histological study suggested that although cell phase progressed from cell division to cell expansion in the stalk is earlier than in the fruit, the cell division was more active in fruit, thus the fruit diameter was twice as large as diameter compared with the stalk (Zhao et al., 2016). As the opposite part of stalk, fruit apex is also an important part of cucumber fruit. In this study, we found that the cell division patterns in fruit apex seems slightly different from fruit stalk, the SFA cucumber developed more cell lays along the vertical direction of fruit apex than the RFA cucumber while the cell density of apex tissues showed no difference between the RFA and SFA cucumbers suggesting the variation of fruit apex was mainly determinate by cell division.

5. Conclusions

In conclusion, evaluation of fruit apex in different cucumber ecotypes revealed variation between different cucumber ecotypes. Twenty-four fruit apex related QTLs were detected across multiple genetic backgrounds and environments which has different distribution in different cucumber ecotypes. Epistatic interaction was revealed between the major-effect QTL pairs *fai4.1* and *fai6.1*. These finding suggested that diversity of fruit apex may be attributed to the different distribution of the QTLs in the cucumber germplasm. Histological analysis further suggested that variation of fruit apex shape in cucumber maybe owed to the different frequency and orientation of cell division in apex structures of fruit.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hpj.2021.12.001.

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