Relationship between Key Environmental Factors and the Architecture of Fruit Shape and Size in Near-Isogenic Lines of Cucumber (Cucumis sativus L.)

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Abstract: Fruit shape and size are complex traits influenced by numerous factors, especially genetics and environment factors. To explore the mechanism of fruit shape and size development in cucumber, a pair of near-isogenic lines (NIL) Ln35 and Ln37 were used. The fruit length and diameter, cell length and diameter, and related gene expression were measured. Both the fruit length, diameter, and cell length and diameter showed sigmate curves in the two lines. The cell length and diameter were significantly positively correlated with fruit length and diameter both in two lines. The expression of CsACS2 and CsLNG showed significant positive correlations with fruit length and diameter increment in Ln35, and there was no correlation in Ln37. Furthermore, there were significant positive correlations between fruit size and thermal effectiveness (TE), as well as between fruit size and photosynthetic active radiation (PAR), both in two lines. Two models using logistic regression were formulated to assess the relationships among fruit length and diameter in Ln35 and Ln37, respectively, based on thermal effectiveness and photosynthetic active radiation (TEP). The coefficient $R^2$ values of the models were 0.977 and 0.976 in Ln35, and 0.987 and 0.981 in Ln37, respectively. The root mean square error (RMSE) was 12.012 mm and 4.338 mm in Ln35, and 5.17 mm and 7.082 mm in Ln37, respectively, which illustrated the accurate and efficient of these models. These biologically interpreted parameters will provide precision management for monitoring fruit growth and forecasting the time of harvesting under different temperatures and light conditions.

Keywords: cucumber; fruit shape and size; fruit cell; thermal effectiveness; photosynthetic active radiation; logistic regression

1. Introduction

In recent years, fruity vegetable production in greenhouses has played a very important role in the development of the regional economy. Fruit is the main product of fruity vegetables, and it is also an important factor in appealing to consumers. Consumer preferences were determined greatly by the fruit appearance quality to some extent [1]. The cucumber (Cucumis sativus L.) is one of the most important fruity vegetables mainly cultivated in the greenhouse, and diversification of fruit shape can meet the requirement of consumers to more varieties and specialization with the improvement of living standards; in addition, the appropriate size of fruits will add additional value of products [2]. However, fruit shape and size were greatly influenced by numerous factors, especially temperature, light, and humidity conditions [3].

During cucumber fruit development, the range of cell expansion determined the final cell size of the fruits, which was considered as the major determinant factor for fruit size [4], while the cell number also exhibited a positive correlation with the growth of fruit length and diameter [5–7]. Both cell size and number altered the fruit shape and size in cucumber [8,9]. However, the mechanism that determined the fruit cell number and size was still unclear. Related genes that participated in the regulation of cucumber...
fruit shape and size were considered important factors. CsLNG was a homologous gene of LONGIFOLIA1 (LNG1), which regulated slender leaves in Arabidopsis, and was considered as the candidate gene of cucumber fruit length QTL loci FS2.1 [10,11]. Researchers reported that the CsLNG regulated cucumber fruit length by controlling the cell numbers using NILs with CsLNG genetic background [4]. CsSUN gene encoded IQ-domain protein of IQD gene family controlled cucumber fruit length growth by influenced the fruit cell numbers [4,10]. Enzymes, transcription factors and proteins were reported to involve in the regulation of fruit size [12]. For example, dysfunction of CsACS2 reduced ethylene production and repressed cell division, and resulted in shorter fruit in cucumbers [13]. Knockdown of the CsFUL1 transcription factor caused further fruit elongation and larger cell size than in wide-type lines [8]. Furthermore, CsSUP, which was regulated by CsFUL1A, participated in the reduction in fruit length and smaller and disorganized cells compared with the wide-type line [8]. CsFNL7.1, which encoded a late embryogenesis abundant protein, was considered to control cucumber fruit neck length via modulated fruit cell expansion [4]. There were some major gene loci to regulate the formation of cucumber fruit shape together with some others [14–17].

Environmental factors were the main external factors that affected fruit shape and size the most. It was believed that temperature and light were two important factors in fruit development [18]. The key environmental such as temperature, light intensity, and irrigation played important roles in fruit growth [19]. Fruit expansion rates of apples were affected by the daily temperatures, and smaller fruit were produced in low-temperature conditions [20]. The largest pepper fruits were obtained under optimal light and temperature conditions, and small fruits were obtained at low temperatures [21]. Higher fresh weight was obtained in high light intensities conditions for tomatoes [22,23]. Supplemental lighting, as an important light-controlling technology, was widely used in the production of fruity vegetables under the protection structure [24,25]. In addition, obviously, solar radiation and temperature played important roles in cucumber fruit architecture, development, and fruit shape. It is essential to regulate environmental factors to form better fruit size and shape to increase the additional value of products [26]. However, it was unclear whether to regulate fruit shape and size through precision management of environmental factors, especially the most important factors, temperature and light. Our findings suggest that the proposed workflow aids in the identification of key metabolites in the central metabolism that responds to monochromatic red-LED treatment and contributes to increasing the fruit size of tomato plants.

The logistic regression method was helpful to understand the development of fruit size and shape [27,28]. Models of effective fruit growth benefited the upgrades of management of key environmental factors [29]. The effects of temperature and light on the architecture of fruit shape were always comprehensive. However, the simulation models established were mainly focused on a single environment, such as light or temperature [30,31]. Recently, thermal effectiveness and photosynthetic active radiation (TEP) were considered as comprehensive parameters that played the important roles in the development of crops [32,33]. The TEP-based models provided a more accurate method to forecast the dynamics of plant growth [32].

In order to explore a clearer mechanism of regulation in cucumber fruit architecture, provide precision management, and exploit the full potentials quality of certain cultivars, the relationship among the architecture of fruit size, cell size, gene expressions, and the environmental factors in cucumber near-isogenic lines (NIL) were probed, logistic regression between fruit size and temperature and light were analyzed. It will provide some basis for precise management through the management of temperature and light.

2. Results

2.1. Changes in the Key Environmental Factors in the Plastic Tunnel

The environmental temperature and light intensity in the plastic tunnel were showed in Figure 1. The average daily light intensity ranged from 3.52 Klux to 38.40 Klux, increased
from 26 May to 31 May, and decreased from 4 June to 15 June. The average daily air temperature ranged from 22.45 °C to 27.97 °C. The highest temperature appeared at 14:00 o’clock on 3 May, which is 43.25 °C. The lowest temperature appeared on 28 May 2020 at nighttime and was 11.55 °C. Both the accumulation of RTE and accumulation of PAR showed linear patterns from 23 May to 24 June 2020.

Figure 1. Changes in temperature and light intensity in the plastic tunnel.

2.2. Changes in Fruit Shape and Size during the Fruit Development

The changes in fruit length and diameter of Ln35 and Ln37 were measured, and the fruit shape index was calculated (Figure 2). It showed that the most significant difference in fruit shape between Ln35 and Ln37 was observed at 3 dpa (Figure 2A,B). The growth dynamics of fruit length in both Ln35 and Ln37 showed sigmate curves, and it increased rapidly from 3 dpa (Figure 2C), and then the increase in fruit length in Ln35 was faster than that in Ln37, and the length of Ln35 was 1.94–2.58 times of Ln37 from 0 dpa to 18 dpa, which represented the ovary stage, picked stage and mature stages. The growth dynamics of fruit diameter in both Ln35 and Ln37 also showed sigmate curves (Figure 2D), and there were no significant differences between the fruit diameter of both Ln35 and Ln37 before 6 dpa. The fruit diameter of Ln37 had a rapid increase at 6 dpa, and it was significantly larger than that in Ln35 from that time on. There was also a significant difference in fruit shape index between the two lines from −12 dpa to 18 dpa. The fruit shape index of Ln35 ranged from 1.78 to 3.78 and the fruit shape index of Ln37 ranged from 1.02 to 1.44.
Figure 2. Changes of fruit shape during fruit development in two NILs lines. (A, B) is the dynamic changes of fruit in Ln35 and Ln37, respectively. (C–E) was the dynamic changes in fruit length, fruit diameter and fruit shape index in the two lines.

2.3. Changes of Fruit Cell Shape in NILs

The fruit cell length, diameter, and cell density of Ln35 and Ln37 were observed and showed in Figure 3. The growth dynamics of cell length and cell diameter in the fruit of Ln35 and Ln37 showed sigmoid curves and it increased rapidly from the 3 dpa (Figure 3A,B). From then on, the fruit cell length and diameter increased rapidly before 18 dpa. The cell length of Ln35 at 18 dpa was 7.62 times of that at 3 dpa, the cell length of Ln37 at 18 dpa was 5.89 times of that at 3 dpa, the cell diameter of Ln35 at 18 dpa was 5.78 times of that at 3 dpa, and the cell diameter of Ln37 at 18 dpa was 5.45 times of that at 3 dpa, indicating that the cell length increased more fastly in Ln35 than that in Ln37 during this period, whereas the cell diameter increased similarly in both lines.

There was a significant difference in cell size between the two lines from 6 dpa to 18 dpa. At 6 dpa, the cell length and diameter of Ln37 fruit were significantly longer than that of Ln95. At 18 dpa, the fruit of Ln97 had a decreasing trend, which resulted in shorter cell length and diameter than that of Ln95. The cell density in Ln35 and Ln37 decreased rapidly from −15 to 6 dpa, while slowly decreasing were observed from 9 to 18 dpa. The rapidly increasing of fruit length and diameter from 3 to 12 dpa was mainly caused by both the rapidly increasing of the cell length and diameter, which implied that cell expansion plays important role in fruit size changes from 3 to 12 dpa.
Figure 3. Fruit mesocarp cells shape in NILs. (A–C) are the changes in cell length, cell diameter, and cell density in NILs, respectively. (D) is the dynamic cellular changes of fruit cells in Ln35, (E) is the dynamic cellular changes of fruit cells in Ln37.

2.4. The Relative Expression of Genes Related to Fruit Shape

The gene expressions of CsSUN, CsFNL, CsSUP, CsACS2, CsFUL, and CsLNG were examined during fruit development in Ln35 and Ln37 (Figure 4). CsSUN, CsFNL, and CsSUP had similar expression patterns in both lines during fruit development. They exhibited a high level at pre-anthesis stages and decreased to a low level after 0 dpa, except that the CsSUN gene had a higher expression level at 12 dpa in Ln37 than that in Ln35. Whereas CsACS2, CsFUL, and CsLNG had different expression patterns in two lines. The expression of CsACS2 peaked at 6 dpa in Ln37, while it had a rather low expression level in Ln35. The expression pattern of CsFUL was similar to that of CsACS2, which peaked before 0 dpa in Ln37, while its expression was rather low in Ln35. The CsLNG gene had a significantly high expression level in Ln35, while it had a rather low expression level and exhibited little change in Ln37. The expression level of CsLNG increased rapidly from 0 dpa to 9 dpa in Ln35 and peaked at 9 dpa, which was 8.2 times Ln37 at 9 dpa. The difference in CsLNG expression in two lines indicated that CsLNG might play an important role in the increment of fruit length of Ln35.
The cell density value was determined by cell size and cell number. However, the fruit shape index had negative correlations with cell length and cell diameter in Ln37, and it had no significant correlation in Ln35. Moreover, the fruit shape index was negatively correlated with cell density in Ln35 and no significant correlation in Ln37. These results implied that the cell number might contribute to the difference in fruit shape index between Ln35 and Ln37.

2.6. The Relationship between Gene Expression and Fruit Increment

The relationships between fruit shape increment and gene expression and fruit cell increment and gene expression in both lines were analyzed (Table 1). There was a significant positive correlation between fruit CsLNG expression and fruit length increment in Ln35, and it had no significant correlation in Ln37. As well as, CsACS2 expression significantly positively correlated with fruit length increment and fruit diameter increment in Ln35, and
it had no significant correlation in Ln37. The other four genes, CsFLU, CsSUN, CsFNL, and CsSUP, showed no significant correlations in both two lines. In addition, CsLNG expression significantly positively correlated with cell diameter increment in Ln35, and it had no significant correlation in Ln37. CsACS2 expression is significantly positively correlated with cell length increase in Ln37. CsFLU, CsSUN, CsFNL, and CsSUP showed no significant correlations with cell increment in both two lines. These results indicated that CsLNG and CsACS2 genes might be hereditary factors for the difference in cell size and fruit size between Ln35 and Ln37. CsLNG and CsACS2 contributed to fruit elongation by affecting the cell size.

2.7. The Correlation between Key Environmental Factors and Fruit Shape and Size Increment

For fruit length and diameter, which were the cumulative variables and cannot be explored, the relationship with environmental factors at one time point. Thus we analyze the relationship between the product of thermal effectiveness (TE) and fruit shape and size traits and between the photosynthetic active radiation (PAR) and fruit shape and size traits. The results are exhibited in Table 2. Both the TE and PAR were significantly positively correlated with fruit length and fruit diameter in the Ln35 and Ln37. The results indicate that the TE and PAR values influenced the fruit size.

Table 2. The correlation analysis between fruit shape and TE and PAR.

|                  | Ln35  | Ln37  |
|------------------|-------|-------|
| **Fruit Length** |       |       |
| TE               | 0.967 ** | 0.969 ** |
| PAR              | 0.918 ** | 0.924 ** |
| **Fruit Diameter** |   |       |
| TE               | 0.96 **  | 0.953 ** |
| PAR              | 0.907 ** | 0.899 ** |

Asterisks indicate statistical difference of the values at $p < 0.01 (**).$

Two-way analysis of variance (ANOVA, Table S2) was used to determine the effect of cucumber materials and seasons on fruit length, diameter, and fruit shape index. There were no significant interactions found between cucumber materials and seasons on fruit length and fruit diameter. Ln35 has a significantly longer fruit length and a larger fruit shape index value than Ln37. However, there was significant interaction found between cucumber materials and seasons on the fruit shape index. Furthermore, there was no significant effect of season on fruit length, diameter, and fruit shape index. These results suggest that the autumn of 2020 fruit shape data can be used to validate the models that model in the spring of 2020. Then, there were significant differences in fruit shape development between Ln35 and Ln37, which need to be represented by different fruit shape development models.

2.8. Logistic Regression of the Fruit Shape Based on TEP

To probe the relationship between fruit shape traits and environment, the product of thermal effectiveness and photosynthetic active radiation (TEP) value was adopted to probe the relationship of light and temperature with cucumber fruit length, diameter, and volume.

The logistic equation was performed using Formulas (1)–(4). The results are shown in Figure 5 and Table 3. The fruit length and diameter increased slowly when the TEP was lower than 20 MJ/m$^2$ in both Ln35 and Ln37. Fruit length increased rapidly when the TEP was between 20 MJ/m$^2$ and 40 MJ/m$^2$. Both Ln35 and Ln37 had maximum growth rates when the TEP was about 31.2 MJ/m$^2$. However, there were significant differences between fruit length in the two lines. Ln35 increased by 17.42 mm per day, while Ln37 increased by 7.46 mm per day at the maximum growth rate time. The TEP accumulated from 20 MJ/m$^2$ to 40 MJ/m$^2$, and the fruit length increased by 143.68 mm in Ln35 and 49.31 mm in Ln37. When TEP was higher than 40, the growth speed of fruit length slowed down.
when the TEP was about 31.2 MJ/m². However, there were significant differences between fruit length in the two lines. Ln35 increased by 17.42 mm per day, while Ln37 increased by 7.46 mm per day at the maximum growth rate time. The TEP accumulated from 20 MJ/m² to 40 MJ/m², and the fruit length increased by 143.68 mm in Ln35 and 49.31 mm in Ln37. When TEP was higher than 40, the growth speed of fruit length slowed down.

Figure 5. Relationship between fruit shape and TEP under Ln35 and Ln37. (A) is the model of fruit length in Ln35; (B) is the model of fruit diameter in Ln35; (C) is the model of fruit length in Ln37; (D) is the model of fruit diameter in Ln37.

Table 3. Logistic regression equations of fruit shape for two inbred lines.

| Fruit Traits   | Model                                      | $R^2$ | F     | P     | RMSE |
|---------------|--------------------------------------------|-------|-------|-------|------|
| Fruit length  | Ln35 $y = 1/(0.005 + 0.930 \times 0.832^x)$ | 0.977 | 205.866 | 0     | 12.012 |
|               | Ln37 $y = 1/(0.013 + 0.991 \times 0.856^x)$ | 0.976 | 200.464 | 0     | 5.17  |
| Fruit diameter| Ln35 $y = 1/(0.016 + 1.384 \times 0.863^x)$ | 0.960 | 117.786 | 0     | 4.338 |
|               | Ln37 $y = 1/(0.014 + 1 \times 0.859^x)$    | 0.987 | 422.541 | 0     | 7.082 |
| Fruit volume  | Ln35 $y = 1/(0.002 + 522.51 \times 0.708^x)$ | 0.981 | 261.447 | 0     | 43.07 |
|               | Ln37 $y = 1/(0.004 + 593.002 \times 0.712^x)$ | 0.987 | 418.87  | 0     | 19.19 |

Fruit diameter increased rapidly when the TEP was between 20 MJ/m² and 42 MJ/m² in both lines. Similar to fruit length, the maximum growth rate of fruit diameter is the time when TEP was accumulated to 31.2 MJ/m² in both Ln35 and Ln37. At this time, Ln35 increased by 4.76 mm per day, while Ln37 increased by 7.34 mm per day. The TEP accumulated from 20 MJ/m² to 42 MJ/m², and the fruit diameter increased to 41.80 mm in Ln35 and 47.57 mm in Ln37. When TEP was higher than 42 MJ/m², the growth speed of fruit diameter slowed down both in Ln35 and Ln37.

$R^2$ and RMSE were tested to determine if these models were suitable or not for the growth of fruit shape in Ln35 and Ln37. It showed that the $R^2$ of fruit length and fruit diameter were 0.977 and 0.960 in Ln35, and 0.976 and 0.987 in Ln37, respectively. RMSE
was 12.012 mm and 0.263 mm in Ln35, and 5.17 mm and 7.082 mm in Ln37, respectively. It indicated that the models of fruit length and diameter based on the TEP in both Ln35 and Ln37 were accurate and efficient (Table 3). These models can provide an accurate harvesting time, which is helpful information for productive behavior.

### 2.9. Validation of the Model

The data of fruit length and diameter of Ln35 and TEP during fruit development were collected in 2020 autumn to verify the models (Table 1). It was found that the determination coefficient $R^2$ was 0.930 and 0.891 in fruit length and diameter, respectively (Figure 6). The value of RMSE in fruit length and diameter during development in 2020 autumn was 10.01 mm and 0.263 mm, which meant suitable conformity between predicated value and measured values in fruit length and diameter, and these models of fruit shape traits were reasonable representations for the actual system.

![Figure 6. Comparison between predicted and measured values of cucumber fruit length (A) and diameter (B).](image)

### 3. Discussion

#### 3.1. Cell Division and Expansion in Fruit Shape and Size Morphogenesis

The appearance quality of cucumber fruit not only enhanced the aesthetic degree but also improved its commodity values. Previous studies have been carried out on various cucumber lines for fruit and cell growth [5–7,34]. These results showed that cell division occurs before anthesis and continues for about 3-6 days after anthesis in cucumber fruit, remaining more or less constant in further growth. Cell expansion occurs after cell division or at the end of cell division stages and with rapid fruit growth [5,6,34]. However, many of the prior studies on cucumber fruit development have examined growth post pollination or the ovaries’ approach to anthesis [35,36]. Here, we explored the morphological and cellular changes during fruit development, which ranged from −15 dpa to 18 dpa in two CsLNG cucumber NILs. Consistent with earlier work [5,6], cell size had changed little before 3 dpa, implying the fruit length increased by cell division at the early stages. The peak of fruit length and diameter increase in Ln35 and Ln37 coincided with the peak of cell expansion from 3 dpa to 15 dpa, indicating that cell expansion significantly promoted the fruit size increase in the two lines. There was no difference in fruit cell size and shape between Ln35 and Ln37 before the 6 dpa point. However, a greater difference in fruit length and fruit shape index between Ln35 and Ln37, and the difference became apparent at −15 dpa. These results implied that the differences in fruit length and shape index before 6 dpa in two lines were influenced by cell division. After 6 dpa, it exhibited a consistently greater difference between Ln35 and Ln37 in fruit diameter and cell shape index. Ln35 fruits diameter and cell shape index were larger than Ln37. It revealed that the difference of NILs in fruit diameter after 6 dpa was influenced by fruit cell shape.
3.2. Effects of Genetic and Environmental Factors on Cucumber Fruit Development

The related gene expression can determine the development of fruit appearance quality, especially for fruit shape and size [37]. In cucumber fruit development, the related genes that participated in the regulation of fruit shape and size have been reported for many years [4,10–12]. However, the development of fruit shape and size was regulated by many different genes for different cucumber germplasms.

Environmental conditions would affect the growth of any plant organ under which it develops [38]. The environmental conditions were generally considered temperature regimes, light availability, soil characteristics, water availability, and so on. There have many plant organ growth influenced by the environment have been reported [39–42]. It is an essential factor for plant growth as well as fruit products. During cucumber development, the environmental elements, especially solar radiation and temperature, were regarded as important factors [43,44]. However, research on the relationship between environment and cucumber fruit shape and size development is still lacking.

In this study, a pair of NILs Ln35 and Ln37, which carry gene CsLNG for fruit length QTL loci FS2.1 in cucumber, were adopted to analyze the effects of genetic and environmental factors on cucumber fruit development. The results showed that CsACS2 and CsLNG gene expression were the main internal factors that affected the different fruit shapes in this study between the two lines. The environmental factors were the main external factors that determined the fruit development and maturity period. The correlation analysis showed that the CsACS2 and CsLNG gene expression were not significantly correlated with environmental factors. That is, the gene expression of CsACS2 and CsLNG was not affected by environmental factors. This explains that the effect of environmental factors on fruit development and maturity period had almost no difference between Ln35 and Ln37.

3.3. The Number of Days Needed for the Fruit of Commercial Maturity

The growing number of days needed for the fruit of commercial maturity might be forecasted according to the model. Usually, the average daily light intensity and average daily air temperature in local plastic tunnels were 24.73 °C and 11.86 Klux in the spring and 23.98 °C and 9.02 Klux in the autumn when it was suitable for the production of cucumber. It needed 9.63 day in the spring and 12.56 d in the autumn to reach the suitable length of commercial maturity fruits for Ln35 and Ln37, respectively. It needed 10.69 d and 10.28 d in the spring and 13.96 d and 13.42 d in the autumn to reach the suitable diameter of commercial maturity fruits for Ln35 and Ln37, respectively. It can provide precise management for round-shape cultivars and long-shape cultivars under different environmental conditions.

Generally, cucumber fruits were consumed immaturely, and it needed different times for cucumber fruit from the ovary to grow into a commercial one in the spring and autumn. Based on the average fruit length and diameter at the commercial stage in two lines, it needed 12.47 MJ/m² TEP in Ln35 and 12.86 MJ/m² TEP in Ln37 for the fruit length to grow a suitable size from 0 dpa to the commercial stage. It needed 13.91 MJ/m TEP and 13.75 MJ/m² TEP in Ln37 for the fruit diameter to grow suitable size from 0 dpa to the commercial stage (Supplementary Table S3). In the application of a facility greenhouse, the temperature of cucumber fruit development is often set at 25–32 °C during the daytime and 20 °C at nighttime. For the light intensity, it needed 99.92 Klux in Ln35 and 103.04 Klux in Ln37 for the fruit length to grow suitable size from 0 dpa to commercial stage, and it needed 111.46 Klux and 110.18 Klux light intensity, respectively, for the fruit diameter to grow suitable size from 0 dpa to commercial stage. Under the natural light conditions, it needed 8.43 d and 8.69 d in spring and 9.78 d and 10.09 d in autumn to reach the suitable length of commercial maturity fruits for Ln35 and Ln37, respectively. It needed 9.40 d and 9.29 d in the spring and 10.91 d and 10.78 d in the autumn to reach the suitable diameter of commercial maturity fruits for Ln35 and Ln37, respectively. Supplemental lighting will shorten the time for the fruit to reach commercial maturity. Under the optimum temperature (25–32 °C at daytime and 20 °C at nighttime) and maximum light intensity (50 Klux) conditions for
eight hours in the daytime, it only takes about 6 to 7 days for cucumber fruit growth to harvest stage. Therefore, in cucumber production, artificial heating or additional light supplementation can be used to promote the rate of cucumber fruit growth, shorten the fruit production cycle, and obtain greater economic value for the fruit diameter to grow a suitable size from 0 dpa to commercial stage.

4. Material and Methods
4.1. Plant Materials
A pair of near-isogenic lines (NILs), Ln35 and Ln37, carrying gene CsLNG for fruit length QTL loci FS2.1 in cucumber, were developed from a recombinant inbred lines population (RILs) [10,11,17], which were created by our research group. The Ln35 carrying the wild-type CsLNG gene had a long cucumber shape with a fruit shape index of 3.48 at 12 days post anthesis (dpa), while Ln37 carrying the mutant cslng gene had an ovate shape with a fruit shape index of 1.04 at 12 dpa. No significant difference between these two cucumber lines was found except for the fruit shape (Figure S1).

4.2. Field Experiments
Two inbred cucumber lines, including Ln35 and Ln37, were planted in two seasons. The seeds were sowed and cultured in substrate using 50-hole trays in a culture room, and seedlings with two fully spread true leaves were transplanted in a plastic tunnel at the Horticulture Experimental Station (34°16′ N, 108°4′ E) of the College of Horticulture, Northwest A&F University, Yangling, Shaanxi Province, China, 5 April 2020 in spring, and 4 August 2020 in autumn. A total of 3 replicates were grown for each inbred line, and 64 cucumber plants per replicate were included. Six rows were designed as the protection rows on both sides of the experiment site. The area of every plot was 4 × 2.4 m² (length × width). The seedlings were planted on a plain rectangular pieces according to wide/narrow row alternation in which wide row spacing was 70 cm and narrow row spacing was 50 cm, and the plant spacing was 25 cm. One drip irrigation pipe was laid along the ridge before transplanting. A total of 64 seedlings were planted in each plot. A total of 10 m³ water per 667 m² was irrigated to the plants from transplanting to recovering of seedlings. Fertilizers were applied five times with watering at the stage 10 days after transplanting, 20 days after transplanting, 50 days after transplanting, and 70 days after transplanting according to the growth characteristics of cucumber plants [45,46], which were shown in red font in the manuscript.

Two-time pre-experiments in the spring and autumn of 2019 were performed separately, in which we carefully observed the ovary size changes and the corresponding days before and after anthesis. Fruit sizes were measured from the beginning of the ovaries appearance until to the day of the fruit anthesis, and the corresponding days of fruit development were all recorded. The data were recorded and provided the reference for the formal statistics of fruit sizes before anthesis in 2020. The pre-experiment in 2019 provided us with a rather clear number of days about the approximate fruit sizes in autumn and spring 2020.

In the formal experiments, field experiments were conducted in two seasons, and one was performed from the early April to the late of June in 2020, and the other one was carried out from the early August to the late October in 2020. The data about fruit shape in spring were collected from the mid-May to the late of June, and which in autumn were collected from the early September to the late September. The data from the spring of 2020 were used as the model establishment and the data from the autumn of 2020 were used as the model verification.

To observe the fruit growth dynamics during the development, two hundred ovaries in Ln35 or Ln37 plants were marked once ovaries appeared at about the 14th node on 22 May 2020 and 3 September 2020. The ovaries with opening female flowers at the flowering day were selected and marked as 0 days post anthesis (0 dpa), and the ovaries with unopened female flowers before 0 dpa were selected and marked according to the number of days
away from 0 dpa, which were recorded as $-15$, $-12$, $-9$, $-6$, and $-3$ day pre-anthesis ($-15$, $-12$, $-9$, $-6$, and $-3$ dpa, respectively), and the fruits after the flowering day were selected and marked according to the number of days away from 0 day post anthesis were recorded as 3, 6, 9, 12, 15, and 18 days post anthesis (3, 6, 9, 12, 15, and 18 dpa, respectively). The fruit shape was measured every three days, along with the development of the fruit. When the fruits developed until the day of anthesis, the values of the fruit shape before anthesis were obtained. Excluding the data of fruits that did not flower on 6 June 2020 and 18 September 2020, the measurement and counting of the fruit shape after anthesis was continued to obtain the fruit shape data after flowering.

The marked ovaries were measured carefully at 9:00–10:00 o’clock in the morning at $-15$, $-12$, $-9$, $-6$, $-3$, 0, 3, 6, 9, 12, 15, 18 dpa. The uniform and disease-free fruits were collected at 9:00 in the morning and transported to the laboratory immediately in the icebox. The fruits were divided into 3 parts: one part was stored at $-80 \degree$C, the other part was used for RNA extraction, and the third part was used for paraffin section analysis.

4.3. Methods
4.3.1. Data Collection about the Key Environmental Factors

The data of aerial temperature and light intensity were collected using the automatic environmental recorder (YM-18, Changmeng Electronic Science and Technology Limited Company, Handan, Hebei, China). The data were recorded automatically for every ten minutes. The aerial recorders were put on the place of the 2/3 plant height of cucumber plants. The daytime was defined from 07:00 to 19:00 and the nighttime was defined from 19:00 to 07:00 of the next day according to the local time of sunrise and sunset.

4.3.2. Measurement of Fruit Length, Diameter and Volume

Fruit length and fruit diameter were used to describe the fruit’s shape and size. To observe the dynamic changes of fruit shape and size in NILs, cucumber fruits were measured at $-15$, $-12$, $-9$, $-6$, $-3$, 0, 3, 6, 9, 12, 15, and 18 dpa. The fruit length and diameter were measured using a digital caliper very carefully. The fruit shape index was the ratio of fruit length to diameter. The fruit length of the cucumber was the cumulative variable, which was the sum of fruit length elongation on the $i$-th day and the length on the $i-1$ of the previous day. As well as the fruit length and diameter were cumulative variables in cucumber. The fruit length increment and fruit diameter increment were adopted in this study, and every three days were calculated.

4.3.3. Measurement of Cell Size during Cucumber Fruit Development

To observe the changes in fruit cell size during fruit development. Fruit cell length and fruit cell diameter were described as the fruit cell shape or size. The fruit longitudinal paraffin sections of $Ln35$ and $Ln37$ were prepared according to Guidarelli et al. [47]. The whole fruit of $-9$, $-6$, and $-3$ dpa, respectively, were used for embedding, and the fruits of 0, 3, 6, 12, 15, and 18 dpa were chopped into about 0.5 cm$^2$ in the middle of the mesocarp and immediately fixed in 70% FAA solution. The tissues were then transferred into a graded ethanol series (10–100%) and chloroform series (10–100%) and embedded in paraffin. Then the samples were cut into 8 µm thick sections and, stained with safranine, sealed with gum. The sections were observed under an OLYMPUS BX51 microscope (OLYMPUS, Tokyo, Japan).

The cell size of fruits at different development stages was measured, and the cell density was counted in a 1 mm$^2$ section using the software of Image J (National institutes of health, Bethesda, MD, USA). Five sections were selected randomly and measured. Five different fruits from different plants were used for every biological replication, and the experiment was repeated three times.
4.3.4. qRT-PCR

The expression patterns of key genes related to the architecture of fruit size and shape in cucumbers were analyzed with quantitative real-time PCR. The primers of the six genes were provided in Supplementary Table S1. The six genes associated with the formation of fruit shape were chosen, including CsSUN, CsLNG, CsFUL, CsSUP, CsACS2, and CsFNL. RNA was extracted from the fruits of RILs at −9, −6, −3, 0, 3, 6, 12, 15, and 18 dpa using AG RNAex Pro Reagent (Accurate Biology, Hunan, China). The cDNA fragments were synthesized using the RNA by Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany). The qRT-PCRs were performed using SYBR Green Master Mix (GeneStar, Beijing, China) with 20 µL reaction system on a QuantStudio5 (Life Technologies, Gaithersburg, MD, USA), and the data were analyzed using the \(2^{-\Delta\Delta CT}\) method and normalized by ubiquitin [48].

4.3.5. Calculation of the Accumulated TEP

Thermal effectiveness and photosynthetic active radiation (TEP) were the product of thermal effectiveness (TE) and photosynthetic active radiation (PAR). TE was the accumulated value of relative thermal effectiveness (RTE), and RTE was proportional to actual temperature to optimal temperature under plant growth conditions. RTE was calculated according to Hang et al. [49] as follows:

\[
RTE(T) = \begin{cases} 
0 & (T < T_b) \\
\frac{T - T_b}{T_{ab} - T_b} & (T_b \leq T < T_{ab}) \\
\frac{T_{ab} - T}{T_{ab} - T_{ou}} & (T_{ab} \leq T < T_{ou}) \\
0 & (T > T_{ou}) 
\end{cases}
\]  

(1)

where \(T\) corresponded to the mean of actual temperatures in one hour, \(T_b\) was the lower limit temperature of the cucumber fruiting period, \(T_{ab}\) was the lower limit of optimum temperature in the cucumber fruiting period, \(T_m\) was the upper limit temperature, and \(T_{ou}\) was the upper limit of optimum temperature in cucumber fruiting period. The \(T_b\), \(T_{ab}\), \(T_{ou}\), and \(T_m\) temperatures in the cucumber fruiting period were defined at 16 °C, 25 °C, 32 °C, and 40 °C, respectively.

PAR (MJ/(m² × d)) was the total solar radiation with a 0.5 photosynthesis coefficient in the solar light during one day [50]. The daily TEP (DTEP) was calculated according to Hang et al. [49] as follows the formula:

\[
DTEP(i) = \left(\frac{\sum RTE(i,j)}{24}\right) \times PAR(i) \quad (j = 1, 2, 3 \ldots, 24)
\]  

(2)

where \(DTEP(i)\) was the accumulated TEP on the \(i\)th day, \(RTE(i,j)\) was the RTE in the \(j\)th hour on the \(i\)th day, and \(PAR(i)\) was the PAR on the \(i\)th day. TEP (MJ/m²) was the accumulation of RET and PAR supply in the cucumber fruit growth period, which was calculated according to Formula (3):

\[
TEP = \sum_{i=1}^{N} DTEP(i)
\]  

(3)

In this study, TEP was calculated from −18 days pre-anthesis, which was regarded as the smallest fruit development stage visually and cannot be measured by vernier caliper.

4.3.6. Statistical Analysis

Data from this study were subjected to statistical analysis, and significance tests were performed using one-way ANOVA analysis with Duncan’s multiple range test (\(p < 0.05\)). The significant difference in data was presented by an asterisk. Logistic regression was employed to analyze the relationship between the environmental TEP and fruit shape, including fruit length, diameter, and volume. The root mean squared error (RMSE) was
conducted to analyze the conformity of the predicted value and measured value. RMSE values were calculated according to Nijssen [51] and Formula (4):

\[
RMSE = \sqrt{\frac{\sum_{i=1}^{n} (OBSi - SIMi)^2}{n}}
\]  

(4)

where \(OBSi\) was the measured value, \(SIMi\) was the predicted value, and \(n\) was the sample number. Linear regression was used to verify the models between the predicted value and measured value using 2020 autumn data. Model development and mathematical data analysis were performed by SPSS 22.0 (Statistical Package for the Social Sciences, Chicago, IL, USA) on Windows PC. The histograms were generated using Graph Pad Prism 6 (Graph Pad Software Inc., San Diego, CA, USA).

5. Conclusions

The study presented the relationship between fruit shape and size increment, cell size increment, and gene expression on the growth and development of a pair of CsLNG NIL cucumber lines. The correlations analysis revealed that cell number might contribute to the difference in fruit shape index between \(Ln35\) and \(Ln37\), and CsLNG and CsACS2 genes might act as the hereditary factors for the difference in cell size and fruit size between the two lines. The significant correlations between TE and fruit size and between RAC and fruit size indicated a potential capacity to apply mathematical models to predict cucumber fruit size development. Four models via logistic regression analysis were formulated, and the models were well validated using next year’s fruit size data and environmental data.

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Abbreviations

TEP thermal effectiveness and photosynthetic active radiation
QTLs quantitative trait locus
Dpa days post anthesis
NIL near-isogenic line
TE thermal effectiveness
PAR photosynthetic active radiation
RMSE root mean squared error
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