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FLOWERING LOCUS T **(*FT*) gene regulates short-day flowering in low latitude Xishuangbanna cucumber (*Cucumis sativus* var. *xishuangbannanesis*)**

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Abstract

Xishuangbanna (XIS) cucumber is a semi-wild cucumber originating from a low latitude. XIS cucumbers are strictly short-day plants, while cultivated cucumbers are day-neutral plants. The length of the light phase has a dosage effect on flowering time and the day length requirement for flowering is 8−11.5 h. Out-of-range photoperiod conditions and weak light conditions are unfavorable for blooming. Transcriptomic and gene expression analysis indicate circadian pathway genes as well as *CONSTANS* (*CO*) did not show a differential response to photoperiod treatment between XIS and cultivated cucumbers. The *FLOWERING LOCUS T* (*FT*) gene is activated from 10:00 to 18:00, and long- (16 h) and medium- (12 h) day length suppressed this diurnal rhythm expression. We designed Kompetitive allele specific PCR (KASP) markers based on genomic SNPs between the mapping parents (XIS49 and CL) to genetically map the short-day flowering gene. Field investigation was performed after long-day photoperiod (16 h) treatment. Finally, we detected a strong quantitative trait locus (QTL) signal at a 540-kb segment (chr1: 29.08–29.62 Mb) that carries the *FT* gene. We found a 30-kb TE-rich insertion with a distance of 15 kb to the *FT* gene in XIS49, which may contribute to the gain of the short-day flowering trait in XIS49. The 30-kb insertion endowed the *FT* gene and the inserted *polygalacturonase* (*PG*) gene with a photoperiod-dependent expression manner. Our study indicates that the *FT* gene, but not its upstream circadian clock genes, regulate short-day flowering in the XIS cucumber, and the *cis*-regulation of the *FT* gene is probably due to a TE insertion.

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Introduction

The species *Cucumis sativus* includes a cultivated variety (var. *sativus*) and three wild/semi-wild varieties (var. *hardwickii*, var. *sikkimensis*, and var. *xishuangbannanesis*)[1,2]. Genomic structural analysis based on 119 resequencing data proposed that all cucumber germplasm can be divided into four geographic groups: a Eurasian group, an East Asian group, a Xishuangbanna (XIS) group, and an India group[3]. XIS cucumbers are semi-wild and were found in the 1980s[4], spreading in a narrow area about N20°–N23° around the Xishuangbanna prefecture of China. This cucumber group is characterized by its melon-like fruit, colorful flesh, vigorous growth, large organ size, as well as photoperiod-dependent sex expression[5,6]. One XIS assembly Cuc80 was released recently, which is generated on long-read sequencing technology[7]. There are some reports about the genetic analysis of carotenoid-rich flesh[3], fruit shape[8], and flowering time[9] by using XIS cucumbers. Generally, many characteristic traits of this group need to be determined in the future.

Higher plants can be classified into short-day (SD) plants, long-day (LD) plants, and day-neutral (DN) plants according to their photoperiodism. Short-day plants and long-day plants are found mainly in low latitude and high latitude areas, respectively. Higher plants can obtain the trait of photoperiodic flowering through simple mutation. For example, long photoperiodic barley and wheat can flower earlier at SD conditions when they have the *ppd-H1* (*PRR7*) mutation[10]. Common bean (*Phaseolus vulgaris* L.) transformed from short-day plants to day-neutral plants when they migrated from southern to higher latitudes; such a flowering habit change is obtained from mutations at *PHYA3* and *COL2*[11,12]. Cucumbers originated from the low latitude Indian subcontinent 10.1 million years ago[13] and a south-to-north adaptation must have occurred for temperature and photoperiod responses to change. During this evolutionary process, vegetative growth usually became shorter and flowering time became earlier for adaptation[14]. Nowadays, predominant cultivated cucumbers are day-neutral plants, except for XIS cucumbers and some Indian cucumbers. XIS cucumbers develop only lateral branches instead of flowers in the spring when moved from their original habitat and growers have to extend their growth into autumn for propagation purposes[15,16]. However, a recent study controversially reported that XIS and wild cucumbers are insensitive to photoperiod treatment[17].

There are already some studies focused on the flowering traits of XIS cucumbers. Three major QTLs, *FT1.1*, *FT5.1* and *FT6.2*, are proposed to control late flowering under a long photoperiod in XIS cucumber, respectively[8]. Fine mapping of

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early flowering locus 1.1 indicates that a large deletion of the *FLOWERING LOCUS T* (*FT*) upstream should account for higher expression of *FT* and therefore earlier flowering during domestication[17]. A recent pan-genome analysis proposed the association of sequential variance positioned upstream of the *FT* coincides with flowering time in cucumber germplasm[7]. From these QTL mapping works, we can get a consensus locus that controls cucumber flowering time on the end of the long arm of chromosome 1 carrying the *FT* gene.

Previously, studies usually ignored the photoperiod influence and carried out the field investigation in natural field conditions, labeling the XIS cucumber as a late-flowering plant. In fact, our experiment indicates that the XIS cucumber can bloom as quickly as a cultivated cucumber when under SD conditions. In this study, we get a linear correlation between day length and flowering time, which explains why XIS cucumbers become late-flowering when moved to a higher latitude area. From QTL mapping, transcriptome analysis, and gene expression analysis, we reveal the regulation mechanism of short-day flowering in XIS cucumbers.

Results

Short-day-dependent flowering in XIS cucumber

We performed photoperiod treatments to XIS49 cucumber seedlings. After the sixth leaves unfolded, the seedlings were then moved into the greenhouse and were subject to investigation of flowering traits. Only those flowers formed within the first 15 nodes were examined since cucumber seedlings with six unfolded leaves have already formed male and female flowers within 16.75 ± 1.21 nodes as observed under a microscope. Under weak light conditions, flowers were only induced by a light phase of 10 h; while under strong light conditions, plants can bear flowers at light phase of 8 h and 10 h (Fig. 1a). Weak light, under required day length (≤ 6 h), and a long day length (≥ 12 h) are unfavorable to flower formation.

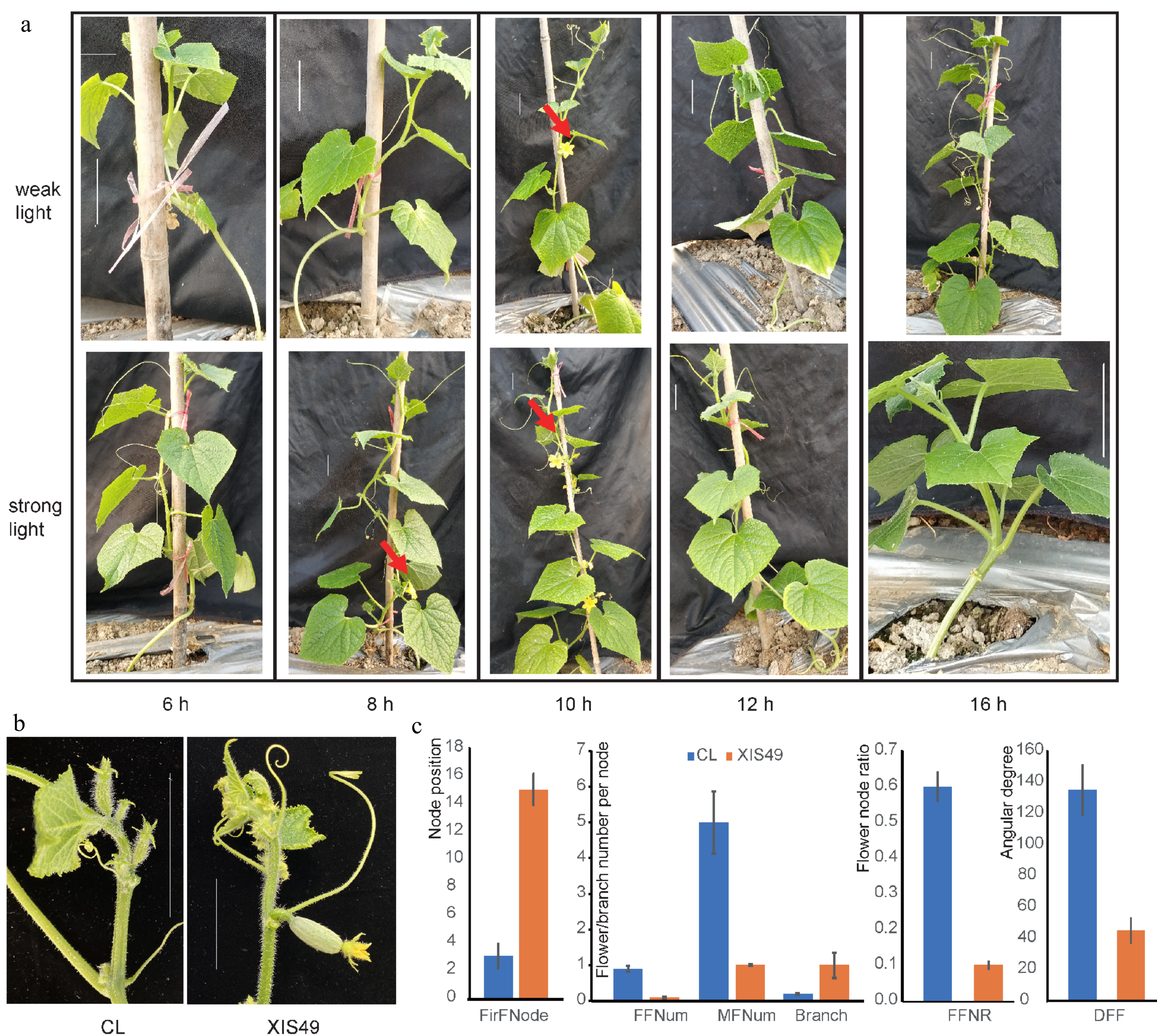

Fig. 1 Characteristic flowering-related phenotypes of XIS49. (a) Photoperiod and light intensity-dependent flowering initiation. The bars indicate a scale of 5 cm. (b) Female flower orientation. The bars indicate a scale of 5 cm. (c) XIS49 is significantly different with CL cucumber in first flower node (FirFNode), female flower number (FFNum) per node, male flower number (MFNum) per node, branch number per node, female flower node ratio (FFNR), and downward female flower (DFF) denoted by the angle at the stem.

In addition to flowering time, XIS49 is also characterized by downward female flower (DFF), fewer female and male flowers, a lower female-to-male flower ratio, and more branches (Fig. 1b & 1c).

To determine the minimum demand of a short-day condition, XIS49 seedlings were subjected to a treatment of a gradient day length change with a particularly short interval of 0.5 h between 10 and 12 h (Table 1). Here, flowers within 25 nodes were objection of field investigation. It was observed that a light phase longer than or equal to 12 h does not induce flowering within the first 15 nodes, and a 11.5 h light phase clearly meets the minimum demand of a SD condition. Interestingly, the flowering time (denoted as the first flower node) was gradually pushed back when day length increased, which means there is a negative dosage effect of light phase length on XIS49 flowering. The arrest of flowering under LD conditions is GA-independent since exogenous GA could not make amends (data not shown).

Table 1. Influence of photoperiod on flowering of XIS cucumber.

Photoperiod	First flower node[1]	First FF node[1]	FF number[1]
8H	3.5 ± 0.2 e	8.9 ± 0.3 c	1.8 ± 0.2 a
10H	4.7 ± 0.3 d	11.3 ± 0.3 b	2.1 ± 0.3 a
10.5H	6.0 ±0.4 c	12.6 ± 0.3 ab	1.5 ± 0.4 a
11H	5.2 ± 0.2 cd	12.9 ± 0.8 a	1.6 ± 0.2 a
11.5H	10.4 ± 0.4 b	–	–
12H	17.6 ± 0.6 a	–	–
16H	–	–	–

[1] Average flower number ± SE within 25 nodes. The different letters following the numbers indicate significant difference between different photoperiod, $P \le 0.05$. FF, female flower; "–" indicates no flowers observed.

Transcriptomic analysis under photoperiod treatment

Plants sense photoperiodic change with their leaves. To profile the global response of transcriptome to photoperiod condition change, we divided night time into trisection (N1-N3) and daytime into quinquesection (D1–D5). Leaf samples of XIS49 were collected at the midpoint of night time (N2) and daytime (D3). Then we compared transcriptome between different photoperiod conditions of 8 h (SD), 12 h (MD), and 16 h (LD). More than 6.21 Gb clean data of RNA-seq were generated for each sample by Illumina sequencing (Supplemental Table S1). The biological triplicates of each sample clustered together indicate a good reproducible (Supplemental Fig. S1). The difference of transcriptome between day and night is much bigger than the contrast between different photoperiod treatments. There were 6,205, 4,932, and 3,894 day-night differently expressed genes (DEGs) under SD, MD, and LD conditions, respectively (Supplemental Fig. S2). The numbers of the day genes (upregulated in daytime) and the night genes (upregulated in night time) are almost the same. As expected, the most significantly enriched process/pathway in Gene Ontology (GO) and KEGG enrichment analysis is about chlorophyll and photosynthesis metabolism, followed by amino acid and plant hormone-related processes and pathways.

We are more interested in those genes that show a different response to photoperiod treatments. For the daytime gene set, there are a total of 5,269 DEGs and 363 of these genes are conserved in different comparisons (Supplemental Fig. S3); for the night time gene set, there are a total of 4,976 DEGs and 293 of these genes are conserved in different comparisons (Supplemental Fig. S4). To summarize the response pattern, GO and KEGG enrichment analysis was performed on those DEGs (Supplemental Fig. S3 & S4). This was repeated with day-night DEGs and a short-long photoperiod caused DEGs involved with assimilation, amino acid/protein biosynthesis, and primary and secondary metabolites. Interestingly, ribosome genes show both circadian and photoperiodic expression mannerisms. For day-night DEGs, overwhelming predominant ribosome genes are downregulated at night under SD; in contrast, MD and LD treatment upregulate almost all the ribosome DEGs at night (Fig. 2a). On the other hand, SD enhanced the expression level of ribosome gene in the daytime, but decreased their expression in nighttime (Fig. 2b). In fact, in addition to flowering time, long photoperiod also causes serious abnormal growth, which is probably ascribed to the disturbed expression of ribosome genes.

As there is a dosage effect of day length on flowering time (Table 1), we generated a dot plot analysis between 16-8 (16 h vs 8 h) and 12-8 (12 h vs 8 h). Although 12 h and 16 h exert the same influence on DEGs expression, there is not any expected dosage effect on global gene expression (Fig. 2c). The dosage effect on phenotype is probably a result of a single gene regulation rather than a response by the global transcriptome. Among the photoperiodic DEGs, 10 DEGs are annotated (GO annotation) to be flowering-related. *FT* and *EID1* gene are suppressed by LD condition (Fig. 2d). For *GIGANTEA* (*GI*), *EARLY FLOWERING 3* (*ELF3*), and *EARLY FLOWERING 4* (*ELF4*), clearly, longer day length correlates with a higher expression level.

Response of circadian rhythm genes to photoperiod change

Plants sense day length change through circadian rhythm. Therefore, we profiled the expression change of genes annotated in a circadian rhythm plant (KEGG, ko04712) during the photoperiod treatment (Fig. 3a). Quantified by FPKM in RNA-seq and transcriptome analysis, there are 27 photoperiodic DEGs encoding 16 enzymes showing a significant response to photoperiod treatment in the circadian pathway. Clearly, most of these DEGs do display a circadian fluctuation in expression level as expected. For example, *SPA1* is a night gene in cucumbers, and was decreased by LD; *GI* is a day gene and was increased by LD. The most important two genes are the *FT* and the *CO* gene. The *FT* gene directly regulates initiation of a decrease in shoot meristem while the *CO* gene is the outflow of circadian rhythm and modulates the *FT* gene. Impressively, the *FT* gene is detected by RNA-seq only under SD conditions, while the induction of *CO* by SD is ambiguous and unclear.

To precisely profile the expression response of circadian genes, leaf samples were collected every two hours in a 24 h period and gene expression was quantified by qRT-PCR (Fig. 3b). Alternatively, daytime and night time length were equally divided into five and three sections, respectively. The *FT* gene, the central small molecule that transmits the flowering signal from leaf to meristem, is only expressed under SD conditions from 10:00 to 18:00, and is sharply suppressed by MD conditions; under LD conditions, the *FT* gene is totally inactive (Fig. 3b and Supplemental Fig. S5). The *CO* gene 'bridges' circadian rhythm and the downstream *FT* gene. The *CO* gene is extensively expressed from 8:00 to 16:00, which coincides well with the *FT* genes. However, no clear substantial

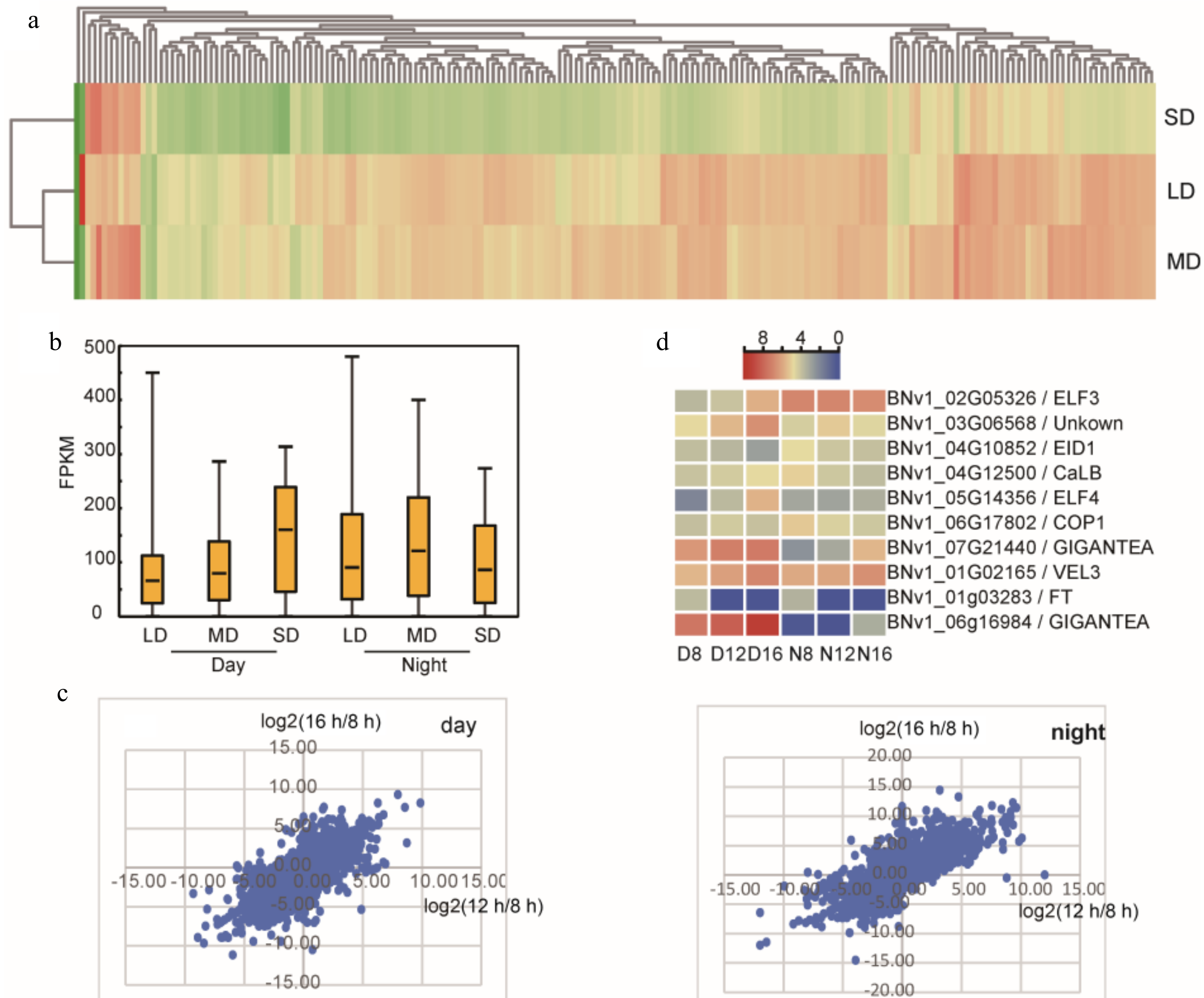

Fig. 2 Influence of photoperiod treatment on XIS49 transcriptome. (a) A great number of ribosome genes show diurnal rhythm, and these day-night ribosome DEGs (day vs night) are upregulated at night under LD and MD but downregulated at night under SD. (b) Photoperiodic DEGs (LD vs SD, MD vs SD, LD vs MD) show much higher expression level under SD in daytime but not in nighttime. (c) Plotting the value of log2(16 h-FPKM/8 h-FPKM) against log2(12 h-FPKM/8 h-FPKM) by using the photoperiodic DEG set. (d) Flowering-related photoperiodic DEGs annotated by GO term.

difference was observed for *CO* and those circadian clock genes between photoperiod treatments.

QTL mapping confirmed the central role of *FT* genes

We constructed an F2 population by crossing XIS49 with CL cucumber to map the QTL of first flower node (FFNode) that reflects the flowering time, as well as other flowering-related traits. All seedlings were grown under a LD condition until the six-leaf stage to ensure that the shoot apices had finished the process of sex determination within the first 15 nodes before moving them to a greenhouse. A total of 23 KASP markers screened out from 30 KASP markers were applied in the QTL mapping work (Supplemental Fig. S6). We successfully detected QTLs for all the targeted phenotypes twice in 2020 and 2021 with LOD > 5; particularly, the QTLs of the FFNode and the MFNum can explain 54.8% and 47.09% of the inheritance, respectively (Table 2). For the FFNode trait, XIS49 could not initiate flowering within the first 15 nodes, while the average value of first flower node of CL cucumber is 3.15 ± 0.55.

To shorten the QTL region, nine more markers positioned from 28,294,532 to 30,080,987 were applied. As a result, the QTLs were detected twice around 29,795,488 and the final QTL was positioned between 29.08 and 29.62 Mb on chromosome 1 (Fig. 4a; Table 2). At this locus, there are two big polymorphic fragments between the parent plants: a sequence variant (SV) cluster and the big insertion in XIS49 (Fig. 4b). The *FT* gene is positioned between the SV cluster and the big insertion. Since there is no sequential difference of the *FT* genes in term of both gene body and promoters between parents, we focused on the two big polymorphic fragments. The SV cluster region consists of serious SVs within a segment of 29.1MB-29.4Mb. These SVs caused polymorphic promoters of 16 genes, which contribute all the SV genes in the QTL region (Table 3). The big insertion (with a length of 39.88kb) was formed during the evolutionary period when cucumbers separated from melons and is found in wild cucumbers (WI22) and semi-wild cucumbers (XIS49), but later was lost in cultivated cucumbers (Supplemental Fig. S7). A *polygalacturonase* (*PG*) gene and four TIR transposon with high quality (length > 1,000; score > 2,000) were positioned in the big insertion. The closest ITR TE was 976 bp upstream of coding

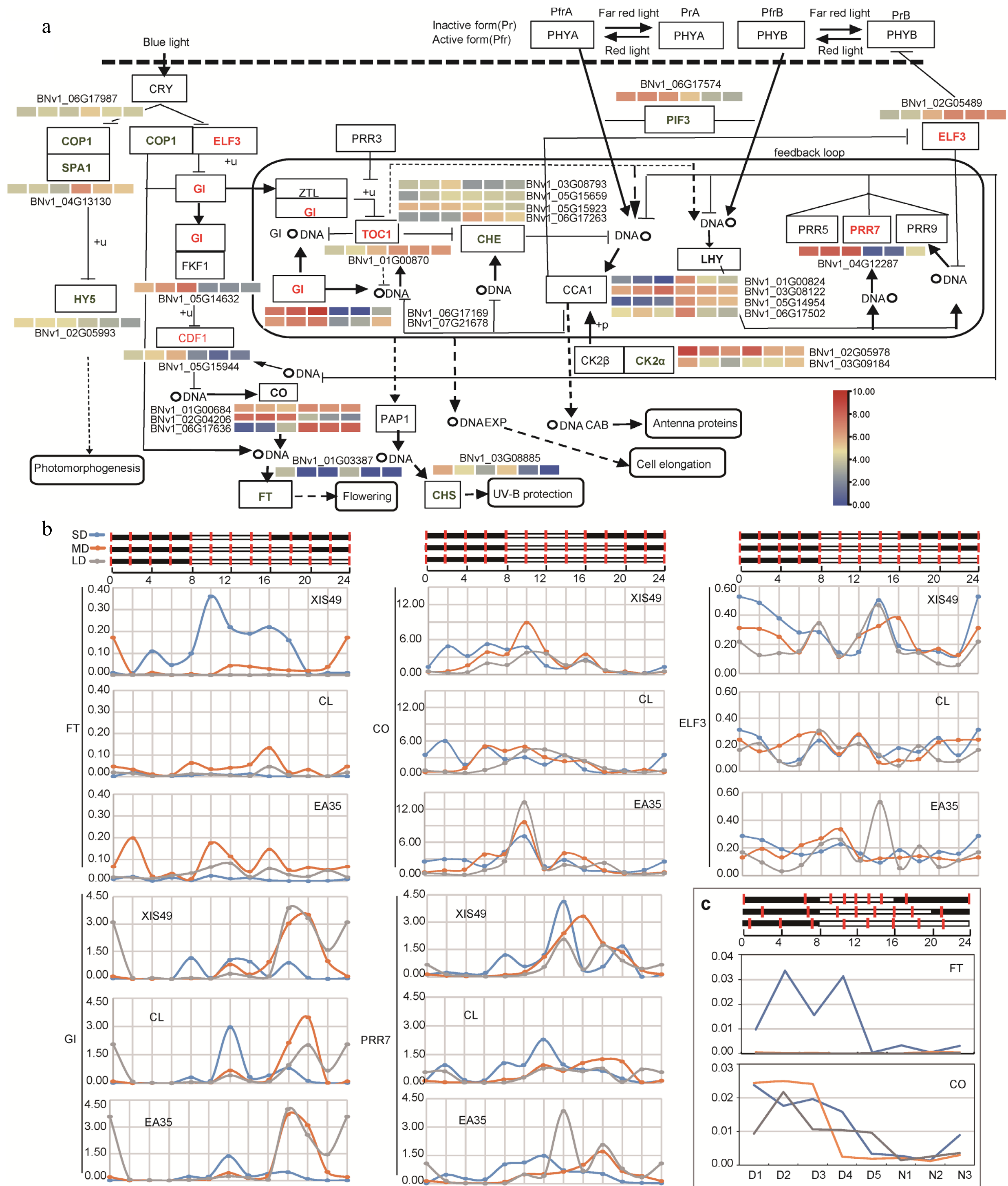

Fig. 3 Circadian expression manner of DEGs (a) Influence of photoperiod treatment on circadian pathway genes (KEGG, ko04712). Upregulated and downregulated genes by long photoperiod is denoted in red and green. A heat map is present to show transcriptional change; from left to right, 8 h-day, 12 h-day, 16 h-day, 8 h-night, 12 h-night, 16 h-night. (b) Samples were collected every two hours during a 24 h period and the expression was quantified by qRT-PCR. (c) The circadian expression of CO and FT was confirmed again by using leaf samples collected at nighttime-trisection timepoints (N1-N5) and daytime-quinquesection timepoints (D1-D5).

start site of *PG* gene. Interestingly, *PG* genes in the big insertion also showed a photoperiod-dependent expression response, which is the same as the *FT* gene.

Discussion

XIS cucumbers were reported as late-flowering plants and as having no different response between SD and LD conditions[18].

Table 2. QTL mapping of several flower traits by KASP.

	Chr.	Start	End	LOD	PVE%	Size (Mb)
SF1.1	1	29,795,488	29,931,423	32.09	46.90	0.14
SF6.1	6	12,580,505	21,116,696	6.65	7.90	8.54
FirFFNode1.1	1	190,063	10,053,157	8.97	15.72	9.86
FFNum1.1	1	190,063	10,053,157	6.03	8.70	9.86
FFNum2.1	2	12,405,113	22,000,804	5.81	11.55	9.60
MFNum1.1	1	29,369,853	29,795,488	18.10	14.11	0.43
MFNum6.1	6	2,507,906	12,580,505	14.14	16.15	10.07
MFNum6.2	6	12,580,505	21,116,696	14.85	12.18	8.54
FFNR1.1	1	190,063	10,053,157	7.89	13.20	9.86
DFF3.1	3	135,870	11,923,221	6.76	11.00	11.79
branch1.1	1	29,795,488	29,931,423	5.19	7.17	0.14

Short-day flowering (*SF*) was mapped based on the phenotype of first flower node; FirFFNode, first female flower node; FFNum, female flower number; MFNum, male flower number; FFNR, female flower node ratio; DFF, downward female flower; branch, branch number per node.

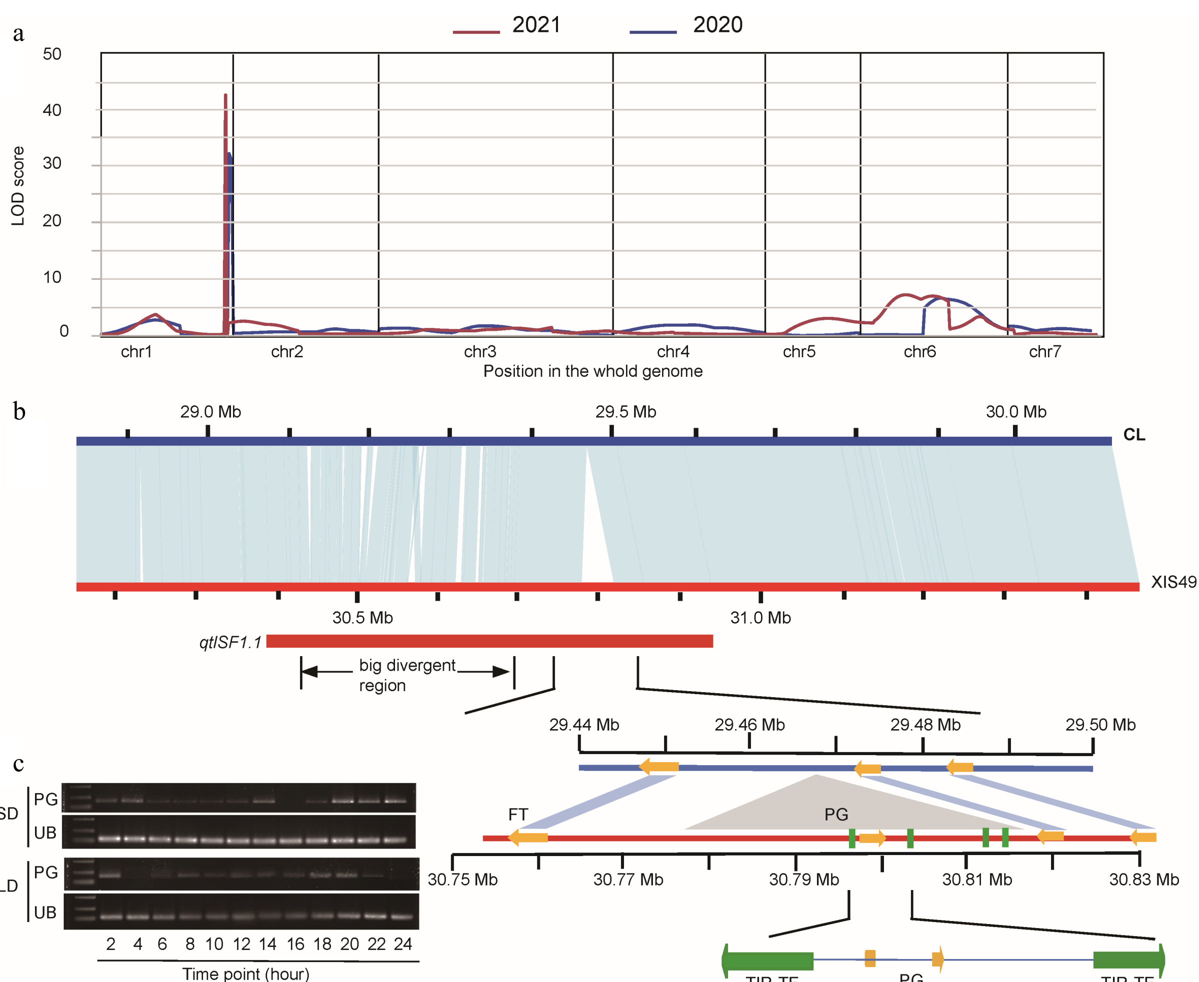

Fig. 4 QTL mapping of short-day flowering in XIS49. (a) LOD profiles of QTL for FFNode (first flower node). Plants were pretreated by LD condition. (b) A syntenic analysis of 1 Mb segment that carries the QTL between XIS49 and CL cucumber. The qtlSF1.1 carry two major sequence variants nearing the FT gene, 'the bid divergent region' and 'the big insertion'. The orange arrows indicate protein coding genes. The four green rectangles indicate TIR-TE insertion. (c) The expression profile in a 24-h period of the inserted polygalacturonase (PG) gene shows photoperiodic manner. UB, ubiquitin.

However, flowering time of the XIS cucumber is deeply influenced by photoperiod condition in many other studies[15,16]. Moreover, CG9192 (an XIS cucumber) was reported to be about 80 days later than '404' (a cultivated cucumber) in terms of flowering time, regardless of photoperiod condition[18]. Our study indicates that XIS49 can form blooms as quickly as a cultivated cucumber when grown under SD conditions. The negative dosage effect and the linear correlation of day length and FFNode explains why these cucumbers perform as late-flowering plants when introduced to a middle latitude. In summary, XIS cucumbers are short-day plants, not late-flowering. In addition to day length, light

intensity also affects FFNode; weak light and day length that is too short (≤ 6 h) both suppress flower initiation even under SD conditions, which must be due to deficient vegetative growth (Fig. 1). Indeed, there is no difference in flowering time between SD and LD treatment when light is inadequate.

There have been a few reports about QTL mapping of a late-flowering trait in XIS cucumbers[8]. In a recent QTL mapping work, an XIS cucumber CG9192 and an East Asian cucumber '404' were used as a donor parent and a recruit parent to construct an NIL population[18]. All these studies identified the same locus that carry the *FT* gene. These studies did field investigation under natural photoperiod conditions and focused on "flowering time". Here, flowering traits were all investigated under a photoperiod treatment of 16h. Interestingly, we detected the same *FT*-QTL on chromosome 1 but with a much higher LOD value (30−40) than previous reports. In XIS49, the circadian expression of the *FT* gene during 10:00−18:00 (SD conditions) was totally suppressed by LD conditions. Such an influence of photoperiod on *FT* expression is not observed in CL cucumber. These results confirm that the *FT* gene contributes to short-day flowering in XIS cucumber.

Despite of the success of QTL mapping and the consensus of the *FT* gene, the detailed mechanism remains unknown because there is no sequence and gene structure diversity of the *FT* gene. A recent pan-genome analysis summarized that the big insertion around the *FT* gene associates with late flowering[7]. The big insertion which carries a *PG* gene and four TEs was also detected in our study (Fig. 4). The *PG* gene shows a similar photoperiodic-dependent expression patten with the *FT* gene, which may imply *cis*-regulation from the big insertion. However, since the big insertion is too far from the *FT* gene (>15 kb), a detailed regulation mechanism needs to be clarified in the future. It is still too early to exclude the possible regulation from the SV cluster region as well as the SV genes (Fig. 4; Table 3).

Since photoperiodic flowering is determined by the length of light phase, the circadian pathway plays a critical role in flowering regulation. Nowadays, the circadian clock has been

Table 3. Genes at the locus *LF1.1* with sequential divergent between CL and XIS49.

Gene ID	Position/Mb	SVs in promoter	SNP		Gene function
			CDS	Promoter[2]	
CsaV3_1G043880	29.04	HDR			Unknown
CsaV3_1G043890	29.04	DEL; *DEL*		T(-31)A; C(-30)G; A(-5)C	Unknown
CsaV3_1G043980	29.14	INS; *INS*	T18I; D214N; I304Y		Choline monooxygenase
CsaV3_1G043990	29.15	HDR	A51T	A(-26)C	Unknown
CsaV3_1G044000	29.15	INS	Y158S		Phosphatidylglycerol transfer protein
CsaV3_1G044010	29.17	DEL			Unknown
CsaV3_1G044020	29.18	CPL			DnaJ protein
CsaV3_1G044060	29.23	HDR; *INS*	L23H; K127R; A130S; S202L	T(-43)A	Cytochrome P450
CsaV3_1G044110	29.33	HDR			YTH domain, RNA binding
CsaV3_1G044160	29.38	HDR			Thioredoxin F-type
CsaV3_1G044440	29.78	CPG; *INS*	A27T		Protein TIC 20-II
CsaV3_1G043900	29.05	*DEL*			Unknown
CsaV3_1G043920	29.06	*DEL*		T(-43)G; A(-22)T	DnaJ protein
CsaV3_1G043930	29.08	*DEL*	A572T		Serine/threonine-protein phosphatase
CsaV3_1G043960	29.12	*DEL*			Glycosyl transferase
CsaV3_1G044070	29.25	*DEL*			S locus-related glycoprotein 1 binding pollen coat protein
CsaV3_1G044130	29.36	*INS*			Glycoside hydrolase
CsaV3_1G044170	29.39	*DEL*			Unknown
CsaV3_1G044180	29.43	*DEL*			Activator of Hsp90 ATPase
CsaV3_1G043940	29.10		F281I; Q332R; I441T; I574L		Unknown
CsaV3_1G043950	29.11		E13D; K34E; P26L; Y29F; L67S		Unknown
CsaV3_1G043970	29.13				Protein IQ-DOMAIN
CsaV3_1G044030	29.19		D47N; C81R; V113A; A119G; H145Y; Q160K; F207L; Y211N		23 kDa jasmonate-induced protein
CsaV3_1G044040	29.20		F208L; G381D; I424M; S753F; G790D		beta-galactosidase 7
CsaV3_1G044050	29.22		G431R; L569S		Microtubule-associated protein
CsaV3_1G044080	29.28		S20A		Pentatricopeptide repeat-containing protein tubulin-folding cofactor E
CsaV3_1G044090	29.28				
CsaV3_1G044100	29.32				Heavy metal-associated domain
CsaV3_1G044120	29.35		S90A		Unknown
CsaV3_1G044140	29.37		D317E		Aspartic proteinase
CsaV3_1G044150	29.37				Unknown
CsaV3_1G044210	29.45		G82R		FT
CsaV3_1G044450	29.80		R64G		Phosphoglycolate phosphatase

[1] Sequential/Structural variance (SV) including (HDR), (CPL), (CPG), and insertion (INS) and deletion (DEL); Regular INS and DEL, SV length > 50 bp; italic *INS* and *DEL*, SV length < 50. [2] T(-31)A, the nucleotide with a distance of upstream-31 bp to the transcription start site is T in CL, and A in XIS49.

experimentally determined and well-reviewed by many researchers[19,20]. Taking *Arabidopsis* for example, a complex CO-FT module is suggested to control its long photoperiod flowering[21−23]. One mechanism is that the SPAs-COP1 complex degrades the CO protein in the dark phase, while longer nighttime in SD conditions covers the critical evening expression of the *CO* in LD conditions, which subsequently results in suppression of *FT*. One other mechanism relies on GI-FKF1 activator and CDF repressor of the *FT* gene. LD conditions upregulated many circadian pathway genes like morning gene *CDF1*, evening genes *ELF3* and *GI*, afternoon gene *PRR7*, and night gene *TOC1*. Night genes *SPA1*, *COP1*, *HY5*, and *CHE*, are downregulated (Fig. 2). Clearly some of the circadian clock is disturbed as with the *GI* gene. However, these changes might not account for short-day flowering since these disturbances seem to appear in both the XIS cucumber and cultivated cucumbers.

Conclusions

As demonstrated in this study, XIS49 is a restrictive short-day plant, a distinct divergence, as it's been previously reported as late-flowering. The flowering time was gradually decreased with the increase of day length and the minimum light phase demand for flowering is 11.5 h. LD conditions can completely suppress the flowering process in XIS49. Our study indicates that the *cis*-regulation of the *FT* gene accounts for short light phase-dependent flowering and the photoperiodic *cis*-regulation probably comes from a 30 kb insertion. Outflow of circadian clock disturbance caused by photoperiod change cannot explain the acquisition of short-day flowering and there is likely not any *trans*-regulation mechanism. This conclusion is based on gene expression analysis during a diurnal cycle and QTL mapping work. The mapped locus *FFNode1.1* can explain 46.9% of the flowering time trait with an LOD value of 32.09. We also mapped QTLs controlling other flowering-related phenotypes at the same time.

Materials and methods

Plant material

The homozygous cucumber XIS used in this study was collected by Sichuan Agricultural University from Wenshan prefecture, Yunnan Province (China) and was assigned ID number XIS49 after being purified for six generations. Chinese long cucumber, also known as '9930', is the sequencing material. EA35 is a pure line of the East Asian group, the same type as '9930'. XIS49 and EA35 were sequenced by nanopore sequencing technology and the assemblies and annotations were downloaded from Gene Bank with the project ID of PRJNA956325.

Photoperiod treatment and sample collection

Photoperiod treatment was started at seed-sowing and once six leaves were unfolded, the seedlings were then moved to a greenhouse in April. Photoperiod conditions were controlled by a plant incubator with long-day length (LD, 16 h/8 h, day/night), medium-day length (MD, 12 h/12 h, day/night), and short-day length (SD, 8 h/16 h, day/night). The photoperiod treatment was carried out under moderate temperatures (30 °C/25 °C, day/night). We also studied the effect of light density inanition in regards to day length, with strong light (1,700 $\mu mol \cdot m^2 \cdot s^{-1}$) and weak light (200 $\mu mol \cdot m^2 \cdot s^{-1}$). The entire third unfolded leaf samples were collected for total RNA extraction for RNA-seq and qRT-PCR. Leaves from three seedling individuals were homogenate as one repeat and there were triplicates for each sample. Leaf samples were collected every 2 h over a 24 h period at the stage of four unfolded leaves. While, night time and daytime were divided into trisection (N1−N3) and quinquesection (D1−D5), respectively. Samples collected at N2 and D3 were used for RNA-seq and transcriptome analysis.

RNA-seq and transcriptome analysis

Total RNA was extracted using the Trizol method as previously described[24]. A total amount of 1 μg RNA per sample was used. Purity of the RNA sample was examined by NanoDrop, while Agilent 2100 (Agilent Technologies, De Novo Santa Clara, CA, USA) was used to assay RNA integrity. Qualified RNA was processed for library construction and illumine sequencing by Biomarker (Beijing, China).

Sequencing bases quality score was calculated to evaluate the probability of an incorrect base[25]. After trimming adapter contaminations and removing low quality-score nucleotides, clean data were obtained. Clean reads were mapped to the XIS49 genome by using an alignment tool HISAT2[26]. StringTie was used to assemble the mapped reads[27]. The mapped reads were compared with original annotation; novel transcripts were determined and annotated by searching against databases[28]. The expression level of a gene or transcript was quantified by StringTie in a form of FPKM (Fragments Per Kilobase of transcript per Million fragments mapped)[27]. Reproducibility of biological replicates was determined by Pearson correlation coefficient R (Pearson's Correlation Coefficient)[29]. DESeq2 was used in differential expression analysis and the criteria for differentially expressed genes was set as Fold Change (FC) ≥ 2 and FDR < 0.01[30]. Functional annotation and enrichment analysis were done by using a database of GO (Gene ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes).

Gene expression was quantified by qRT-PCR

Relative gene expression was quantified by qRT-PCR. Total RNA was extracted using the MolPure TRIeasy Plus Total RNA Kit (Yeasen Biotechnology, Shanghai, China). Reverse transcription was carried out by Hifair V one-step RT-gDNA digestion SuperMix for qPCR (Yeasen Biotechnology, Shanghai, China). For qRT-PCR, the PCR mix regent is SYBRPRIME qPCR Kit（Fast HS）(BIOGROUND Biotechnology, ChongQing, China), and the apparatus was Bio-Rad CFX96 (BIO-RAD, USA). The inner reference gene is ubiquitin (CsaV3_7G003730). The primer sequence and annealing temperature is listed in Supplemental Table S2.

Phenotyping of important trait in the F2 population

An F2 population was constructed by crossing XIS49 and CL. All the F2 seedlings were treated with LD conditions before being removed to a greenhouse in April. The parental genotypes and a segregating population were grown for two years (2020 and 2021) in Chengdu (China) N30.6°. For each time period, the population size was 250 individuals. Six important traits were investigated. (1) First flower node (FFNode), the first node bearing a flower, regardless of whether it is a male or a female flower. (2) Female flower number per node (FFNum) within 15 nodes. (3) Male flower number per node (MFNum)

within 15 nodes. (4) Female flower node ratio (FFNR), the ratio of nodes (< 15 nodes) that bear any female flowers. (5) Female flower angle (FFA), the angle between the female flower and the stem. There are four values assigned to describe the angles during investigation: 0–45°, 1; 45°–90°, 2; 90°–135°, 3; 135°–180°, 4. Branch number (BN) per node (< 15 nodes). For the above traits, we investigated flowers and branches within 15 nodes.

QTL mapping

Comparative genomic analysis between XIS49 and CL cucumber genome has been carried out. We used BWA software to align clean reads of XIS49 to the reference genome (CL cucumber), and only uniquely mapped reads were used for the following analysis. SNPs were called using the Samtools software[31], and low quality SNPs were filtered using Coval scripts[32]. Finally, polymorphic SNPs between XIS49 and CL were identified after sequence alignment and variation calling.

To design SNP-KASP markers, we retrieved the upstream and downstream sequences of selected SNPs and used BLASTN to determine their specificity in genomic sequences. Newly designed KASP markers were validated for polymorphisms and genotyping quality by application on the parents. Only those KASP-SNP markers having correct and clear genotyping results were used to genotype the entire mapping population. PCR reaction reagents were purchased from GENTIDES Biotech Co. Ltd. (Wuhan, China) and the SNP genotyping technique is penta-primer amplification refractory mutation system (PARMS). In this detection system, the report fluorophores were FAM and HEX KASP, while ROX was used to normalize the fluorescent reporter signal. PCR amplification and fluorescence detection was performed by Bio-Rad CFX96 Touch^{TM} system (Bio-Rad Laboratories, Inc., Irvine, USA). Soft QTL IciMapping4.2 was used to estimate the genetic distance, LOD values, and finally QTLs[33].

Data availability

RNA-seq data of LD-D3, MD-D3, SD-D3, LD-N2, MD-N2, SD-N2 were deposited in the NCBI sequence read archive (SRA) under the accession number PRJNA951446.

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Conflict of interest

The authors declare that they have no conflict of interest.

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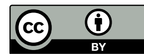
References

1. Jeffrey C. 1980. A review of the Cucurbitaceae. *Botanical Journal of the Linnean Society* 81:233−47
2. Kirkbride JH, Jr. 1993. *Biosystematic monograph of the genus* Cucumis *(Cucurbitaceae): botanical identification of cucumbers and melons*. pp x + pp159. New York: Parkway Publishers. pp 1−12.
3. Qi J, Liu X, Shen D, Miao H, Xie B, et al. 2013. A genomic variation map provides insights into the genetic basis of cucumber domestication and diversity. *Nature Genetics* 45:1510−15
4. Qi C, Yuan Z, Li Y. 1983. A new type of cucumber - Cucumis sativus L. var. xishuangbannanesis. *Acta Horticulturae Sinica* 10:259−63
5. Lai Y, Zhang X, Zhang W, Shen D, Wang H, et al. 2017. The association of changes in DNA methylation with temperature-dependent sex determination in cucumber. *Journal Experimental Botany* 68:2899−912
6. Lai Y, Shen D, Zhang W, Zhang X, Qiu Y, et al. 2018. Temperature and photoperiod changes affect cucumber sex expression by different epigenetic regulations. *BMC Plant Biology* 18:268
7. Li H, Wang S, Chai S, Yang Z, Zhang Q, et al. 2022. Graph-based pan-genome reveals structural and sequence variations related to agronomic traits and domestication in cucumber. *Nature Communications* 13:682
8. Pan Y, Qu S, Bo K, Gao M, Haider KR, et al. 2017. QTL mapping of domestication and diversifying selection related traits in round-fruited semi-wild Xishuangbanna cucumber (*Cucumis sativus* L. var. *xishuangbannanesis*). *Theoretical and Applied Genetics* 130:1531−48
9. Bo K, Ma Z, Chen J, Weng Y. 2015. Molecular mapping reveals structural rearrangements and quantitative trait loci underlying traits with local adaptation in semi-wild Xishuangbanna cucumber (*Cucumis sativus* L*var. xishuangbannanesis* Qi et Yuan). *Theoretical and Applied Genetics* 128:25−39
10. Turner A, Beales J, Faure S, Dunford RP, Laurie DA. 2005. The pseudo-response regulator *Ppd-H1* provides adaptation to photoperiod in barley. *Science* 310:1031−34
11. Weller JL, Vander Schoor JK, Perez-Wright EC, Hecht V, González AM, et al. 2019. Parallel origins of photoperiod adaptation following dual domestications of common bean. *Journal Experimental Botany* 70:1209−19
12. Boden SA. 2021. Evolution: replicated mutation of *COL2* contributed long-day flowering in common bean. *Current biology* 31:PR384−R398
13. Xie D, Xu Y, Wang J, Liu W, Zhou Q, et al. 2019. The wax gourd genomes offer insights into the genetic diversity and ancestral cucurbit karyotype. *Nature Communications* 10:5158
14. Roux F, Touzet P, Cuguen J, Le Corre V. 2006. How to be early flowering: an evolutionary perspective. *Trends in Plnat Science* 11:375−81
15. Bo K, Chen L, Qian C, Zhang S, Chen J. 2010. Short-day treatments induce flowering of Xishuangbanna cucumber. *China Cucurbits and Vegetables* 23:1−3
16. Shen D, Li X, Song J, Wang H, Qiu Y. 2011. Effects of different sowing dates on sex type and lateral stem development of Xishuangbanna cucubmer. *China Vegetables*22−7
17. Wang S, Li H, Li Y, Li Z, Qi J, et al. 2019. *FLOWERING LOCUS T* Improves Cucumber Adaptation to Higher Latitudes. *Plant Physiology* 182:908−18
18. Wang S, Li H, Li Y, Li Z, Qi J, et al. 2020. *FLOWERING LOCUS T* improves cucumber adaptation to higher latitudes. *Plant Physiology* 182:908−18
19. Harmer SL. 2009. The circadian system in higher plants. *Annual Review of Plant Biology* 60:357−77
20. Inoue K, Araki T, Endo M. 2018. Circadian clock during plant development. *Journal of Plant Research* 131:59−66

21. Song YH, Shim JS, Kinmonth-Schultz HA, Imaizumi T. 2015. Photoperiodic flowering: time measurement mechanisms in leaves. *Annual Review of Plant Biology* 66:441−64
22. Shim JS, Imaizumi T. 2015. Circadian clock and photoperiodic response in *Arabidopsis*: from seasonal flowering to redox homeostasis. *Biochemistry* 54:157−70
23. Johansson M, Staiger D. 2015. Time to flower: interplay between photoperiod and the circadian clock. *Journal Experimental Botany* 66:719−30
24. Feinbaum RL, Ausubel FM. 1988. Transcriptional regulation of the *Arabidopsis thaliana* chalcone synthase gene. *Molecular and Cellular Biology* 8:1985−92
25. Ewing B, Hillier LD, Wendl MC, Green P. 1998. Base-Calling of automated sequencer traces using *Phred*. I. accuracy assessment. *Genome Research* 8:175−85
26. Kim D, Langmead B, Salzberg SL. 2015. HISAT: a fast spliced aligner with low memory requirements. *Nature methods* 12:357−60
27. Pertea M, Pertea MG, Antonescu CM, Chang TC, Mendell TJ, et al. 2015. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nature Biotechnology* 33:290−95
28. Buchfink B, Xie C, Huson DH. 2015. Fast and sensitive protein alignment using DIAMOND. *Nature Methods* 12:59−60
29. Brent E, Phil G. 1998. Base-Calling of Automated Sequencer Traces Using Phred. *II. Error Probabilities. Genome Research* 8:186−194
30. Wang L, Feng Z, Wang X, Wang X, Zhang X. 2010. DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics* 26:136−38
31. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, et al. 2009. The sequence alignment/map format and SAMtools. *Bioinformatics* 25:2078−79
32. Kosugi S, Natsume S, Yoshida K, MacLean D, Cano L, et al. 2013. Coval: improving alignment quality and variant calling accuracy for next-generation sequencing data. *PLoS ONE* 8:e75402
33. Meng L, Li H, Zhang L, Wang J. 2015. QTL IciMapping: integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *The Crop Journal* 3:269−83



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