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ORIGINAL PAPER

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)

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Abstract

Key message **Comparative genetic mapping revealed the origin of Xishuangbanna cucumber through diversification selection after domestication. QTL mapping provided insights into the genetic basis of traits under diversification selection during crop evolution.**

Abstract The Xishuangbanna cucumber, *Cucumis sativus* L. var. *xishuangbannanesis* Qi et Yuan (XIS), is a semi-wild landrace from the tropical southwest China with some unique traits that are very useful for cucumber breeding, such as tolerance to low light, large fruit size, heavy fruit weight, and orange flesh color in mature fruits. In this study, using 124 recombinant inbred lines (RILs) derived from the cross of the XIS cucumber with a cultivated cucumber inbred line, we developed a linkage map with 269 microsatellite (or simple sequence repeat) markers which covered 705.9 cM in seven linkage groups. Comparative analysis of orders of common marker loci or marker-anchored draft genome scaffolds among the wild (*C. sativus* var. *hardwickii*), semi-wild, and cultivated cucumber genetic maps revealed that the XIS cucumber shares major chromosomal rearrangements in chromosomes 4, 5, and 7 between the wild and cultivated cucumbers suggesting that the XIS cucumber originated through diversifying selection after cucumber domestication. Several XIS-specific minor structural changes were identified in chromosomes 1 and 6. QTL mapping with the 124 RILs in four environments identified 13 QTLs for domestication and diversifying selection-related traits including 2 for first female flowering time (*fft1.1*, *fft6.1*), 5 for mature fruit length (*fl1.1*, *fl3.1*, *fl4.1*, *fl6.1*, and *fl7.1*), 3 for fruit diameter (*fd1.1*, *fd4.1*, and *fd6.1*), and 3 for fruit weight (*fw2.1*, *fw4.1*, and *fw6.1*). Six of the 12 QTLs were consistently detected in all four environments. Among the 13 QTLs, *fft1.1*, *fl1.1*, *fl3.1*, *fl7.1*, *fd4.1*, and *fw6.1* were major-effect QTLs for respective traits with each explaining at least 10 % of the observed phenotypic variations. Results from this study provide insights into the cytological and genetic basis of crop evolution leading to the XIS cucumber. The molecular markers associated with the QTLs should be useful in exploring the XIS cucumber genetic resources for cucumber breeding.

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Introduction

Cucumber, *Cucumis sativus* L. ($2n = 2x = 14$), is an economically important specialty crop and a system of choice for studying several important biological processes (Weng and Sun 2012). Cucumber is native to the Southern Asia continent (Candolle 1959; Sebastian et al. 2010). Classical taxonomic investigations have identified several botanical varieties of *C. sativus* which include the cultivated

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cucumber *C. sativus* var. *sativus*, the wild cucumber *C. sativus* var. *hardwickii* (Royle) Alef. (Royle 1835; Duthie 1903), the Sikkim cucumber, *C. sativus* var. *sikkimensis* Hook. f. (Hooker 1876; Renner and Pandey 2013), and the Xishuangbanna cucumber, *C. sativus* var. *xishuangbannanesis* Qi et Yuan (Qi 1983).

The Xishuangbanna (XIS hereinafter) cucumber, first described in 1983 (Qi 1983), is distributed in the mountainous Xishuangbanna region of Yunnan Province in southwest China (21°09′ to 22°36′N altitude, and 99°58′ to 101°50′E longitude, elevation 800–1,200 m) bordering Myanmar. The region has a typical tropical monsoon climate. The XIS cucumber is grown by local ethnic groups and is often intercropped with upland rice without trellis support. In general, XIS cucumber plants grow more vigorously than commercially cultivated cucumbers; the main vine may reach 8 m with 20–40 lateral branches and more than 900 nodes (Chen et al. 1994). The growth period may last up to 6 months, and the plant requires short day length for flowering (Bo et al. 2011). This short day length requirement has been lost in commercial cucumbers. On average, eight to ten fruits are set on each plant, and a single mature fruit weighs 2–3 kg (with the maximum of 5 kg) and contains over 1,000 seeds (Qi 1983). The mature fruits vary in shape [long, oblong, oval, or round with length to diameter ratio (*L*/*D*) from 1 to 4] and rind color (white, creamy, yellow, or brown) (Qi 1983; Shen 2009). Probably the most unique trait of the XIS cucumber is its orange flesh color (Qi 1983) which is due to the accumulation of high level of β-carotene in mature fruits (Bo et al. 2012). Unlike wild cucumbers but similar to the cultivated cucumber, the fruit of XIS cucumber is bitter-free and can be consumed immature or mature. The mature fruits are also stored as an important off-season vegetable. Because of these characteristic traits, the XIS cucumber was classified as a semi-wild variant of *C. sativus* (Qi 1983).

Several studies have investigated the relationship of XIS cucumber with other botanical variants of cucumber. The XIS cucumber is readily intercrossed with and shares the same 14 chromosomes as cultivated cucumber (Qi 1983). There are seven ring bivalents from chromosome pairing during meiosis, although occasionally a chromosome bridge was observed in meiotic anaphase I of XIS cucumber (Qian et al. 2003). The distribution of repetitive DNA sequences revealed by florescence in situ hybridization suggested that XIS is phylogenetically closer to the cultivated cucumber than to wild cucumber (Zhao et al. 2011). Molecular marker-based clustering analysis placed XIS cucumber in the same clade as cultivated cucumber (Zhuang et al. 2004). Further phylogenetic analysis (Lv et al. 2012; Qi et al. 2013) on worldwide cucumber collections found that the XIS cucumber is affiliated with the Indian population which has the highest genetic diversity as compared to the East Asian and Eurasian cucumber groups. In addition, the XIS accessions were genetically close to each other suggesting that a single dispersal occurred from India in the evolution of the XIS cucumber (Lv et al. 2012). While these studies have provided good information on the population structure of the XIS cucumber, the degree of differentiation between the XIS cucumber and other cucumber variants and the crop evolution history of the XIS cucumber are largely unknown. Therefore, one objective of the present study was to conduct comparative genetic mapping between the XIS and cultivated cucumbers to reveal possible chromosomal differentiations between the two botanical varieties. In cucumber, this approach has been employed to reveal structural rearrangements between cultivated and wild cucumbers (Ren et al. 2009; Yang et al. 2012), and study the syntenic relationships between cucumber and melon (*C. melo* L.) (Li et al. 2011).

From the cucumber breeding perspective, several traits possessed by the XIS cucumber are attractive for improving commercial cucumbers of different market classes, which may include short hypocotyl, tolerance to low light, large fruit, and high β-carotene content (orange flesh color) (Bo et al. 2012). However, due to its semi-wild nature, some traits in XIS cucumber such as photoperiod sensitivity, low percentage of female flowers, and long growth period may be potential obstacles for efficient use of this germplasm source. Understanding the genetic basis of these traits will provide insights into crop evolution and domestication processes leading to the XIS cucumber, thus facilitating its efficient use in cucumber breeding. Since most domestication or diversifying selection-related traits are quantitative in nature, the QTL (quantitative trait loci) mapping strategy has been extensively used for genetic dissecting of such traits in major crop plants such as rice, maize, sorghum, wheat, millet, and sunflower (reviewed by Doebley et al. 2006; Alonso-Blanco et al. 2009; Gross and Olsen 2010; Meyer and Purugganan 2013; Olsen and Wendel 2013; Abbo et al. 2014).

In cucumber, due to application of next-generation sequencing (NGS) technologies, draft genome assemblies for two cultivated cucumber inbred lines (9930 and Gy14) have been released and many other lines have been re-sequenced (Huang et al. 2009; Yang et al. 2012; Qi et al. 2013). Hundreds of simple sequence repeat (SSR) markers have been genetically mapped (Ren et al. 2009; Cavagnaro et al. 2010; Yang et al. 2013). These genetic and genomics resources have greatly facilitated genetic mapping, molecular tagging, and gene cloning in cucumber. However, only a limited number of QTLs have been reported in this crop for disease resistance (Sakata et al. 2006; Liu et al. 2008; Zhang et al. 2011, 2013; Fukino et al. 2013; He et al. 2013), flowering time, fruit size, fruit bitterness, or fruit epidermal features (Kennard and Havey 1995; Dijkhuizen

and Staub 2002; Fazio et al. 2003; Yuan et al. 2008; Cheng et al. 2010; Miao et al. 2011, 2012; Qi et al. 2013). These studies utilized cultivated or wild cucumbers, but not XIS semi-wild cucumber. Therefore, the second objective of the present study was to conduct QTL mapping of domestication, or diversifying selection-related traits in XIS cucumber. We first developed an SSR-based linkage map using 124 recombinant inbred lines (RILs) derived from the cross between the semi-wild XIS cucumber and a cultivated cucumber. The resulting linkage map was compared with published cucumber genetic maps to reveal possible chromosome differentiations among the XIS, cultivated, and wild cucumbers. We next conducted multi-year and multi-location phenotyping for flowering time, fruit length, fruit diameter, and fruit weight in this RIL population and identified QTLs underlying these traits.

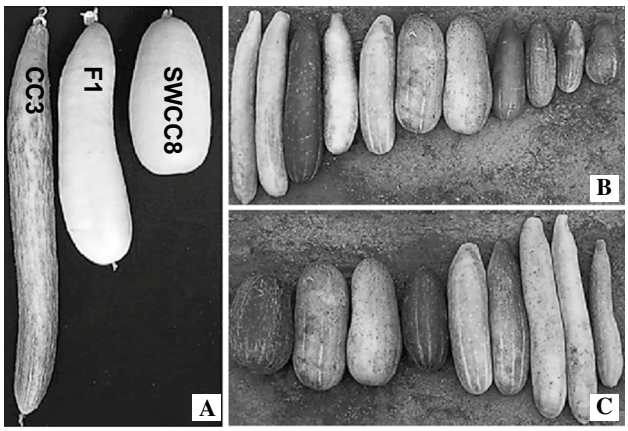


Fig. 1 Fruit size (length and diameter) variations of CC3, SWCC8, and their F_1 (**a**, taken from greenhouse studies) and F_9 recombinant inbred lines (**b** and **c**, taken from WI2012H experiment). **b**, **c** Fruit length and diameter distributions

Materials and methods

Plant materials

For linkage map development and QTL mapping, 124 F_9 RILs were developed from the cross between two cucumber inbred lines CC3 and SWCC8 through single seed descent. CC3 (maternal parent, P_1) was derived from a north China fresh market type landrace 'Beijing Jietou' that flowers early and bears long, slim fruits (~65 cm). SWCC8 (paternal parent, P_2) is a semi-wild XIS cucumber originating from southwest China which requires short day length for female flower development and bears short (~28 cm) but blocky fruits (Fig. 1).

An F_2 population from the CC3 × SWCC8 cross was also used to validate QTLs identified with the RIL population.

Phenotypic data collection and analysis

Phenotypic data were collected from 124 RILs plus two parental lines and their F_1 in four field trials across 3 years at two locations. Details of the four experiments, NJ2009F, NJ2012S, WI2012H, and WI2013H, are presented in Table 1. Briefly, NJ2009F and NJ2012S were conducted in plastic houses at the Jiangpu Experiment Farm of Nanjing Agricultural University in Nanjing, China (32°03′N and 118°47′E, 30/18 °C day/night temperature, 14/10 h day/night photoperiod in May and June; 30/17day/night temperature, 11/13 h day/dark photoperiod in September and October). On each plant, only one self-pollinated fruit was allowed to develop, and fruit data were collected approximately 40 days after pollination. The experiments WI2012H and WI2013H were performed in an open field at the University of Wisconsin Experiment Station in Hancock, Wisconsin, USA (44°08′N, 89°31′W; 15/9 h day/

Table 1 Details of four environments used in QTL mapping for domestication or diversifying selection-related traits in the semi-wild Xishuangbanna cucumber

Environments	Location	Season	Experimental design[a]	Traits investigated[b]
NJ2009F	Nanjing, China	2009 fall (August to November)	14 plants per entry without replication; grown in plastic houses with data from one fruit per plant	FFT, FL, FD and FW
NJ2012S	Nanjing, China	2012 spring (April to July)	For each entry, three replications with four plants per rep, grown in plastic houses with data from one fruit per plant	FFT, FL, FD and FW
WI2012H	Hancock, WI, USA	2012 summer (June to September)	For each entry, three replications with five plants per rep, grown in open field; data collected from two to five fruits per plant	FFT, FL, FD and FW
WI2013H	Hancock, WI, USA	2013 summer (June to September)	Same as WI2012H but with 15 plants per entry and no replication	FFT, FL, FD and FW

[a] For each experiment, there were 127 entries including 124 RILs, the parental lines SWCC8 (P_1), and CC3 (P_2) and their F_1

[b] *FFT* first female flower date, *FL* mature fruit length, *FD* mature fruit diameter, *FW* mature fruit weight

dark photoperiod in August and 28/15 °C day/night temperature in July that is the warmest month of the year). The WI2012H experiment also included 540 CC3 × SWCC8 F_2 plants of which 436 were able to grow to maturity and bear fruits on which data were collected. Individual RIL or F_2 plants were spaced 40 cm apart in rows placed 80 cm apart. A honey bee colony was placed near the experimental field and fruits (3–10 per plant) were produced from open pollination. Fruit data were collected from ten or more mature fruits from each RIL, or two to five fruits from each F_2 plant. In all experiments, no female flowers were observed for SWCC8 at the time of data collection. For comparison purpose, fruit data of SWCC8 used in the present study were obtained from historical data.

In each experiment, data were collected for the flowering date of the first female flower (FFT), the length (FL), diameter (FD), and weight (FW) of mature fruits from each plant. For each trait, family means were calculated from four plants in NJ2009F, three plants per replication in NJ2012S, and five plants per replication in WI2012H and WI2013H (no replication) (total 15 plants per RIL). For FFT, CC3 was the earliest among the two parents, F_1 and 124 RILs. FFT was calculated as days after the flowering date of CC3 which was set as 1. Statistical analysis of phenotypic data was performed using SAS v9.3 (SAS Institute Inc., Cary, NC, USA). Pearson's correlation coefficients among FFT, FL, FD, and FW data were estimated with the PROC CORR function based on grand means of each RIL across all four experiments, or measurement of individual F_2 plants.

Linkage map development and comparative analysis

Cucumber or melon SSR markers described in Ren et al. (2009), Cavagnaro et al. (2010), and Yang et al. (2012) were used for polymorphism screening between CC3 and SWCC8. Polymorphic markers were used to genotype 124 RILs. Selected markers were also used to genotype F_2 plants to validate a major-effect QTL in chromosome 1 for fruit length. DNA extraction, PCR amplification of molecular markers, and gel electrophoreses followed Li et al. (2011). For each marker, χ^2 test for goodness of fit was performed against the expected 1:1 segregation ratio in the RIL population. Linkage analysis was carried out using JoinMap 4.0 software. Linkage groups were determined with a minimum LOD score of 4.0. Genetic distance was calculated with the Kosambi mapping function.

The physical locations of all mapped markers in the Gy14 scaffold and draft genome assemblies (Version 1.0, Yang et al. 2012) were used to verify their genetic map locations. Inference of chromosomal locations of molecular markers on the map was performed with BLASTn or in silico PCR according to Cavagnaro et al. (2010).

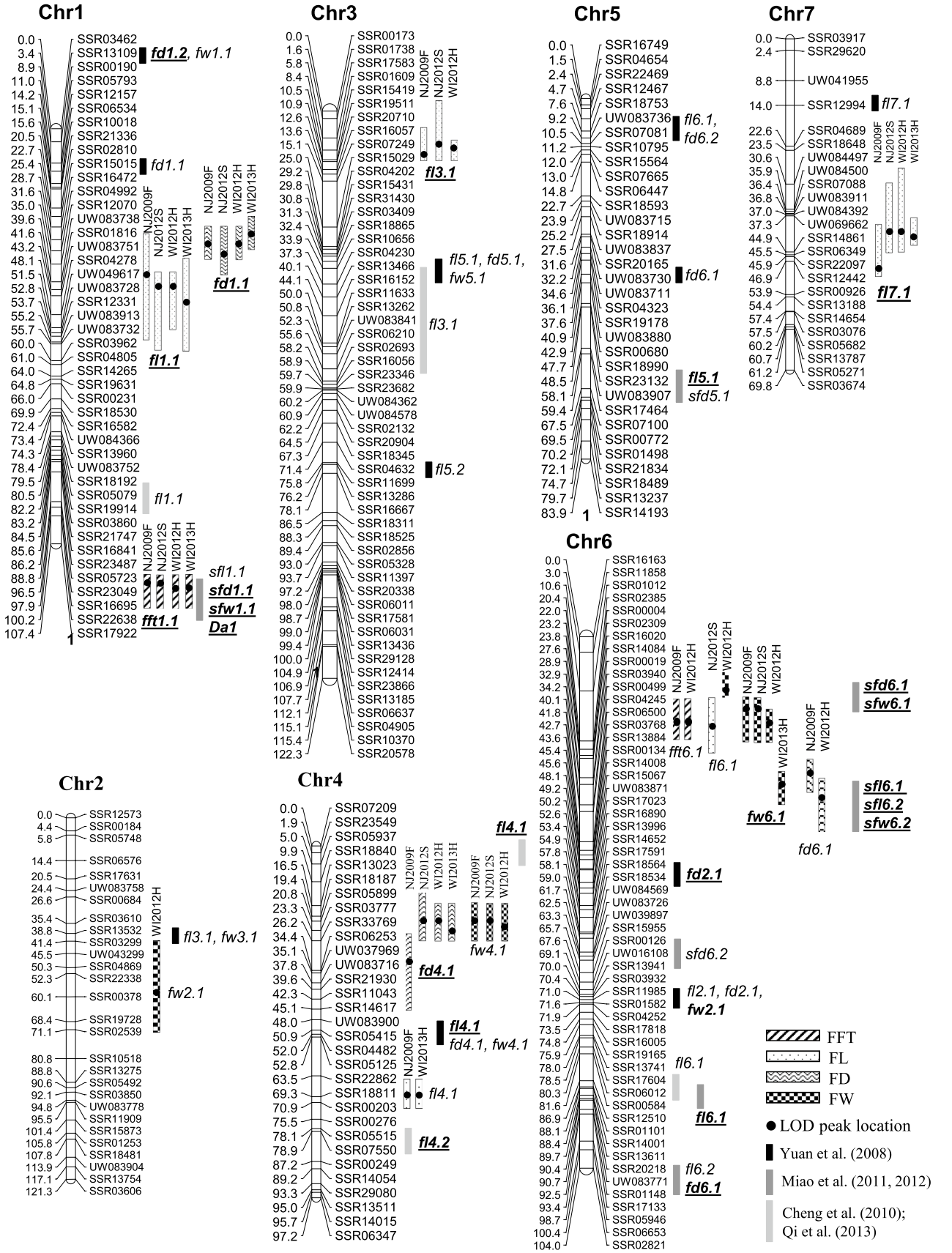
Fig. 2 The SSR-based CC3 × SWCC8 linkage map and chromosomal locations of QTLs for female flowering time (FFT, stripped), length (FL, dotted), diameter (FD, waved), and weight (FW, checked) of mature fruits from four experiments (NJ2009F, NJ2012S, WI2012H, and WI2013H) over 3 years. QTLs for FL, FD, and FW detected in Yuan et al. (2008) (*solid filled black bars*), Miao et al. (2011, 2012) (*solid filled dark gray bars*), Cheng et al. (2010), and Qi et al. (2013) (*solid filled light gray bars*) are also shown. Numbers to the left of each chromosome (Chr) are map length in centiMorgan (cM). For QTLs detected from the present study, *vertical bars* represent 2-LOD support interval of each QTL and *black filled circles* are QTL peak locations. The experiment detecting the QTL is listed above each *bar*; the name of consensus QTL detected across multiple years (environments) is below or alongside the *vertical bar(s)*. LOD support intervals of QTLs in all other studies are not available; thus the lengths of *vertical bars* for these QTL do not represent the confidence intervals. QTL symbols from original publications were used. For all studies, *bold-faced and underlined* QTLs are major-effect QTLs that explained more than 10 % genotypic variations. Map locations of QTLs from other publications were inferred from in silico PCR or BLASTn using primer sequences of QTL-associated molecular markers and are approximations

Chromosome assignment (Chr1 to Chr7) of the seven linkage groups followed Yang et al. (2012).

To examine possible chromosome structural rearrangements between cultivated cucumber and the XIS semi-wild cucumber, the order of mapped loci on the CC3 × SWCC8 RIL map from this study was compared with that of previously developed SSR-based cucumber genetic maps including the inter-subspecific Gy14 × PI 193967 (*C. s.* var. *hardwickii*) RIL map (Ren et al. 2009, 995 SSR loci) and the intra-varietal Gy14 × 9930 F_2 map (Yang et al. 2012, 781 SSR loci). The cultivated cucumber consensus map (1,681 loci) (Yang et al. 2013) was also used to validate marker or scaffold orders in the regions of interest. Alignment of each linkage group was based on shared markers and marker-associated scaffolds of the Gy14 draft genome assembly (Yang et al. 2012).

QTL analysis

A whole genome scan was performed to map the QTLs using the composite interval mapping (CIM) function of WinQTL Cartographer Version 2.5 (Zeng 1994; Wang et al. 2012) with the default settings (Model 6 with a walking speed of 1 cM, a window size of 10 cM, and the inclusion of 6 maximum background marker loci in a stepwise forward regression procedure). The significance of each QTL interval was tested by a likelihood-ratio statistic (LOD). The LOD threshold for declaring significant QTLs for each trait ($P = 0.05$) was determined using a permutation test with 1,000 repetitions. The QTL was named according to its chromosome location and trait name [female flowering time (FFT), fruit length (FL), fruit diameter (FD), fruit weight (FW)]. For example, *fft1.1* and *fl3.1* designated the first


Chr1
SSR03462
SSR13109
fd1.2, fw1.1
SSR00190
SSR05793
SSR12157
SSR06534
SSR10018
SSR21336
SSR02810
SSR15015
fd1.1
SSR16472
SSR04992
SSR12070
UW083738
SSR01816
UW083751
SSR04278
UW049617
UW083728
SSR12331
UW083913
UW083732
SSR03962
SSR04805
SSR14265
SSR19631
SSR00231
SSR18530
SSR16582
UW084366
SSR13960
UW083752
SSR18192
SSR05079
SSR19914
fl1.1
SSR03860
SSR21747
SSR16841
SSR23487
SSR05723
SSR23049
SSR16695
SSR22638
fft1.1
SSR17922
NJ2009F
NJ2012S
WI2012H
WI2013H
fd1.1
fl1.1
sfl1.1
sfd1.1
sfw1.1
Da1
Chr3
SSR00173
SSR01738
SSR17583
SSR01609
SSR15419
SSR19511
SSR20710
SSR16057
SSR07249
SSR15029
SSR04202
fl3.1
SSR15431
SSR31430
SSR03409
SSR18865
SSR10656
SSR04230
SSR13466
SSR16152
SSR11633
SSR13262
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SSR13185
SSR06637
SSR04905
SSR10370
SSR20578
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fl5.2
Chr5
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SSR13237
SSR14193
fl6.1, fd6.2
fd6.1
fl5.1
sfd5.1
Chr7
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SSR18840
SSR13023
SSR18187
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SSR33769
SSR06253
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SSR05515
SSR07550
SSR00249
SSR14054
SSR29080
SSR13511
SSR14015
SSR06347
fl4.1
fw4.1
fd4.1
fl4.1
fd4.1, fw4.1
fl4.1
fl4.2
FFT
FL
FD
FW
LOD peak location
Yuan et al. (2008)
Miao et al. (2011, 2012)
Cheng et al. (2010); Qi et al. (2013)

QTL for female flowering time and fruit length in cucumber chromosomes 1 and 3, respectively.

Results

Linkage map construction

We screened 1,344 cucumber or melon SSR primer pairs between CC3 and SWCC8 and identified 303 polymorphic ones (22.5 %), of which 269 were mapped with 124 RILs. The resulting genetic map is illustrated in Fig. 2, and the main statistics of the map are presented in supplemental Table S1 (online materials). A majority of the marker loci fitted the expected 1:1 segregation ratio, while 55 markers (20.4 %) (those with asterisks in Table S2) showed distorted segregation in χ^2 tests ($P < 0.05$). Nearly one-third (81/269) markers on this map were not mapped in previous studies, which may be useful for the cucurbit research community. Therefore, the detailed information (marker names, map locations, Gy14 cucumber scaffold and draft genome assembly locations, and primer sequences) of all mapped markers is provided in supplemental Table S2 (online materials).

This genetic map covered 705.9 cM in seven linkage groups, which is similar to previously published cucumber linkage maps (for example, Yang et al. 2012; He et al. 2013). Considering Gy14 draft genome scaffolds (Yang et al. 2012) anchored by these markers, this map seemed to physically cover the majority of the cucumber genome (data not shown). The marker orders were also highly consistent with their physical locations in the Gy14 scaffolds (Table S2). The mean marker interval of this map was 2.6 cM with only one gap larger than 10 cM in chromosome 4 from 52.8 to 63.5 cM (Table S2). Therefore, this high-quality genetic map was suitable for subsequent QTL mapping.

Comparative analysis of linkage maps among cultivated, semi-wild, and wild cucumbers

To investigate possible chromosome structural rearrangements between the XIS cucumber and cultivated (*C. s.* var. *sativus*) or wild (*C. s.* var. *hardwickii*) cucumbers, we aligned the XIS RIL map developed herein with the Gy14 × 9930 F_2 inter-varietal map (Yang et al. 2012, 2013) and the inter-subspecific Gy14 × PI 183967 RIL map (Ren et al. 2009). The order of shared loci or marker-associated Gy14 draft genome scaffolds between the XIS map and the other two maps were compared. Among the 269 markers placed on the XIS map, 62 were shared across all three maps, and 102 and 156 shared with the Gy14 × 9930 F_2 map and the Gy14 × PI 183967 map, respectively. Chromosome by chromosome alignment of shared markers is presented in supplemental Fig. S1 (online materials). Putative structural rearrangements represented by Gy14 draft genome scaffolds or shared SSR markers between XIS and cultivated cucumber, as well as between XIS and the wild cucumber are illustrated in Fig. 3. While no substantial changes were found between the XIS and cultivated

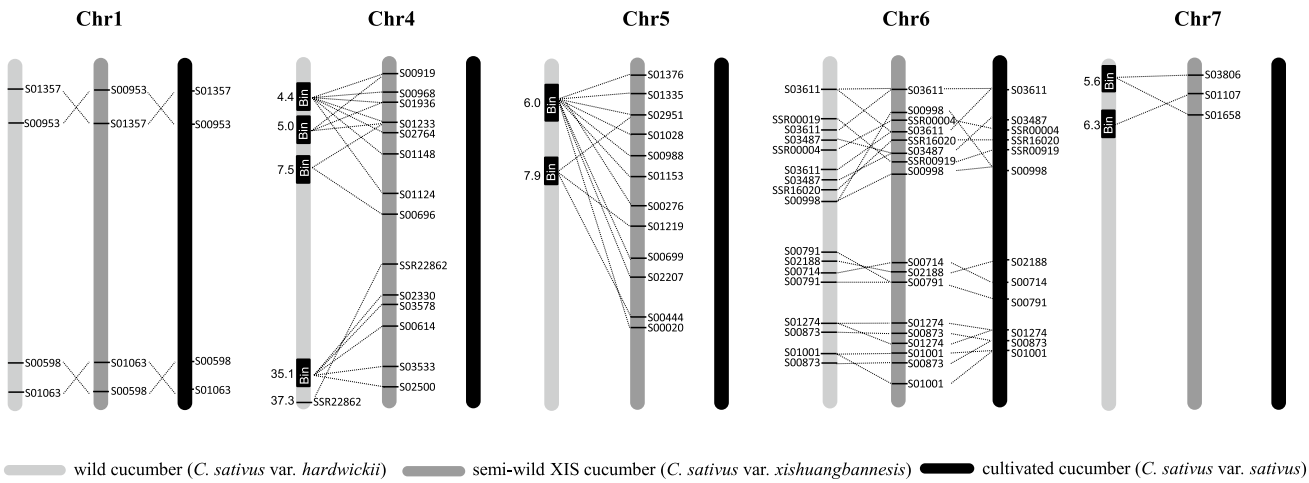

Fig. 3 Putative structural rearrangements in chromosomes 1, 4, 5, 6, and 7 of the semi-wild XIS cucumber (*C. sativus* var. *xishuangbannesis*, *center*, *dark gray*) as compared with the wild (*C. sativus* var. *hardwickii*, *left*, *light gray*) and cultivated (*C. sativus* var. *sativus*, *right*, *black colored*) cucumbers. Structural changes were inferred from orders of Gy14 draft genome *scaffolds* (S, *scaffold*) or shared *markers*. Only *scaffolds* or *SSR markers* involved in the putative rearrangements are listed. The bins are clusters of mapped loci in the wild cucumber, and their map locations (in cM) are shown to the left of the chromosome (Ren et al. 2009). *Dotted lines* connect the same *scaffolds* or *markers* between two maps under comparison. *Markers* or *scaffolds* on the cultivated cucumber chromosomes 4, 5, and 7 are collinear with those in the respective XIS cucumber chromosomes and are not shown

cucumber genomes, there were two putative inversions in chromosome 1 (scaffold00953 vs. scaffold01357 on the top, and scaffold01063 vs. scaffold00598 in the distal end of the long arm) (Fig. 3, Fig. S1). In chromosome 6, there were three blocks in which the orders of scaffolds or molecular markers were inconsistent suggesting possible inversions between the two variants.

Significant non-collinearity of markers and scaffolds was identified in chromosomes 1, 4, 5, 6, and 7 between XIS cucumber and the wild cucumber (Fig. 3). The inversions in chromosomes 1 and 6 seem to be consistent with those between XIS and cultivated cucumber suggesting these rearrangements might be specific to the XIS cucumber. Inversions between the XIS cucumber and the wild cucumber in chromosomes 4, 5, and 7 were evident (Fig. 3). It is known that two, three, and one inversion differentiated the chromosomes 4, 5, and 7 of cultivated and wild cucumbers (Yang et al. 2012). The locations of the rearrangements identified from the present study were consistent with those found between the cultivated and wild cucumbers. This suggests that, except for the putative XIS-specific small inversions in chromosomes 1 and 6, no substantial structural changes occurred during crop evolution of the XIS cucumber.

Phenotypic data analysis

In four environments across 3 years, we recorded the flowering dates for the first female flowers (FFT), the length (FL), diameter (FD), and weight (FW) of mature fruits of CC3, SWCC8, their F_1, and 124 RILs. In WI2012H, 436 F_2 plants were also included in the trial for data collection. The phenotypic means, standard derivation, and range of the four traits in all experiments are presented in Table 2. Since analysis of variance (ANOVA) indicated significant effects of genotypes and genotype × season interactions for all traits examined (data not shown), QTL mapping was conducted on the basis of RIL means of each experiment.

In all experiments, the parental line CC3 flowered earlier than most RIL lines for both male and female flowers. The XIS cucumber SWCC8 (in all experiments) and some F_2 plants (in WI2012H) did not have female flowers, which was due probably to the lack of adequate short day length in the experimental locations. The flowering time of the F_1 was similar to CC3 (Table 2) suggesting early flowering of CC3 was dominant over late flowering in SWCC8. As compared with CC3, SWCC8 had shorter, more cylindrical, and heavier fruits, and the fruit length, diameter, and weight of F_1 plants were in general close to the mid-parental value indicating the quantitative nature of these traits (Table 2; Fig. 1). In the F_2 population, while the FD and FW means among the 436 F_2 plants were almost the same as in F_1, the mean fruit length (FL) of F_2 plants was longer (40.1 cm)

Table 2 Phenotypic means and range of first female flowering time (FFT), fruit length (FL), diameter (FD), and weight (FW) in two parental lines (CC3 and SWCC8), their F_1, F_2, and 124 recombinant inbred lines (RILs) in four experiments (NJ2009F, NJ2012S, WI2012H, WI2013H)

Traits	CC3	SWCC8	F1	RIL (NJ2009F)		RIL (NJ2012S)		RIL (WI2012H)		F2 (WI2012H)		RIL (WI2013H)	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
FFT	1.0 ± 0.0	n/a	2.4 ± 0.6	11.2 ± 5.9	1.0–24.0	11.3 ± 5.9	1.0–25.0	10.2 ± 5.9	1.0–22.0	11.5 ± 8.6	1.0–35.0	20.2 ± 12.5	1.0–42.0
FL	64.6 ± 0.5	28.1 ± 0.7	36.4 ± 0.6	30.9 ± 7.3	16.2–50.6	31.5 ± 7.3	17.6–57.7	31.9 ± 7.5	20.6–59.2	40.1 ± 6.8	21.8–61.0	27.3 ± 6.1	15.2–49.8
FD	6.2 ± 0.2	12.0 ± 0.4	9.1 ± 0.1	9.4 ± 1.4	7.0–12.8	8.3 ± 1.9	5.1–19.7	8.8 ± 1.6	6.1–15.8	9.2 ± 1.6	5.5–17.7	7.8 ± 1.4	5.1–12.4
FW	0.8 ± 0.0	2.8 ± 0.1	2.0 ± 0.1	1.5 ± 0.6	0.5–3.5	1.4 ± 0.6	0.2–3.0	1.4 ± 0.6	0.5–3.4	2.0 ± 0.6	0.6–4.9	0.9 ± 0.3	0.3–2.2

SD standard derivation, *n/a* data not available

than the RIL mean in any experiment. In general, the range of variations among F_2 plants for each trait was also wider than that of the RILs and both parents (Table 2).

The frequency distribution of RIL and F_2 means in the four experiments is illustrated in Fig. 4 (for RILs) and supplemental Fig. S2 (for F_2, online materials), and was largely normal except for FFT of WI2013H. The RIL means and frequency distributions among the three experiments, NJ2009F, NJ2012S, and WI2012H, were highly consistent. For the WI2013H field trial, the temperature was lower than normal in the early growing season, and there were several days with very low night temperatures (<15 °C) in June and July. A few RILs did not flower before conclusion of the experiment. We believe this was the reason for the longer FFT and smaller fruits (lower means of FL, FD and FW) in the WI2013H dataset (Table 2). Despite this, major QTLs were detected with the WI2013H data that were largely consistent with those identified with other data sets (see below).

We analyzed the correlation among the four traits using RIL means across the four environments, as well as the F_2 data from WI2012H. The Pearson's correlation coefficients (r) among FFT, FL, FD, and FW are listed in Table 3. From RIL data, insignificant correlation was found between FFT and FD or FW; FFT was negatively correlated with FL ($r = -0.1667$ at $P = 0.05$). However, with the F_2 data, FFT was significantly and negatively correlated with FL, FD, and FW. In both RIL and F_2 data, no correlation was found between FL and FD; in contrast, FW were highly, significantly, and positively correlated with FL and FD ($P < 0.001$), which seem to be consistent with the QTL locations underlying each trait (Fig. 2; Table 4).

QTL analysis

The RIL means of FFT, FL, FD, and FW from the four experiments were used in QTL analysis. For each trait, the

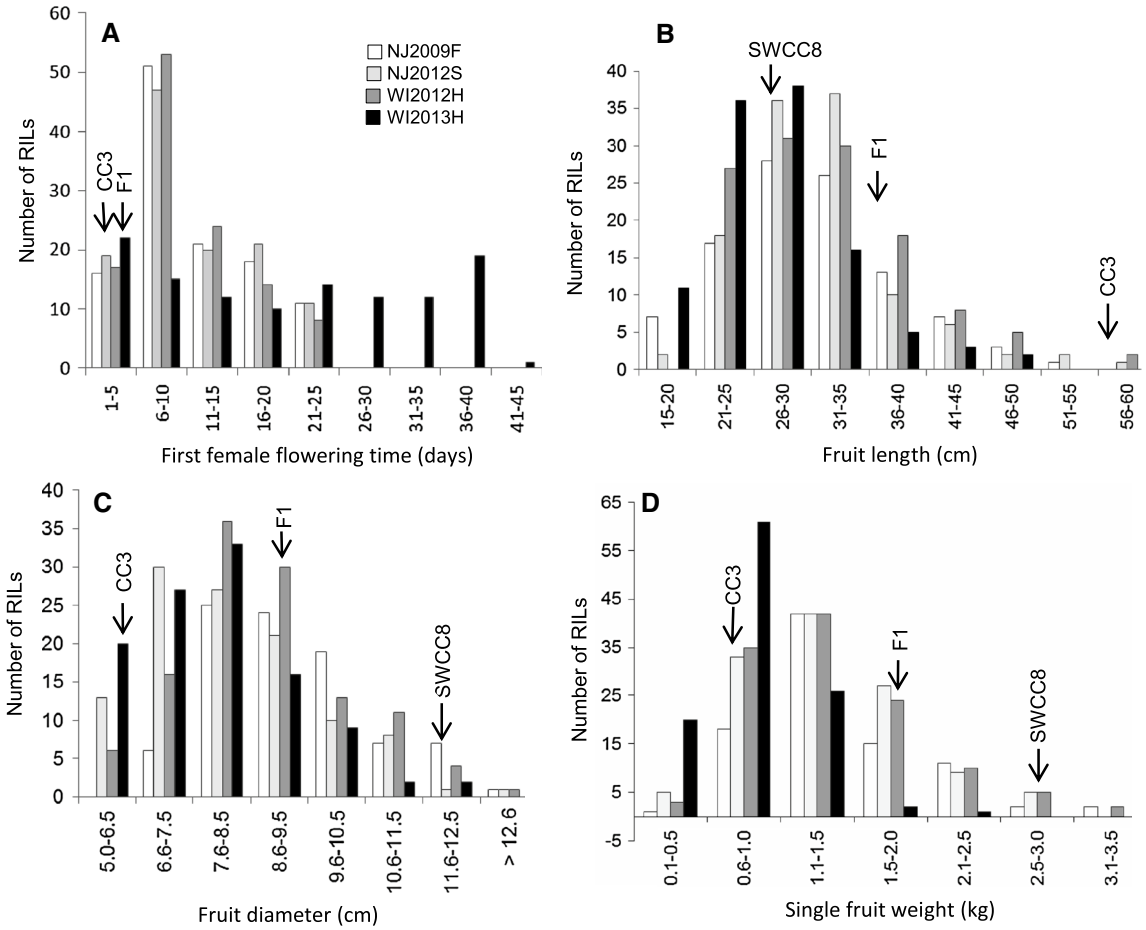

Fig. 4 Frequency distribution of first female flowering time (FFT), fruit length (FL), fruit diameter (FD), and fruit weight (FW) among 124 CC3 × SWCC8 recombinant inbred lines in four experiments (NJ2009F, NJ2012S, WI2012H, and WI 2013H). *Arrows* indicate corresponding values of CC3, SWCC8, and their F_1 in each experiment based on means across all experiments (FFT data for SWCC8 were not available)

Table 3 Pearson's correlation coefficients among first female flowering time (FFT), fruit length (FL), diameter (FD), and single fruit weight (FW) in the CC3 × SWCC8 RIL and F_2 populations

	FFT	FL	FD
FL	−0.1657* (RIL)		
	−0.2630*** (F_2)		
FD	−0.0048 (RIL)	−0.1466 (RIL)	
	−0.1676*** (F_2)	0.04305 (F_2)	
FW	−0.0994 (RIL)	0.4770*** (RIL)	0.6841*** (RIL)
	−0.1302** (F_2)	0.4940*** (F_2)	0.5622*** (F_2)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ ($df = 119$ for RIL and $df = 434$ for F_2)

LOD threshold to declare significance of QTL was determined with 1,000 permutation tests ($P = 0.05$) which ranged from 2.7 to 3.3. A global view of all QTLs detected across the seven chromosomes is provided in supplemental Fig. S3 (online materials). Details of each detected QTL including map location, LOD value, percentages of total phenotypic variances explained (R^2), additive effect, and 2-LOD support interval are provided in Table 4. Their chromosomal locations are visually illustrated in Fig. 2. Among the 39 QTLs identified for 16 traits across four environments, 11, 9, 12, and 7 were detected by NJ2009F, NJ2012S, WI2012H, and WI2013H, respectively. The WI2012H experiment detected the most QTLs which may reflect the most accurate and complete set of data collected among the four due to the large number of plants per RIL and the number of fruits per plant that could be used for data analysis.

QTL of first female flowering time (FFT)

Two QTLs were detected for FFT: one was located in chromosome 1 (*fft1.1*), and the other in chromosome 6 (*fft6.1*) (Table 4). The major-effect QTL *fft1.1* was reproducibly identified in all four environments with highly consistent peak locations on the genetic map (at 96.5 cM, Fig. S3 A1). In NJ2009F, NJ2012S, and WI2012H trials, *fft1.1* could explain >50 % total phenotypic variations. In WI2013H, this QTL had R^2 value of 25.8 %, which was due probably to the lower than normal temperature and slower growth of plants during the growing season. The minor QTL *fft6.1* (at 42.7 cM on chromosome 6, $R^2 \approx 6$ %) was detected in two seasons (NJ2009F and WI2012H). Both QTLs contributed to early flowering (negative additive effects).

QTL of fruit length (FL)

Five QTLs in five chromosomes were identified for fruit length (Table 4; Fig. 1). The QTL *fl1.1* and *fl7.1* were detected in all four experiments; *fl3.1* was detected in three seasons (NJ2012H, WI2012H, and WI2013H); *fl4.1* and *fl6.1* each was identified in two seasons. The three QTLs, *fl1.1*, *fl3.1*, and *fl7.1*, each could explain 7.5–22.5 % phenotypic variations depending on the season. While *fl6.1* had negative additive effect (reduction of fruit length), all other QTLs contributed to increasing fruit length.

Since the major-effect QTL *fl1.1* ($R^2 > 15$ %) had a highly consistent peak location across all four experiments, as a test case we validated this QTL with F_2 data from the WI2012H trial. We genotyped 394 of 436 F_2 plants with the marker SSR12331 at 53.7 cM where *fl1.1* peaked. The frequency distribution of fruit length among the 394 F_2 plants, as well as their corresponding genotypes (AA, AB and BB) at the SSR12331 locus, is presented in supplemental Fig. S4 (online material). It is clear that plants with longer fruits were enriched with the A allele (from CC3) and those with short fruits carried the B allele (from SWCC8). For example, there were 197 plants with an average fruit length of >40.0 cm (the mean FL of F_2, Table 2). Among the 197 plants, 97 carried A allele (genotype AA), 98 were heterozygotes (H, genotype AB), and only two carried the SWCC8 allele (B allele, genotype BB). Among the 93 plants with FL >45 cm, 55 and 48 had the A and H genotypes, respectively (none carried B allele). This result provided further evidence that *fl1.1* was a major QTL for fruit length.

QTL of fruit diameter (FD)

Three QTLs, *fd1.1*, *fd4.1*, and *fd6.1*, were detected; the first two were consistently identified in all four experiments with high LOD support (Table 2, Fig. S3C). All FD QTLs showed negative additive effects (reduction of fruit diameter). The major QTL in chromosome 1 (*fd1.1* at 43.2 cM) had the largest effect: the R^2 varied from 12.3 to 31.7 % among four environments and the peak locations of *fd1.1* in the four trials were highly consistent (Fig. S3C1). The QTL *fd4.1* that was also detected in all four experiments seemed to have moderate effects on fruit diameter ($R^2 \approx$ 5~15 %) (Table 4).

QTL of fruit weight (FW)

Among the three QTLs for fruit weight, the major QTL *fw6.1* (at 42.7 cM, with R^2 ranging from 10.0 to 28.7 %, Fig. S3E1) was identified in all four experiments. The QTL *fw4.1* in chromosome 4 (at 26.2 cM) was detected in three seasons with moderate effects ($R^2 = 5.1$–9.1 %). A minor QTL, *fw2.1* with $R^2 \approx 5.3$ % (peaked at 58.3 cM in chromosome 2), was detected in WI2012H. However, as shown in Fig. S3E, the LOD curves from the data of NJ2009F and NJ2012S also supported the presence of this minor QTL at

Table 4 QTLs for female flowering time (FFT), fruit length (FL), diameter (FD), and weight (FW) detected with 124 RILs of CC3 × SWCC8 in four experiments (NJ2009F, NJ2012S, WI2012H, and WI2013H)

Traits	QTL detected	Chr	Peak (cM)	LOD value	R^2 (%)	Additive effects	2-LOD support interval	
							Left (cM)	Right (cM)
Female flowering time (FFT)								
NJ2009F	*fft1.1*	1	94.8	15.2	51.0	−4.4	SSR05723 (92.8)	SSR16695 (97.5)
NJ2012S	*fft1.1*	1	94.8	16.3	51.3	−4.4	SSR05723 (92.8)	SSR16695 (97.3)
WI2012H	*fft1.1*	1	96.5	20.6	52.6	−4.4	SSR05723 (94.8)	SSR16695 (98.8)
WI2013H	*fft1.1*	1	96.5	8.3	25.8	−6.6	SSR05723 (94.8)	SSR16695 (97.9)
NJ2009F	*fft6.1*	6	42.7	4.0	6.1	−1.6	SSR06500 (40.9)	SSR13884 (43.6)
WI2012H	*fft6.1*	6	42.7	4.3	5.9	−1.5	SSR06500 (41.6)	SSR13884 (43.6)
Fruit length (FL)								
NJ2009F	*fl1.1*	1	51.5	3.1	7.5	2.1	UW083738 (39.4)	SSR03962 (59.6)
NJ2012S	*fl1.1*	1	52.8	8.6	19.2	3.1	UW049617 (51.5)	SSR03962 (57.9)
WI2012H	*fl1.1*	1	52.8	10.0	17.7	3.2	UW049617 (51.5)	UW083732 (55.5)
WI2013H	*fl1.1*	1	53.7	7.0	16.4	2.5	SSR04278 (49.6)	SSR03962 (57.6)
NJ2009F	*fl3.1*	3	21.1	3.8	12.3	2.6	SSR16057 (14.6)	SSR15029 (25.0)
NJ2012S	*fl3.1*	3	15.1	3.6	7.2	2.0	SSR19511 (10.9)	SSR15029 (23.1)
WI2012H	*fl3.1*	3	17.1	8.4	15.7	3.1	SSR07249 (16.5)	SSR15029 (21.8)
NJ2009F	*fl4.1*	4	69.3	4.1	10.4	2.4	SSR22862 (65.9)	SSR00203 (70.9)
WI2013H	*fl4.1*	4	69.3	3.9	8.8	1.9	SSR22862 (63.6)	SSR00203 (70.6)
NJ2012S	*fl6.1*	6	42.7	3.0	6.0	−1.7	SSR04245 (40.1)	SSR00134 (45.4)
WI2012H	*fl6.1*	6	38.2	4.2	7.3	−2.1	SSR03940 (32.2)	SSR04245 (40.1)
NJ2009F	*fl7.1*	7	46.0	5.4	13.9	2.9	UW069662 (40.1)	SSR12442 (46.7)
NJ2012S	*fl7.1*	7	41.3	4.4	10.8	2.4	SSR07088 (36.4)	SSR06349 (45.5)
WI2012H	*fl7.1*	7	41.3	6.7	12.2	2.7	UW084500 (35.8)	SSR06349 (45.5)
WI2013H	*fl7.1*	7	43.3	7.7	22.5	3.0	UW069662 (37.4)	SSR14861 (44.9)
Fruit diameter (FD)								
NJ2009F	*fd1.1*	1	43.2	6.0	12.3	−0.5	SSR01816 (41.6)	SSR04278 (47.9)
NJ2012S	*fd1.1*	1	45.2	8.4	19.0	−0.8	SSR01816 (42.1)	UW049617 (50.1)
WI2012H	*fd1.1*	1	43.2	9.2	15.2	−0.6	SSR01816 (41.6)	SSR04278 (47.8)
WI2013H	*fd1.1*	1	41.6	13.3	31.7	−0.8	UW083738 (39.6)	UW083751 (43.2)
NJ2009F	*fd4.1*	4	37.1	4.6	9.3	−0.4	SSR06253 (34.4)	SSR14617 (42.8)
NJ2012S	*fd4.1*	4	26.2	5.6	12.0	−0.7	SSR05899 (20.8)	SSR06253 (34.4)
WI2012H	*fd4.1*	4	26.2	8.8	14.6	−0.6	SSR03777 (25.1)	SSR06253 (34.0)
WI2013H	*fd4.1*	4	30.2	6.1	5.3	−0.5	SSR03777 (25.3)	SSR06253 (34.2)
NJ2009F	*fd6.1*	6	48.1	9.8	27.4	−0.7	SSR14008 (47.6)	UW083871 (49.2)
WI2012H	*fd6.1*	6	52.2	5.3	8.7	−0.6	SSR15067 (48.4)	SSR13996 (53.4)
Fruit weight (FW)								
WI2012H	*fw2.1*	2	58.3	3.3	5.3	−0.1	UW043299 (46.4)	SSR02539 (70.5)
NJ2009F	*fw4.1*	4	26.2	4.0	9.1	−0.2	SSR03777 (24.3)	SSR06253 (32.2)
NJ2012S	*fw4.1*	4	28.2	3.5	7.5	−0.2	SSR03777 (23.3)	SSR06253 (34.2)
WI2012H	*fw4.1*	4	26.2	3.9	5.8	−0.1	SSR03777 (23.5)	SSR06253 (32.2)
NJ2009F	*fw6.1*	6	41.8	9.6	28.7	−0.3	SSR04245 (40.4)	SSR13884 (43.6)
NJ2012S	*fw6.1*	6	41.8	10.6	25.3	−0.3	SSR04245 (40.6)	SSR13884 (43.6)
WI2012H	*fw6.1*	6	42.7	12.8	24.6	−0.3	SSR06500 (41.9)	SSR13884 (43.6)
WI2013H	*fw6.1*	6	49.2	3.4	10.0	−0.1	SSR15067 (48.1)	SSR17023 (50.2)

Chr, chromosome; R^2, phenotypic variations explained by specific QTL of total variances

Table 5 Consensus map locations of QTLs for female flowering time (FFT), fruit length (FL), fruit diameter (FD), and fruit weight (FW) detected from four experiments over 3 years

Traits	QTL	Chr.	Map locations (cM)[a]	R^2 (%)[b]	Experiments detecting the QTL
FFT	*fft1.1*	1	96.5	52.6	NJ2009F, NJ2012S, WI2012H, WI2013H
	fft6.1	6	42.7	5.9	NJ2009F, WI2012H
FL	*fl1.1*	1	52.8	17.7	NJ2009F, NJ2012S, WI2012H, WI2013H
	fl3.1	3	17.1	15.7	NJ2009F, NJ2012S, WI2012H
	fl4.1	4	69.3	8.8	NJ2009F, WI2013H
	fl6.1	6	38.2	7.3	NJ2012S, WI2012H
	fl7.1	7	41.3	12.7	NJ2009F, NJ2012S, WI2012H, WI2013H
FD	*fd1.1*	1	43.2	15.2	NJ2009F, NJ2012S, WI2012H, WI2013H
	fd4.1	4	26.2	14.6	NJ2009F, NJ2012S, WI2012H, WI2013H
	fd6.1	6	52.2	8.7	NJ2009F, WI2012H
FW	*fw2.1*	2	58.3	5.3	WI2012H
	fw4.1	4	26.2	5.8	NJ2009F, NJ2012S, WI2012H
	fw6.1	6	42.7	24.6	NJ2009F, NJ2012S, WI2012H, WI2013H

[a] Approximate location based on WI2012H data

[b] Data are taken from WI2012H experiment except for *fl4.1* which was from WI2013H data

the same location despite the non-significant LOD support scores.

Consensus QTL for FFT, FL, FD, and FW across the four experiments Among the 39 QTLs for the four traits, all but one (*fw2.1*) (but see Fig. S3E) were detected in at least two environments. QTLs for the same trait often peaked at the same or closeby map locations (Table 4) suggesting that these QTLs probably belong to the same locus for the trait. As such, by synthesizing information from Table 4, 13 QTLs could be recognized with 2 (*fft1.1*, and *fft6.1*), 5 (*fl1.1*, *fl3.1*, *fl4.1*, *fl6.1*, and *fl7.1*), 3 (*fd1.1*, *fd4.1*, and *fd6.1*), and 3 (*fw2.1*, *fw4.1*, and *fw6.1*) for FFT, FL, FD, and FW, respectively. Information on these 13 QTLs is summarized in Table 5.

Among the 13 QTLs, 3, 3, and 4 were located in chromosomes 1, 4, and 6, respectively; chromosomes 2, 3, and 7 each harbored one QTL, and chromosome 5 had none. The QTLs for FD and FW were co-localized in chromosomes 4 and 6 (Fig. 2), which may explain the significant correlation between the two traits in the present study (Table 3).

Discussion

Chromosome differentiation in XIS cucumber

Classical taxonomic studies recognized four cucumber botanical variants in *C. sativus* including the cultivated cucumber (var. *sativus*), the wild cucumber (var. *hardwickii* (Royle 1835; Duthie 1903), the Sikkim cucumber (var. *sikkimensis*) (Hooker 1876), and the XIS cucumber (var. *xishuangbannesis*) (Qi 1983). Morphological variations among these variants are large with cultivated and wild cucumbers representing the two extremities (Kirkbride 1993; de Wilde and Duyfjes 2010). Yang et al. (2012) identified significant differences in the amount and distribution of heterochromatins, as well as six inversions in chromosomes 4, 5, and 7 between *C. sativus* var. *sativus* and *C. sativus* var. *hardwickii*; the results support the subspecies status of these two cucumber taxa and *C. sativus* var. *hardwickii* as the progenitor of cultivated cucumber.

In the present study, alignment of the XIS genetic map with the cultivated cucumber map (Yang et al. 2012, 2013) suggested no major gross structural rearrangements between the two variants except for five small inversions in chromosomes 1 and 6 (Fig. 3). Since the orders of shared marker loci or draft genome scaffolds involved in these inversions were consistent between cultivated and wild cucumbers, these structural changes might be specific to the XIS cucumber. Unlike the wild cucumber map (Ren et al. 2009), no clustering of markers was found in chromosomes 4, 5, and 7 on the XIS map (Fig. 3). This is consistent with the cultivated cucumber map (Yang et al. 2012) suggesting that the XIS and cultivated cucumbers shared common ancestors. That is, the origin of the XIS cucumber was after domestication of cucumber from *C. sativus* var. *hardwickii*. Therefore, the characteristic morphological traits such as large and heavy fruits, dark green leaves, vigorous vine growth, and the orange flesh color in the XIS cucumber were

likely the result of diversifying selection for adaptation to the local environment where the XIS cucumber grows today. This may also imply that the XIS-specific inversions in chromosomes 1 and 6 identified herein (Fig. 3) occurred after its divergence with the cultivated cucumber. In plants, lineage- or population-specific inversions are believed to play important roles in evolution (for example, local adaptation and speciation) (Hoffmann and Rieseberg 2008; Kirkpatrick 2010; Lowry and Willis 2010). However, it is not known if the XIS-specific inversions identified herein exist in all XIS populations or these inversions have any adaptive significance. On the other hand, the XIS cucumber requires short day length for flowering. It is appropriate to treat the XIS cucumber as a semi-wild botanic variant (Qi 1983).

QTL mapping of traits under domestication and diversifying selection in XIS cucumber

Our QTL mapping effort focused on four traits that were characteristic of XIS cucumber. Thirteen QTLs were identified for the first female flowering time (FFT), the length (FL), diameter (FD), and weight (FW) of mature fruits (Table 5). Several previous studies have also identified QTLs for fruit length, diameter and weight, as well as flowering time in cucumber (Kennard and Havey 1995; Yuan et al. 2008; Dijkhuizen and Staub 2002; Fazio et al. 2003; Cheng et al. 2010; Miao et al. 2012, 2011). For convenience of discussion, the locations of QTLs detected by those and the present studies are placed onto the linkage map developed herein (Fig. 2); but for some early studies (Kennard and Havey 1995; Dijkhuizen and Staub 2002; Fazio et al. 2003), the chromosomal locations of mapped QTL were difficult to infer due to the nature of markers used and were not included in Fig. 2.

We identified a major QTL, *fft1.1* located at the distal end of cucumber chromosome 1 that could explain >50 % observed phenotypic variations; the minor QTL *fft6.1* was mapped in chromosome 6 (Fig. 1). Both alleles carried by the XIS cucumber exhibited negative additive effects, which is consistent with its very late flowering nature (photoperiod sensitive for flowering initiation). The QTL *fft1.1* seems to be co-localized with the major QTL *Da1.1* (days to anthesis of first female flower) detected by Miao et al. (2012). Interestingly, the mapping population used by Miao et al. (2012) was RILs derived from a cross between the north China fresh market cucumber 9930 and the European greenhouse type cucumber 9110Gt, which only have 2 days' difference in anthesis of the first female flowers. Since XIS cucumber has the same flowering habit (requirement of short day length for flowering initiation) as the wild cucumber (*C. sativus* var. *hardwickii*), this may suggest that the late flowering QTL *fft1.1* is a trait under selection during domestication of cucumber. Consistent with this, through analysis of genome-wide genetic variations between cultivated and wild cucumbers, Qi et al. (2013) identified 112 putative domestication sweeps, 4 of which (DS14, 15, 16 and 17) were located in the *fft1.1* 2-LOD interval between SSR05723 and SSR16995 (Fig. 2).

Five QTLs were identified for fruit length with three major-effect QTLs (*fl1.1*, *fl3.1*, and *fl7.1*) (Table 5). Kennard and Havey (1995) also detected five fruit length QTLs from an $F_{2:3}$ mapping population derived from Gy14 × PI 183967 (wild cucumber), but the locations of these QTLs in the early study are difficult to infer. None of the three major-effect QTLs showed consistent map locations with previously detected fruit length QTLs (Yuan et al. 2008, Cheng et al. 2010; Miao et al. 2011) (Fig. 2). The locations of the two minor-effect QTLs (*fl4.1* and *fl6.1*) seem to be consistent with those detected by Yuan et al. (2008) (*fl4.1* major-effect QTL on chromosome 4) and Miao et al. (2011) (major-effect QTLs *fl4.1*, *sfl6.1* in chromosomes 4 and 6, respectively) (Fig. 2). In an RIL population developed from 981 (north China type) × PI 183967, five fruit length QTLs were detected in four chromosome regions (1, 3, 4 and 6) (Cheng et al. 2010; Qi et al. 2013), which were believed to be under selection during domestication (Qi et al. 2013). Among the five FL QTLs detected in this study, only *fl4.1* showed consistent map location with *fl4.2* by Cheng et al. (2010) (Fig. 2). For the fruit diameter QTLs (*fd1.1*, *fd4.1* and *fd6.1*) detected herein, their chromosome locations were largely consistent with those identified in Yuan et al. (2008) and Miao et al. (2012). Lastly, the locations of fruit weight QTLs *fw2.1*, *fw4.1*, and *fw6.1* (major-effect QTL) from this study were close to those detected by Yuan et al. (2008) (*fw3.1* on chromosome 2, *fw4.1* on chromosome 4) and Miao et al. (2012) (*sfw6.1* and *sfw6.1* on chromosome 6) (Fig. 1). The significant positive correlation of FD and FW (Table 3) could be explained by the co-localization of FD and FW major-effect QTLs on chromosome 4 and the close linkage in chromosome 6 (Fig. 1).

While the QTLs at the same or close locations across different studies may imply common mechanisms underlying the fruit shape and size during domestication or diversifying selection, the discrepancies in the number, location, and magnitude of effect of QTLs for the same trait in different studies could be explained in several ways. The most reasonable explanation is that the cucumber lines used in these studies belong to different taxonomic groups or market classes. The populations used for QTL mapping were derived from crosses between wild and cultivated (Kennard and Havey 1995; Dijkhuizen and Staub 2002; Cheng et al. 2010; Qi et al. 2013), semi-wild and cultivated (this study), or between cultivated cucumber lines of different marker classes (Yuan et al. 2008; Miao et al. 2011, 2012). It is possible that these traits have undergone domestication

or diversifying selection for specialized market classes, and the genes underlying these traits may be different targets of the selection. In addition, the ability to detect QTLs of fruit-related traits may depend on the growth stages (e.g., commercial harvest stage vs. mature fruits) of data collection (e.g., Miao et al. 2011). Of course, the criteria of trait phenotyping, the seasons (spring, summer or fall) for data collection, and environments (open field, greenhouse or protected plastic houses) may all contribute to the different results in these QTL mapping studies.

Fruit shape QTL in cucumber

The length and diameter of cucumber fruits are economically important traits. For example, at commercial harvest stage, North American pickling cucumbers should have length-by-diameter (*L/D*) ratios of approximately 3.0. In other crops such as tomato (*Solanum lycopersicum*) and melon, the *L/D* ratio is often called fruit shape or fruit shape index. In cucumber, *L/D* has been considered as an independent trait, and QTLs for this trait have been identified in several studies (Kennard and Havey 1995; Dijkhuizen and Staub 2002; Fazio et al. 2003; Yuan et al. 2008; Miao et al. 2011).

In cucumber, the fruit develops from an enlarged inferior ovary. In the pickling cucumber cultivar 'Vlaspik', fruit elongation begins almost immediately after pollination, with the most rapid increase occurring approximately 4–12 days post-pollination (dpp); the rapid increase in cell size mirrors the rapid increase in fruit length. The increase in fruit diameter is somewhat lagging behind the length which occurs primarily between 4 and 16 dpp (Ando and Grumet 2010; Ando et al. 2012). Consistent with these observations, the correlation between length and diameter was not significant in both RIL and F_2 populations ($P = 0.05$) in the present study (Table 3) suggesting that elongation of fruit (FL) and increase of diameter (FD) might be under different genetic mechanisms. The non-significant correlation between FL and FD was also found in melon (Eduardo et al. 2007). *L/D* is a composite trait (calculated from FL and FD) for which the mechanisms and QTL mapping strategy are not well understood (Li et al. 2010). It is obvious that the *L/D* value is influenced by the larger of the two component traits (*L* and *D*). For example, in melon lines with contrasting fruit length, the fruit shape (FS = *L/D*) was often highly correlated with FL (Perin et al. 2002; Monforte et al. 2005; Eduardo et al. 2007). But in tomato when the difference of FD was dominant between two parental lines, the FS is significantly correlated with FD (e.g., Lippman and Tanksley 2001). It is not surprising that, in cucumber, the QTL location for *L/D* often co-localized with either an FL or FD QTL, whichever had the larger effect (Kennard and Havey 1995; Fazio et al. 2003; Yuan et al. 2008; Miao et al. 2011), or in some case, no *L/D* was detected (Miao et al. 2011). In the present study, the locations of *L/D* major QTLs were largely consistent with fruit length QTLs in chromosomes 1 and 7 (Fig. S3D). It is not known if QTLs for *L/D* truly exist. The biological interpretation of *L/D* QTLs is also unknown. This is the reason we did not list *L/D* QTLs in the present study. *L/D* is an important selection criterion in cucumber breeding in all major market classes, but caution should be exercised in using markers linked with *L/D* QTLs in marker-assisted selection. Probably, both fruit length and diameter QTLs should be considered in decision making.

Candidate genes for major-effect fruit shape and size QTLs

In tomato, six genes or QTLs controlling fruit shape and size have been cloned which include *CNR/FW2.2* and *SlKLUH/FW3.2* controlling fruit size (weight), *SUN* and *OVATE* controlling elongated shape, as well as *FASCIATED* (*FAS*) and *LOCULE NUMBER* (*LC*) controlling fruit locule number and flat shape (reviewed in Rodriguez et al. 2011; Monforte et al. 2014). *CNR/FW2.2* encodes a member of the cell number regulator (CNR); *SlKLUH/FW3.2* encodes a member of a subfamily of cytochrome P450 A78 class (CYP78A) and the ortholog of KLUH; *SUN* encodes a protein that is a member of the IQ domain family; *OVATE* encodes a protein in the ovate family protein (OFP); *FAS* encodes a protein that is a member of the YABBY family, whereas *LC* is probably encoded by the ortholog of the *A. thaliana* gene *WUSCHEL*, which is a member of the WOX family (reviewed in Rodriguez et al. 2011; Monforte et al. 2014). Using tomato or *Arabidopsis thaliana* CNR, CYP78A, OFP, SUN, WOX, and YABBY gene family sequences as queries, Monforte et al. (2014) identified 74 homologs of the six gene families in the melon genome and found that QTLs for fruit weight co-localized frequently with members of the *CNR/FW2.2* and *KLUH/FW3.2* families, and fruit shape QTLs co-localized with the OFP family members.

Both cucumber and melon belong to the genus *Cucumis* with similar fruit development patterns, and the genomes of the two species share high degree of chromosomal synteny and sequence homology (e.g., Li et al. 2011; Garcia-Mas et al. 2012; Yang et al. 2013, 2014). We hypothesize that the genes for fruit shape and size cloned in tomato or *Arabidopsis* may also be responsible for QTLs we identified herein in cucumber. We used the 74 melon fruit size and shape-related sequences identified in Monforte et al. (2014) as queries to BLAST against the Gy14 cucumber draft genome (Yang et al. 2012) and found all of them had homologs in the cucumber genome. The melon homologs located within or very close to the region defined by 2-LOD interval of a QTL on the genetic map are shown

in supplemental Table S2. Among the 74 candidate genes, CmOFP-14, CmOFP-15, and CmOFP-17 were co-localized with the fruit length QTL *fl1.1*, and fruit diameter QTL *fl1.1*; CmYABBY-4 was also within the 2-LOD interval of *fl1.1*. In addition, both CmOFP-16 and CmWOX-8 were located in the vicinity of fruit diameter QTL *fd6.1*. As compared with findings in melon (Monforte et al. 2014), co-localization of tomato candidate genes with mapped QTLs was not as common as found in melon. The reason may be multifold. First, the mechanisms underlying fruit length, width, and weight in cucumber may be different from those cloned in tomato or melon. Second, the QTL locations in either melon or cucumber need be refined through fine genetic mapping. Lastly, since we used melon homologs as queries to BLAST the cucumber genome, some sequences not presented in the melon draft genome may not be detected. A more detailed examination of those genes could be performed by looking into the genome annotations in those QTL-residing regions. Nevertheless, these candidate genes provide important clues for future fine mapping and cloning of these fruit shape and size QTLs.

Author contributions YW and JC designed the experiment. KB performed the research. ZM collected data for the NJ2012S experiment. YW and KB analyzed the data and wrote the manuscript with inputs from JC. All authors reviewed and approved this submission.

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Conflict of interest The authors declare no conflict of interest.

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