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Identification and characterization of a *long fruit* mutant in *Cucumis sativus* L.

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Abstract: A *long fruit* (*lf*) mutant was obtained from the cucumber inbred line 'SN5' by ethyl methanesulfonate (EMS)-mediated mutagenesis. We found that plant height and internode length of *lf* were significantly decreased, while leaf area was significantly increased in comparison to wild type (WT) plants. Higher carbohydrate level, together with lower chlorophyll content and photosynthetic parameters, was revealed in *lf* leaves than those of WT. Longer fruits, as well as enhanced fruit quality, were observed for mutant plants in comparison to WT, and the difference in fruit length was continuously increased with the development of fruits. ELISA and real-time PCR analyses demonstrated the significant alterations in not only the CTK and IAA contents but also the expression of key genes related to cytokinin and auxin signalings in the fruits of *lf* when compared to WT plants, evidencing the involvement of cytokinin and auxin in fruit elongation of *lf* cucumber. Our results could benefit subsequent gene mapping and expand our knowledge about cucumber fruit length regulation.

Key words: Cucumber, hormone content, agronomic characteristics, gene expression

1. Introduction

Cucumber (*Cucumis sativus* L.) is a vegetable that is cultivated worldwide. Fruit shape is not only an important appearance trait but also has profound influences on the commercial value and yield of cucumber. It is genetically controlled by quantitative trait loci (QTLs) in cucumber genome and can be evaluated by morphological parameters, including fruit length (FL), fruit diameter (FD), and fruit shape index (FL/FD). Kennard and Havey (1995) first used 100 F3 plants, which were obtained by crossing 'GY14' (North American pickling type) with 'P1432860' (long-fruit type), to locate QTLs for cucumber fruit traits and identified five and three QTLs related to FL and FD respectively. Enormous work has subsequently been carried out to locate QTLs related to fruit shape. For example, Yuan et al. (2008) analyzed F2 and F3 populations obtained by crossing North China-type cucumber 'S94' and European greenhouse cucumber 'S06' and identified 38 QTLs related to fruit weight (FW), FL, FD, and fruit neck length (FNL). Using F2, F3, and recombinant inbred line (RIL) populations that were constructed with 'GY14' and '9930' (North China-type), Weng et al. (2015) detected 12 fruit-related QTLs in different environments and developmental stages. Pan et al. (2017) located 10 fruit-related QTLs with the F2 population, which was constructed with 'WI7238' (long-fruit type) and 'WI7239' (round-fruit type) and further identified *CsSUN* as the candidate gene for *FS1.2*. Wang et al. (2020) detected 21 fruit shape-related QTLs with chromosome segment substitution lines (CSSLs) that were developed from the crossing between 'RNS7' (round-fruit type) and 'CNS21' (long-stick-fruit type). Thus far, many QTLs have been revealed to be associated with fruit shape, while only very few have been successfully cloned. Therefore, the regulatory mechanisms of cucumber fruit shape remain largely unknown (Pan et al., 2017).

Photosynthesis, a basic metabolic process, displays a close association with a series of plant developmental events, such as young leaf expansion (Gratani and Bonito, 2009; Wang et al., 2015), flowering (Micallef et al., 1995; Urban et al., 2004), fruit setting and elongation (Ando and Grumet, 2010; Quentin et al., 2013) by supplying assimilates from source leaves to sink organs. For example, Ando et al. (2012) carried out a comprehensive transcriptomic analysis for cucumber fruits at different developmental phases and observed the predominant expression of photosynthetic genes over the whole investigation course, particularly at 4 DPP (days post pollination), demonstrating the profound

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involvement of photosynthesis in cucumber fruit growth. Miao et al. (2009) found that, on one hand, the levels of exportable sugars in leaves and the assimilate accumulation in fruits are correlated with fruit growth rate, and, on the other hand, the sink fruits can impose the feedback effects on leaf photosynthesis.

Plant hormones have also been revealed as critical players for cucumber fruit growth regulation. Boonkorkaew et al. (2008) reported that pollination activates cell division by stimulating the synthesis of cytokinins and auxin in cucumber fruits. Cui et al. (2014) reported that cucumber auxin receptors *CsTIR1* and *CsAFB2* are important regulators during fruit development. Wang et al. (2017) located the *short-fruit* gene within a 174.6 kb region of chromosome 6 with the short-fruit mutant *sf-1* and further proposed that *sf-1* might regulate cucumber FL through auxin, cytokinin, and gibberellin signalings. Zhao et al. (2019) analyzed 150 cucumber varieties with different fruit shapes, and identified *CsFUL1*^{A} as a negative regulator for FL through auxin signaling.

Ethyl methanesulfonate (EMS) is a commonly used chemical for mutagenesis of field and horticultural crops such as wheat, tomato, and watermelon due to its high mutagenic efficiency, low cost and ability to introduce only single-base mutations in plant genomes (Menda et al., 2004; Galpaz et al., 2013; Wang et al. 2019). In this study, a *long fruit* (*lf*) mutant, which was obtained from the pre-established cucumber EMS mutant library under the genetic background of 'SN5' (Wang et al., 2014), was used to explore the mechanisms underlying cucumber fruit length control. We performed systematical investigation for its morphological and molecular changes, especially during fruit development, in comparison to those of the wild-type (WT) plants. The decreased plant height and internode length, as well as the increased leaf area, were observed for *lf* plants in comparison to WT. Higher carbohydrate level, together with lower chlorophyll content and photosynthetic parameters, was detected in the leaves of *lf* than those of WT. Mutant plants produced longer fruits, and the difference in fruit length was continuously increased with the development of fruits relative to WT. Meanwhile, fruit quality was significantly enhanced in *lf* plants. Using ELISA and real-time PCR (RT-PCR) assays, we found that not only the CTK and IAA contents but also the expression of key genes related to cytokinin and auxin signalings were dramatically altered in *lf* fruits when compared to WT plants, implying the potential involvement of cytokinin and auxin in fruit elongation of *lf* mutant. These analyses could not only shed new insights into the mechanisms underlying fruit shape regulation but also benefit molecular breeding for cucumber fruit shape in future.

2. Materials and methods

2.1. Plant materials

'SN5' is a North China-type cucumber (*Cucumis sativus* L.) inbred line with a commercial FL of 35.33±2.17 cm. The *lf* mutant, for which the FL was increased to approximately 42 cm, originated from EMS-mediated mutation of 'SN5' (WT) followed by 8 rounds of self-crossing. To conduct this study, the *lf* and WT plants were grown in the solar greenhouse of Shandong Agricultural University in spring and autumn, respectively, from 2017 to 2020 under normal fertilizer and water management conditions (Luan et al., 2019).

2.2. Determination of plant agronomic traits

Plant height, which was defined as the distance from the plant base to the top bud, was measured for *lf* and WT cucumbers with a steel ruler at the following five developmental stages: one-leaf [approximately 10 days after sowing (DAS)], two-leaf (approximately 15 DAS), four- or five-leaf (approximately 35 DAS), early flowering (approximately 55 DAS) and fruiting stages (approximately 75 DAS). Length of the first and second internodes, which were counted from the plant base, and stem diameter at the first node were measured with a vernier caliper at fruiting stage (75 DAS). Position of the first female flower node was also recorded. For root comparison between *lf* and WT, cucumbers were grown in sand culture and irrigated with 1/2 strength of Hoagland's solution (Hoagland and Arnon, 1950) every three days, and the seedling roots were sampled at two-leaf stage (15 DAS). Root morphology was analyzed with a MICROTEK root scanner (Shanghai, China) and WinRHIZO software (Regent Instruments Inc., Canada). Fifteen replicate plants were performed for these agronomic traits.

2.3. Investigation of fruit growth and quality

Fruit length of three typical fruits in each *lf* or WT plant was measured with a steel ruler at 7-9 am every day from 12 DBA (days before anthesis) to 21 DAA (days after anthesis), and the mean value was used as the representative fruit length for subsequent statistical analysis. In total, thirty plants for both *lf* and WT cucumbers were measured to monitor fruit development.

To assess fruit quality, the middle sections (approximately 2.0 cm length) of *lf* and WT fruits were collected at 15 DAA. The contents of soluble sugar, soluble protein, ascorbic acid (V_C), and free amino acids were determined according to the methods described by Ji et al. (2018). Eighteen biological repeats were prepared for each quality parameter.

2.4. Leaf morphology and photosynthetic capacity analysis

At the fruiting stage (75 DAS), the first and second functional leaves of *lf* and WT, which were counted

from the plant apex, were selected for evaluation of leaf morphology and photosynthetic capacity. Length and width of the aforementioned leaves were measured with a steel ruler, and leaf area was then calculated according to the previously described protocol (Robbins et al., 1987). Net photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO_2 concentration were determined with a CIRAS-3 portable photosynthetic apparatus (PP-Systems, USA) at 9-11 am on sunny days by following the method of Cheng et al. (2016). At 9-11 am on sunny days, the abovementioned functional leaves were sampled for the determination of photosynthetic pigments according to the method of Wang et al. (2018) and for the assay of carbohydrate contents based on the previously described methods (Ji et al., 2018). Fifteen biological repeats were performed for these analyses.

2.5. Fruit hormone content analysis

Cucumber ovaries/fruits were collected at 0, 8, and 15 DAA for hormone assay. For cucumber fruits, a mixture was further prepared with the top, middle, and bottom sections from each sample before the assay started. The contents of CTK and IAA in ovaries or fruit mixtures were determined with the ELISA kits (Ruixin, China) according to the manufacturer's instructions. There biological repeats were performed.

2.6. Gene expression analysis

Total RNAs were extracted from *lf* and WT fruits, which were sampled at 0, 8, and 15 DAA, with an RNA extraction kit (CWBIO, Beijing). First-strand cDNAs were then synthesized with a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific Fermentas, USA). RT-PCR was carried out with a TransStart TipTop Green qPCR SuperMix Kit (TransGen Biotech Beijing) on a ROCHE LightCycler 480 real-time PCR System (Roche, Switzerland) for detecting the transcription levels of selected genes in cytokinin signaling [*HP* (*histidine phosphotransfer protein*), *RR* (*response regulator*) and *CRF* (*cytokinin response factor*)] and auxin signaling [*AUX1* (*auxin resistant 1*), *AUX/IAA* (*auxin/indoleacetic acids*), *ARF* (*auxin response factor*), *GH3* (*gretchen hagen 3*) and *SAUR* (*small auxin up RNA*)] with *CsActin* as an internal control. Three biological repeats, together with three technical repeats for each biological preparation, were performed. The primers used in RT-PCR were provided in Table 1.

2.7. Data analysis

Relative expression of the select genes from RT-PCR assay was calculated by following the $2^{-\Delta\Delta Ct}$ method (Liu et al., 2019). All data used in this study were processed and visualized with Microsoft Excel 2010 software. The processed results were displayed as mean ± standard deviation, and difference evaluation was carried out at significance levels of 0.05 and 0.01 in related figures and tables.

Table 1. PCR primers used in this study.

Name of the primer	Sequence (5'-3')
CsActin-Forward	ATGGCCGATGCCGAGGATATT
CsActin-Reverse	CTTTTCTCTGTTAGCCTTTGGG
CsHP1-Forward	AACCCAATTCCTTCGCTAC
CsHP1- Reverse	TGCACTTTGTTTGCTCCA
CsHP2-Forward	AGGCAGCAGTTCCAGCATAG
CsHP2- Reverse	CACCAGCATTCAAAATCCG
CsRR1-Forward	CCACCTCTGATTCTGAAG
CsRR1- Reverse	GTCATTCCGGGCATACAG
CsRR2-Forward	CACAACTCTCCCAACATCAG
CsRR2- Reverse	CGCCTTCTTCTAAACACCTG
CsCRF1-Forward	CCTAAGGTTGTGAGGATTTCTG
CsCRF1-Reverse	GCATCTGCTTCAATGAGCC
CsCRF2-Forward	GAGCCATCTTGTTCTGGAG
CsCRF2- Reverse	GCGTTGTCATAAACCATTGC
CsAUX1-Forward	ATTTGGTCCTTCCTTGGC
CsAUX1- Reverse	AGCCCAGTAAACAGCAGAAG
CsAUX/IAA-Forward	TGCCTTTGCTTTCTTGGA
CsAUX/IAA- Reverse	GTTTGCGGAGGTTGAGTG
CsARF1-Forward	GGTCTCGATAATGCTGGC
CsARF1- Reverse	GAGGACAGACTAGTATCAG
CsARF2-Forward	GACGACGATCCTGTACAAG
CsARF2- Reverse	GTTGCACAGGATGTTGAATGAG
CsGH3-Forward	ATGCTGGTGGTGTACTGA
CsGH3- Reverse	TCTCCAATGGGTTGATAG
CsSAUR-Forward	GCTGTGGAAGTAGGGTTG
CsSAUR- Reverse	ATCGGAGTTGGAGAAAGT

3. Results

3.1. Morphological investigation for *lf* mutant

We previously obtained a *lf* mutant, which can produce extraordinarily longer fruits, from the cucumber inbred line 'SN5' by ethyl methanesulfonate (EMS)-mediated mutagenesis (Wang et al., 2014). To further characterize this mutant line, morphological parameters, including plant height, internode length, stem diameter, leaf area, position of the first female flower node and root growth index, were investigated. The results showed that plant height, length of the first and second internodes, were significantly decreased for *lf* mutant in comparison to WT, while the first and second functional leaves were significantly larger than those of WT (Figures 1 and 2). There were no significant differences in stem diameter, first female flower node, and root growth indexes between *lf* mutant and WT (Figure 1; Table 2).

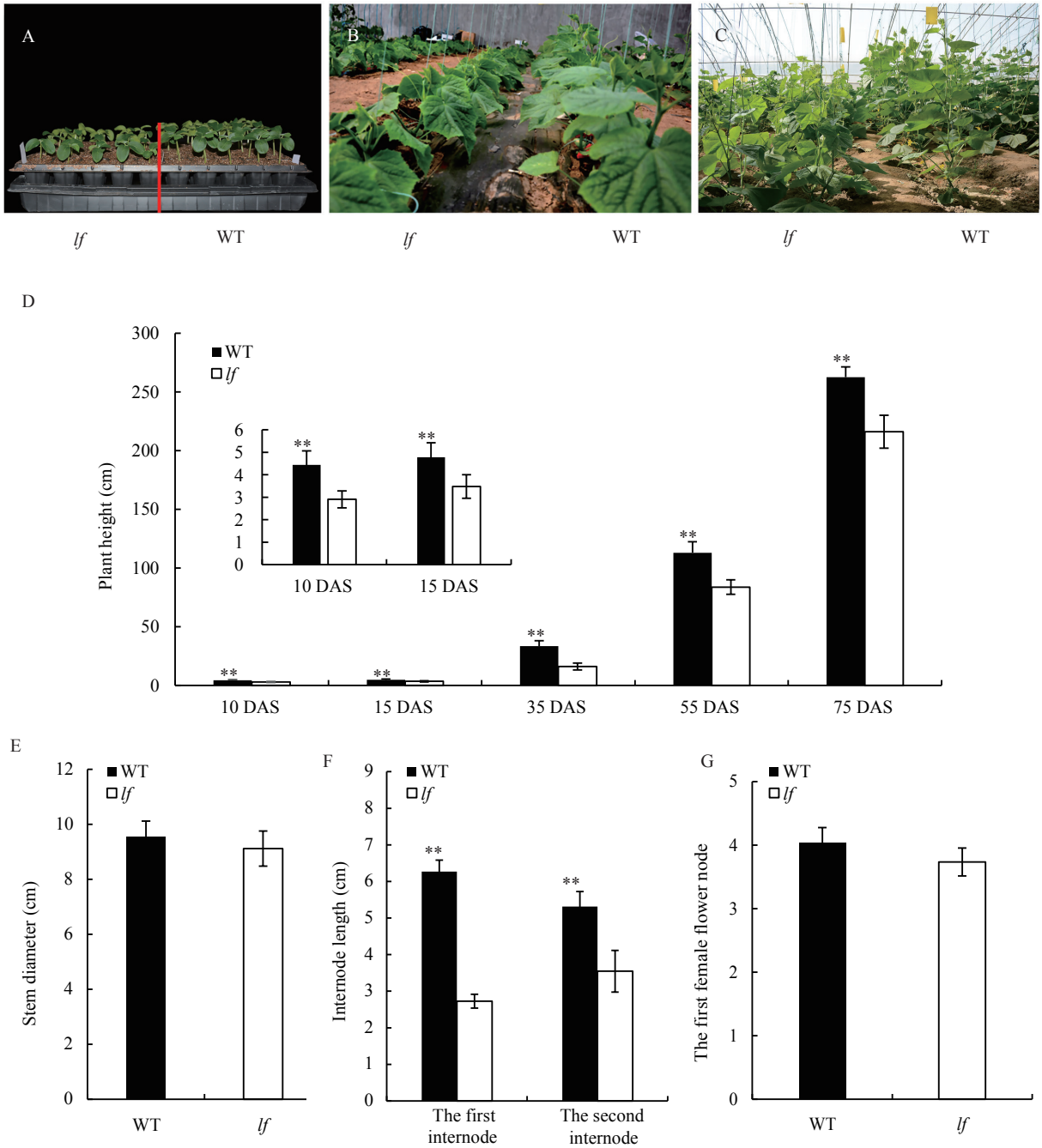

Figure 1. Growth analysis for the *lf* mutant and WT plants. (A) Plants of the *long fruit* (*lf*) mutant and WT at one true-leaf stage [10 days after sowing (DAS)]. (B) Plants of the *lf* mutant and WT at four or five true-leaf stages (35 DAS). (C) Plants of the *lf* mutant and WT at initial flowering stage (55 DAS). (D) Plant height of the *lf* mutant and WT at 10 DAS, 15 DAS, 35 DAS, 55 DAS and 75 DAS. (E-G) Stem diameter (E), the lengths of the first internode, and the second internode (F), and the first flower node (G) in the *lf* mutant and WT. **indicates a significant difference between the *lf* mutant and WT at the 0.01 level. Vertical bars represent standard deviation.

3.2. Physiological investigation for *lf* mutant leaves

As the major source organ, plant leaves play crucial roles in cucumber development. The significant variations in growth parameters and fruit length of *lf* mutant promoted us to carry out additional physiological investigations for its leaves. Being consistent with the light green leaves of *lf* mutant, the much lower contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were revealed in the leaves of mutant plants relative to those of WT plants (Figure 2). Furthermore, in the leaves of

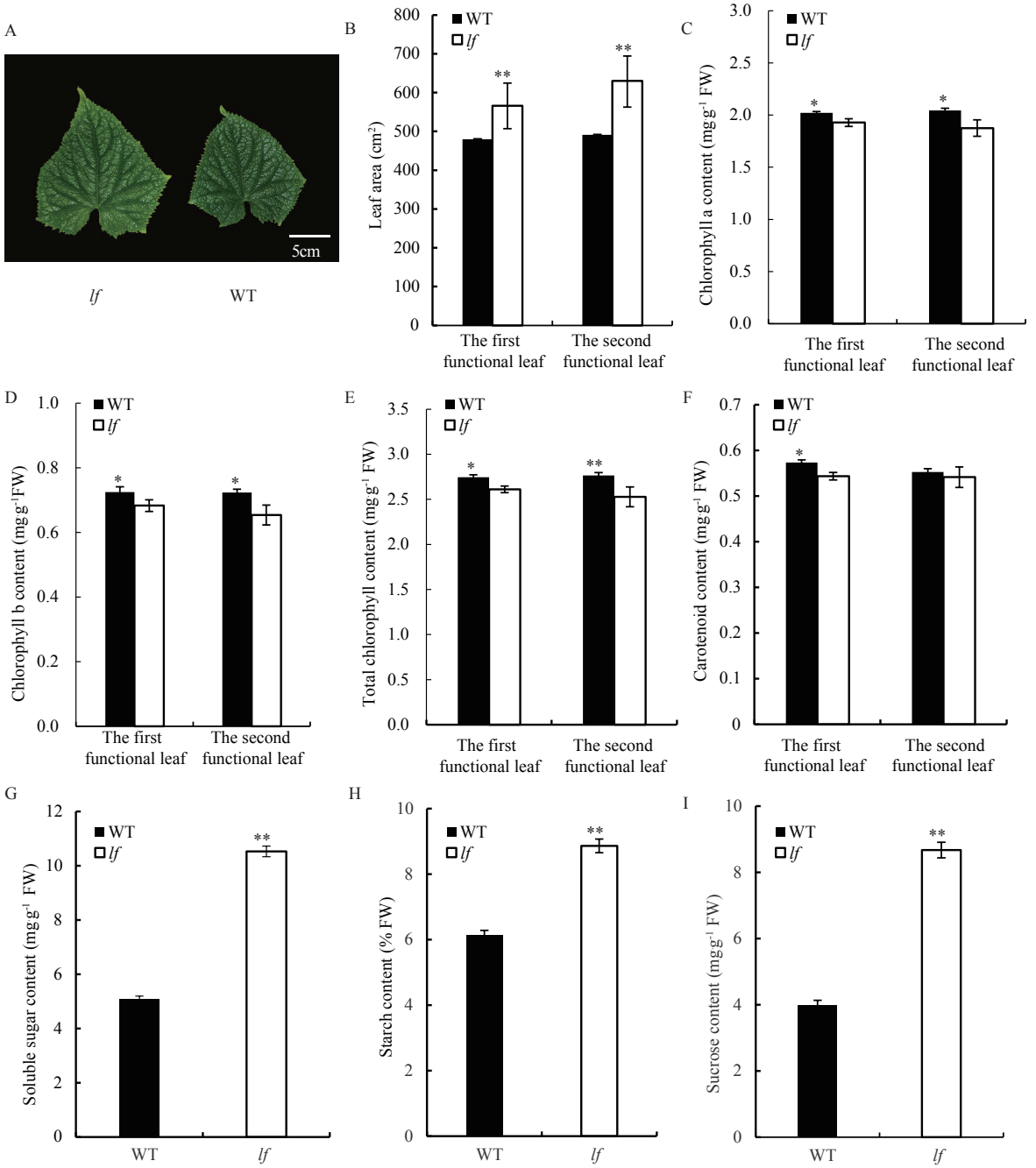

Figure 2. Phenotype, photosynthetic pigment content and carbohydrate content of the *lf* mutant and WT leaves. (A) Leaves of the *long fruit* (*lf*) mutant and WT. (B) The area of the first and second functional leaves counted from the plant apex. (C-F) The contents of chlorophyll a (C), chlorophyll b (D), total chlorophyll (E) and carotenoids (F) in the *lf* mutant and WT leaves. (G-I) Soluble sugar (G), starch (H) and sucrose (I) contents in the *lf* mutant and WT leaves. *indicates a significant difference between the *lf* mutant and WT at the 0.05 level, and **indicates a significant difference between the *lf* mutant and WT at the 0.01 level. Vertical bars represent standard deviation.

lf mutant, soluble sugar, starch, and sucrose contents were 10.53 mg·g^{-1} FW, 8.86 % FW, and 8.47 mg·g^{-1} FW, respectively, which all were dramatically higher than those of WT (Figure 2).

We, then, explored photosynthetic parameters, including photosynthetic rate, transpiration rate, stomatal conductance, and intercellular carbon dioxide (CO_2) concentration in the first and second functional leaves of

lf and WT plants at fruiting stage (75 DAS). The results showed that the photosynthetic rate, transpiration rate, and stomatal conductance were significantly lower in the mutant leaves than those of WT, while no apparent difference was observed in the intercellular CO_2 concentration between *lf* mutant and WT (Table 3), possibly owing to the lowered contents of photosynthetic pigments in the leaves of mutant plants.

3.3. Characterization of fruit development and quality for *lf* mutant

The *lf* mutation results in substantially elongated fruits for mutant line relative to 'SN5' (Wang et al., 2014). We wondered how this mutation influences fruit development. To this end, fruit length from 12 DBA to 21 DAA was measured for *lf* and WT plants every day. Similar growth pattern was observed for the fruits of *lf* mutant and WT over the whole investigation course, while the growth rate was greatly enhanced for *lf* cucumber from 6 DAA, thus, generating longer fruits at the end of investigation course (Figure 3).

Quality parameters including the contents of chlorophyll, soluble sugars, V_C, free proline, and soluble proteins were further explored in the fruits of *lf* mutant and WT plants (Figure 4). The contents of chlorophyll a and chlorophyll b were slightly lower in the pericarp, while they were slightly higher in the sarcocarp of *lf* mutant in comparison to WT; however, these differences did not reach a significant level (Figure 4). The content of soluble sugars was 22.36 mg·g^{-1} FW in the fruits of *lf* mutant, while this parameter was only 16.37 mg·g^{-1} FW for WT fruits. The contents of V_C and free proline in the fruits of *lf* mutant were 17.1 mg·g^{-1} FW and 0.18 mg·g^{-1} FW, respectively, which were significantly higher than those of WT. No significant difference was observed for fruit soluble protein content between *lf* mutant and WT (Figure 4).

3.4. Hormone and gene expression analysis for *lf* mutant fruits

Plant hormones play crucial roles in fruit growth and development (Kumar et al., 2014). We wondered whether hormone contents were influenced in *lf* cucumber. To this end, the ovaries/fruits were sampled from WT and mutant plants at 0, 8, and 15 DAA, and the contents of CTK and IAA, two phytohormones closely associated with fruit development (Deng et al., 2010; Zhao et al., 2010), were determined. The results showed that CTK content was significantly lower in the ovaries/fruits of *lf* mutant than that of WT at 0 DAA. With the development of cucumber fruits, CTK content was incremented and reached a significantly higher level in mutant fruits at the end of investigation course when compared to WT fruits. For IAA, its content was significantly higher in the fruits of *lf* mutant than that of WT at 8 DAA, while it was significantly lower in the ovaries/fruits of mutant than that of WT at 0 DAA and 15 DAA (Figure 5).

To unveil how plant hormones were involved in the elongation of fruit length, the expression levels of key genes in cytokinin and auxin signalings were explored in the fruits of *lf* mutant and WT plants. The lower expression levels of three cytokinin signaling genes (*CsRR1*, *CsRR2*

Table 2. Characteristics of cucumber root growth in the *long fruit* (*lf*) mutant and WT.

Sample	Total root length (cm)	Average diameter (mm)	Total surface (cm^2)	Total volume (cm^3)	Root tip number
WT	450.62 ± 21.50	0.40 ± 0.03	50.79 ± 3.32	0.98 ± 0.23	876.75 ± 104.96
lf	463.99 ± 21.45	0.40 ± 0.02	51.6 ± 0.02	1.07 ± 0.38	855.25 ± 115.94

Note: The results for each root growth parameters were displayed as mean of fifteen biological repeats ± standard deviation.

Table 3. Photosynthetic characteristics in the first and second functional leaves of *long fruit* (*lf*) mutant and WT at fruiting stage (75 DAS).

Sample	Pn (μmol·m^{-2}·s^{-1})	Tr (mmol·m^{-2}·s^{-1})	Gs (mmol·m^{-2}·s^{-1})	Ci (μmol·mol^{-1})
WT	15.7 ± 1.99**	9.42 ± 1.21*	609.75 ± 43.36**	312.75 ± 9.5
lf	12.15 ± 1.08	7.48 ± 0.95	347 ± 62.01	310.25 ± 19.48

Note: Pn: photosynthetic rate; Tr: transpiration rate; Gs: stomatal conductance; Ci: intercellular carbon dioxide concentration. Results for each photosynthetic parameter were displayed as mean of fifteen biological repeats ± standard deviation. *indicates a significant difference between the *lf* mutant and WT at the 0.05 level, and **indicates a significant difference between the *lf* mutant and WT at the 0.01 level.

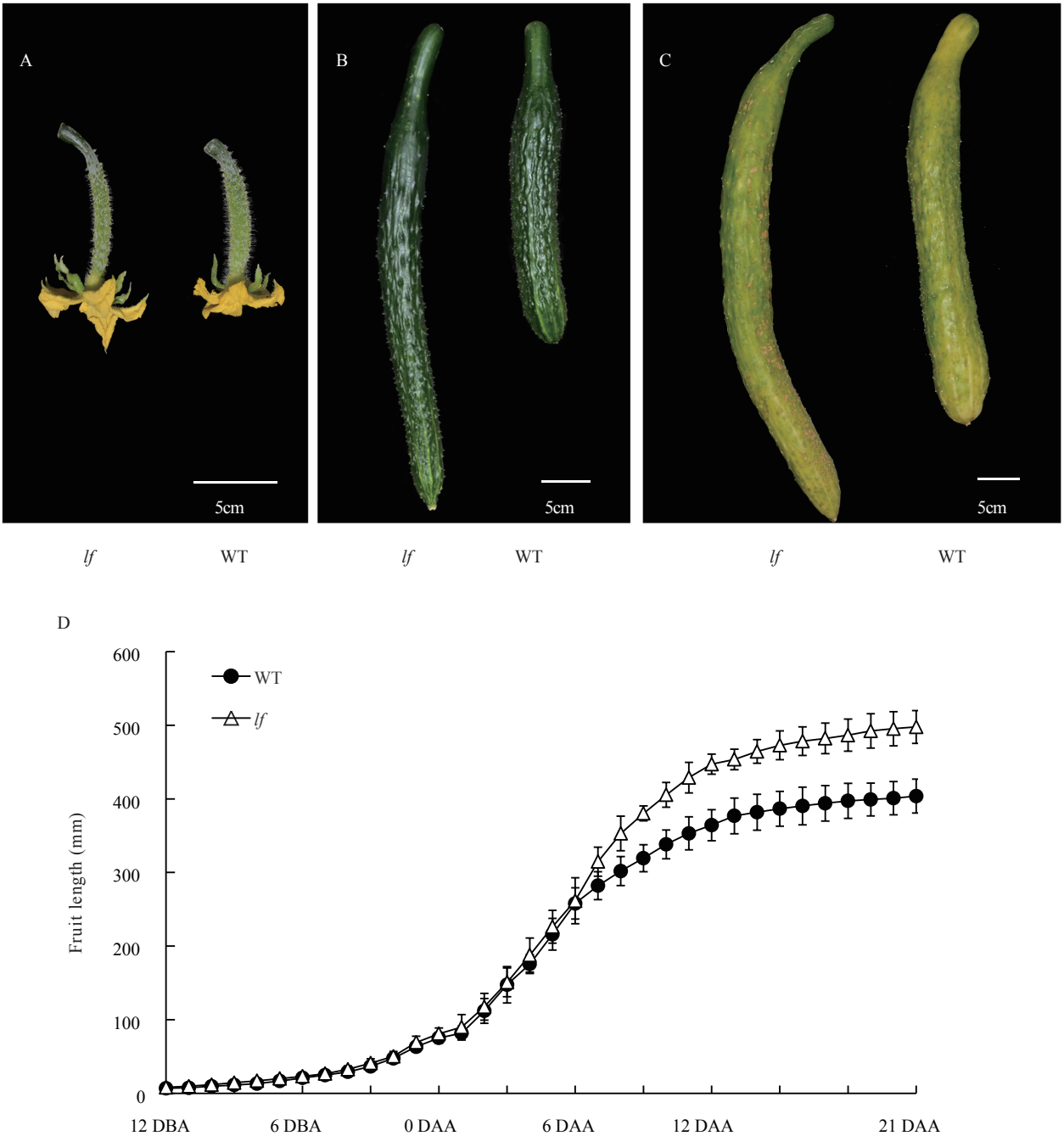

Figure 3. Fruit length of the *lf* mutant and WT. (A-C) Phenotypes of the *long fruit* (*lf*) mutant and WT fruits at 0 days after anthesis (DAA) (A), 15 DAA (B) and 35 DAA (C). (D) Growth curve for fruit length of the *lf* mutant and WT from 12 DBA (days before anthesis) to 21 DAA. Vertical bars represent standard deviation.

and *CsCRF1*) were observed for the ovaries of *lf* mutant relative to WT at 0 DAA, while all select genes (*CsHP1*, *CsHP2*, *CsRR1*, *CsRR2*, *CsCRF1* and *CsCRF2*) displayed significantly higher expression in the fruits of *lf* mutant than those of WT at 15 DAA (Figure 6). For the six select genes in auxin signaling, the expressional fluctuation was observed. As shown in Figure 7, both *CsAUX/IAA* and *CsARF1* displayed significantly lower expression in the ovaries of *lf* mutant than those of WT plants at 0 DAA. With the development of cucumber fruits, the expression of all six select genes (*CsAUX1*, *CsAUX/IAA*, *CsARF1*, *CsARF2*, *CsGH3* and *CsSAUR*) were increased to a significantly higher level for *lf* mutant relative to WT plants at 8 DAA, while the lowered expression was detected for

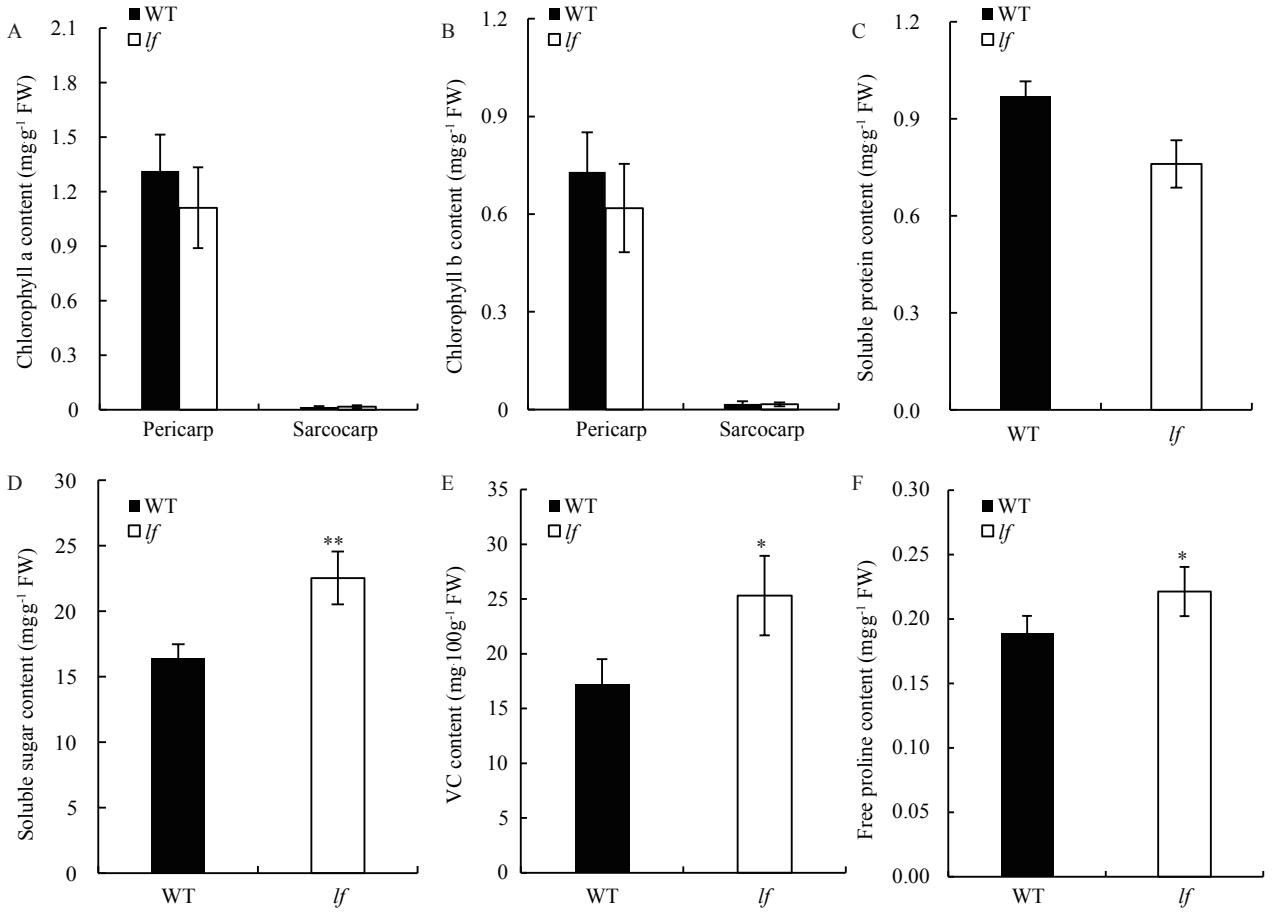

Figure 4. Fruit quality of the *lf* mutant and WT plants. The contents of chlorophyll a (A), chlorophyll b (B), soluble protein (C), soluble sugars (D), V_C (E) and free proline (F) in the *long fruit* (*lf*) and WT fruits. *indicates a significant difference between the *lf* mutant and WT at the 0.05 level, and **indicates a significant difference between the *lf* mutant and WT at the 0.01 level. Vertical bars represent standard deviation.

CsAUX1, *CsAUX/IAA*, *CsARF1* and *CsARF2* in *lf* mutant than those of WT plants at the end of investigation course. These expression results were largely consistent with the variations in CTK and IAA contents in the ovaries/fruits of *lf* mutant over the investigation course.

4. Discussion

Fruit shape is an important trait for evaluating the quality of cucumber appearance. Thus far, the North China-type cucumbers such as 'SN5', whose FL is approximately 35 cm, have become the dominant varieties in horticultural production in China. In contrast, the fruits of *lf* mutant reached about 42 cm, which was over 20 % longer than WT and other North China-type cucumbers and could, thus, enrich cucumber germplasms for future fruit shape breeding.

Plant growth and development are largely dependent on the distribution of photoassimilates between source and sink organs, which follows a principle of preferential transportation to plant growth centers (Quentin et al., 2013). Widders and Kwantes (1995) showed that the decreased canopy photosynthesis has greatly restricted the supply of assimilates from photosynthetic tissues, and, consequently, fruit growth and yield are significantly lowered. In a study regarding the effects of supplemental lighting on cucumber development, Hao and Papadopoulos (1999) observed the significantly increased chlorophyll contents and photosynthesis in plant leaves and the stimulated photoassimilate translocation to fruits, thus, leading to the enhanced fruit yield and quality. The supply of photoassimilates depends on the strength of source organs, which are influenced by not only photosynthetic rate but also leaf area and have a direct impact on fruit yield and quality of cucumber (Wu et al., 2009). In the present study, we discovered that the contents of photosynthetic pigments were obviously reduced in the leaves of *lf* mutant, leading to the lowered photosynthetic rate in the leaves of mutant plants (Figure 2). In contrast, the enlarged leaves

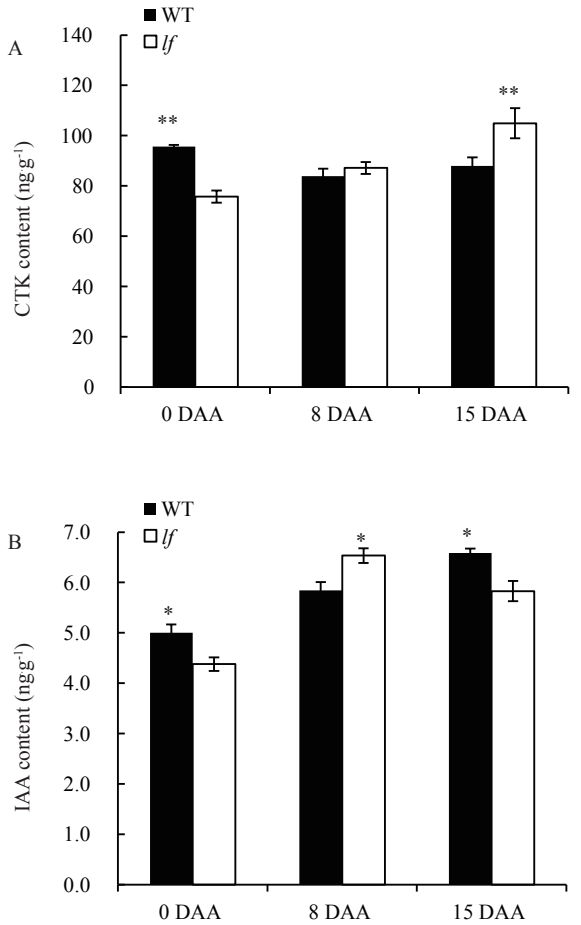

Figure 5. The contents of CTK and IAA in the fruits of the *lf* mutant and WT. The content of CTK (A) and IAA (B) in the fruits of the *long fruit* (*lf*) mutant and WT at 0 DAA, 8 DAA and 15 DAA. *indicates a significant difference between the *lf* mutant and WT at the 0.05 level, and **indicates a significant difference between the *lf* mutant and WT at the 0.01 level. Vertical bars represent standard deviation.

were observed in *lf* mutant, which might compensate the negative influences from the lowered photosynthesis and, thus, could accumulate more photoassimilates in the mutant leaves (Figure 2; Wang et al., 2012; Shi et al., 2014). We speculate that photoassimilate distribution from source leaves might give priority to fruits over other sink organs such as stems in the *lf* mutant, and as a result, fruit length and quality of *lf* mutant were significantly improved, while plant height was lowered (Figures 3 and 4). Yang et al. (2018) revealed that the *LL* (*LITTLELEAF*) gene encoding a WD40 protein in cucumber has pleiotropic effects on both the size of multiple organs, such as leaves, flowers, fruits and seeds, and the lateral branch differentiation. In the present study, *lf* mutant displayed longer fruits, as well as dwarfed plant height and enlarged leaves, though this mutant had been multiselected via several rounds of self-crossing, possibly suggesting the pleotropic nature of the *lf* gene.

Plant hormones can individually or interactively influence plant tissue or organ development (Su et al., 2011; Meng et al., 2015; Ma and Li, 2019). Zhang et al. (2019) found that cucumber fruit length is determined by the combined functions of plant hormones and epigenetic elements. In the present study, the lowered content was detected for CTK at 0 DAA, and for IAA at 0 and 15 DAA, while the obviously enhanced content was detected for CTK at 15 DAA, and for IAA at 8 DAA, in the fruits of *lf* mutant than those of WT plants (Figure 5). Being largely consistent with the altered contents of CTK and IAA upon *lf* mutation, the decreased expression of key genes was observed for cytokinin signaling at 0 DAA, and for auxin signaling at 0 and 15 DAA, while the significantly stimulated gene expression was revealed for cytokinin signaling at 15 DAA, and for auxin signaling at 8 DAA, in the fruits of *lf* mutant when compared to WT plants (Figures 6 and 7). The stimulated fruit length might thus be attributed to these significant differences in the contents of CTK and IAA, as well as the expression of keys genes related with cytokinin and auxin signalings, in *lf* cucumber. Similar results have been demonstrated by Matsuo et al. (2012) and Zhao et al. (2019).

Dwarf is considered as a significant trait for crops with ideal architecture. Therefore, the identification and utilization of dwarf-related genes have become an attractive field in plant breeding (Ji et al., 2006). To date, a number of genetic studies on dwarf plants have been carried out in field and horticultural crops, such as tomato (Lin et al., 1995), cucumber (Bishop et al., 1996), rice (Spielmeyer et al., 2002), apple (Yang et al., 2011), and watermelon (Wei et al., 2019). For example, Nam et al. (2005) identified a dwarf locus *de* (*determinate habit*), which is associated with the dwarf phenotype of a cucumber mutant line, by analyzing two cucumber BAC libraries. Subsequently, Xu et al. (2018) identified a cucumber mutant *Csdw*, which exhibits a dwarf phenotype with a reduced internode length because of the reduction of cell division in the main stem. In the present study, we demonstrated that the shortened plant height of *lf* mutant was mainly related to the significantly decreased length of the first and second internodes (Figure 1). The dwarf phenotype of *lf* cucumber could decrease labor requirement during the cultivation period, thus being advantageous over the nondwarf varieties such as 'SN5'.

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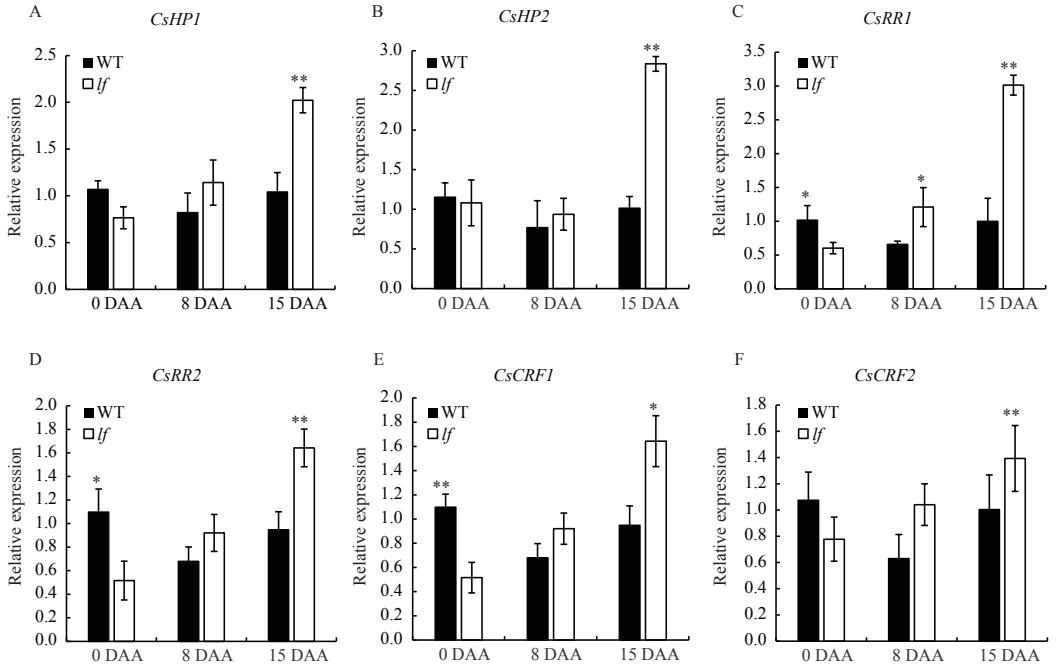

Figure 6. Differentially expressed genes in cytokinin signaling between the *lf* mutant and WT fruits. Expression of cytokinin signaling genes (*CsHP1*, *CsHP2*, *CsRR1*, *CsRR2*, *CsCRF1* and *CsCRF2*) in the fruits of the *long fruit* (*lf*) mutant and WT at 0 DAA, 8 DAA and 15 DAA. Three biological replicates were performed for each select gene. *indicates a significant difference between the *lf* mutant and WT at the 0.05 level, and **indicates a significant difference between the *lf* mutant and WT at the 0.01 level. Vertical bars represent standard deviation.

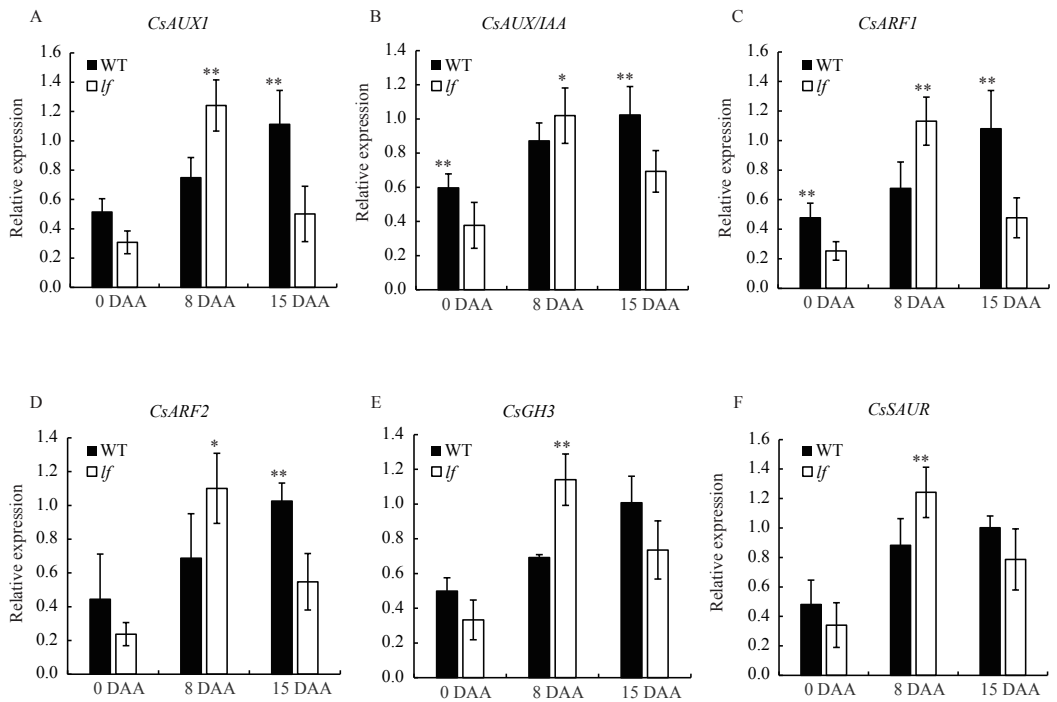

Figure 7. Differentially expressed genes in auxin signaling between the *lf* mutant and WT fruits. Expression of auxin signaling genes (*CsAUX1*, *CsAUX/IAA*, *CsARF1*, *CsARF2*, *CsGH3* and *CsSAUR*) in the fruits of the *long fruit* (*lf*) mutant and WT plants at 0 DAA, 8 DAA and 15 DAA. Three biological replicates were performed for each select gene. *indicates a significant difference between the *lf* mutant and WT at the 0.05 level, and **indicates a significant difference between the *lf* mutant and WT at the 0.01 level. Vertical bars represent standard deviation.

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Contribution

L.W. and Z.R. conceived and designed research. CX.C. provided plant material (wide type). Y.H., X.D., Y.Z., R.T., J.L. and CH.C. performed the experiments. Y.H., Y.Z., X.D. and L.W. analyzed the data. X.D., L.W and Z.R. wrote the manuscript. Y.H. and X.D. had contributed equally to this manuscript.

Conflict of interest

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Abbreviations

CO_2	carbon dioxide
CRF	cytokinin response factor
DAA	days after anthesis
DAS	days after sowing
DBA	days before anthesis
DPP	days post pollination
EMS	ethyl methanesulphonate
FD	fruit diameter
FL	fruit length
FNL	fruit neck length
FW	fresh weight
HP	histidine phosphotransfer protein
lf	*long fruit*
QTL	quantitative trait locus
RIL	recombinant inbred line
RR	response regulator
SEM	scanning electron microscopy
WT	wild type

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