Inheritance and quantitative trait locus analysis of low-light tolerance in cucumber (Cucumis sativus L.)

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ABSTRACT. The low-light tolerance index was investigated in a set of 123 F₂:₃ lines during the seedling stage across 2 seasons, and the heredity of low-light tolerance was assessed via different genetic analysis methods. The results of the classical analysis showed that low-light tolerance is controlled by an additive-dominant polygene, and the polygenic inheritance rate of separate generations was >30%. In addition, 5 quantitative trait loci (QTLs) exhibited a low-light tolerance index across both seasons, including 2 QTLs (Llti1.1 and Llti1.2) on the 1st linkage group (variances of 6.0 and 9.5%) and 3 QTLs (Llti2.1, Llti2.1, and Llti2.1) on the 2nd linkage group (variances of 10.1-14.0%). The classical analysis method and QTL information on the heredity of low-light tolerance showed that it is controlled by several major genes and a mini-polygene. The results
will facilitate the breeding of resistance to low-light stress in cucumber.

**Key words:** Cucumber (*Cucumis sativus* L.); Low-light tolerance; Seedling; Classical genetic analysis; QTLs

**INTRODUCTION**

Cucumber (*Cucumis sativus* L.) is one of the most important vegetable plants grown worldwide, and increasing yields from cucumber plants are necessary. However, low-light intensity is a stressor that leads to major yield losses in northern regions. Plants under low-light stress exhibit slower growth rates, higher fruit abscission rates, and declines in production (Liebig and Krug, 1991). The low-light tolerance of plants is a complex trait, and the mechanisms for expression and/or inheritance are not well understood.

Several attempts have been made to apply both physiological and morphological techniques in cucumber to develop effective screening methods for low-light tolerance (Ma et al., 1997). The tolerance index is a direct evaluation method in plants; for example, the chilling injury index is obtained by classifying the chilling injury level and is used to evaluate chilling tolerance (Liu et al., 2009). The low-light tolerance index is regarded as the most important and intuitive index evaluated (Li et al., 2009a); thus, it is crucial in understanding the genetic characteristics related to the heredity of low-light tolerance in cucumber. Considerable work has been dedicated to breeding large numbers of cucumber cultivars with high yield and quality. There have been many studies investigating the traits related to low-light stress in cucumber, including genetic reports (Li et al., 2009b), physiological research (Li et al., 2006), and quantitative trait locus (QTL) analyses of the chlorophyll content in cucumber seedlings under low-light stress (Li et al., 2010). Moreover, an additional 5 QTLs related to leaf area growth in cucumber seedlings have been detected under low-light stress, including *la-1*, *la-2*, *la-3*, *la-4*, and *la-5* (Zhang et al., 2004). However, there have been no reports on low-light tolerance, and it is necessary to conduct heredity and QTL analyses of low-light tolerance in cucumber.

Recent technological advances have accelerated the development of genetic mapping and QTL analyses in several crop species to include the QTL detection of important horticultural traits (Li et al., 2008), resistance to powdery mildew in cucumber (Liu et al., 2008), stalk tunneling in maize (Krakowsky et al., 2004), and bacterial leaf streak in rice (*Oryza sativa* L.; Tang et al., 2000). For example, the location of *F* and *de* was identified by genetic linkage and associated with the simple sequence repeat (SSR) loci *CSWCT28* and *CSWCTT14* at 5.0 and 0.8 cM, respectively (Fazio et al., 2003). Four QTLs for powdery mildew resistance have been identified (i.e., *pm1.1*, *pm2.1*, *pm4.1*, and *pm6.1*; Liu et al., 2008). A total of 36 QTLs controlling collateral characteristics have been reported, and the total phenotypic variation was 3.1-32.3% (Zhang et al., 2010).

A limited number of QTLs related to low-light tolerance have been reported (Li et al., 2010). In this study, we analyzed the hereditary and QTL effects via classical genetic and mapping methodologies in order to describe, in depth, the genetic characteristics and identify the QTLs related to low-light tolerance. The identification of QTLs for low-light tolerance traits may be useful in marker-assisted breeding of cucumber cultivars.
MATERIAL AND METHODS

Plant materials

The cucumber low-light stress-tolerant line M\textsubscript{22} was crossed with the low-light sensitive line M\textsubscript{14}. M\textsubscript{22} grew normally and exhibited acclimation when exposed to low-light stress. In contrast, M\textsubscript{14} was a shorter plant with severe abnormalities. The parents were selected after screening a large number of cucumber germplasms native to northern China, southern China, and Europe. The generations F\textsubscript{1}, B\textsubscript{1}, and B\textsubscript{2} were obtained from the parents. The F\textsubscript{1} generation obtained from the crossing of M\textsubscript{22} and M\textsubscript{14} was self-pollinated to produce 152 F\textsubscript{2} progeny, which were then self-pollinated by single-seed descent to obtain 123 F\textsubscript{2:3} families.

Field evaluation and plant characteristics

The 2 parental lines (i.e., M\textsubscript{22} and M\textsubscript{14}), their F\textsubscript{1} generation, and the F\textsubscript{2:3} families were evaluated in a greenhouse at the Horticulture School, BaYi Agricultural University, Daqing, China. The F\textsubscript{2:3} families were grown in 2 seasons, including the autumn of 2012 (A) and spring of 2013 (S). The soil media was comprised of 25, 25, and 50% peat, cinder, and perlite, respectively. The 6 generation groups of P\textsubscript{1}, P\textsubscript{2}, F\textsubscript{1}, B\textsubscript{1}, B\textsubscript{2}, and F\textsubscript{2}, and the F\textsubscript{2:3} families were arranged in a randomized complete block design with 3 replications. Each replicate of P\textsubscript{1}, P\textsubscript{2}, and F\textsubscript{1} had 10 plants; there were 20 plants for B\textsubscript{1} and B\textsubscript{2} and 40 plants for F\textsubscript{2} and the F\textsubscript{2:3} families. The individual plants were planted in a bowl (base area: 8 x 8 cm) and spaced 5 cm apart from each other. Evaluation of the low-light tolerance index during the seedling stage was carried out under low-light intensity.

Low-light intensity was simulated by double layers of havelock in the greenhouse, with an average daylight intensity of ~100 \textmu mol·m\textsuperscript{-2}·s\textsuperscript{-1} and a day/night average temperature of 25°/15°C. The temperature was regulated using heating equipment and aeration. A 30-day low-light treatment was carried out during the two-leaf stage of the seedlings.

Design method of the low-light tolerance index

The low-light tolerance index was obtained by design formulas after statistics were performed on 6 generations of plants, with samples from every grade (i.e., 1st-4th, defined below) under the low-light treatment. We divided the degree of damage to cucumber seedlings under low-light stress into 4 (i.e., 1st-4th) grades. The grades were characterized as follows: 1st) a cotyledon that hangs down gently, only a few green leaves gently withdrawn, and excessive plant growth; 2nd) etiolation of 50% of the cotyledons and a chlorotic leaf that can turn green after exposure to light; 3rd) complete necrosis of the cotyledons, the number of chlorotic leaves is >50%, which cannot turn green after exposure to light, and the ability for the top portion of the plant to continue growth; and 4th) complete plant death.

\[
\text{Low-light inhibition index} = \frac{\sum X_a (n \sum x)}{a_1 + a_2 + a_3 + \ldots + a_n (n \sum x)}
\]

where \(X_a\) are the damaged numbers of every grade; \(a_1, a_2, a_3, \ldots, a_n\) are the damaged grades; \(n\) is the highest grade = 4; and \(\sum x\) indicates all plants = 10.

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Marker analysis

SSR marker (Ren et al., 2009) and sequence-related amplified polymorphism (SRAP) (Li and Quiros, 2001) technologies were selected for genomic analyses of the parents. Leaves (weight: approximately 0.5 g) from 2-week-old F₂ (123), F₁, and parent seedlings were collected and preserved in an ultra-low temperature freezer. The tissue was then immediately lyophilized for DNA extraction using the cetyl trimethyl-ammonium bromide method (Zhao and Pan, 2004).

Polymerase chain reaction (PCR) analyses for SRAP (Li and Quiros, 2001) and SSR markers were performed in 10-µL volumes of a uniform reaction mixture [i.e., SRAP: 1 µL 10X PCR commercial buffer, 20 mM MgCl₂, 2 mM dNTPs, 15-20 ng DNA, 2.5 µM of each primer, and 1.75 U Taq DNA polymerase (Shanghai Promega); SSR: 1 µL 10X PCR commercial buffer, 20 mM MgCl₂, 2 mM dNTPs, 15-20 ng DNA, 2.5 µM of each primer, and 1 U Taq DNA polymerase (Shanghai Promega)], incorporating 10 µL light-weight mineral oil overlay. The amplified products were resolved on 4.0% denatured polyacrylamide gels (6.0% SSR; Life Technologies, Gaithersburg, MD, USA) by silver staining. The gels were visualized using an Image Scanner III.

Mapping and QTL analysis

Linkage mapping was performed using the JoinMap 3.0 software (van Ooijen and Voorrips, 2001) based on the F₂ data, with a log-likelihood threshold ≥3.0 and the Kosambi mapping function (Kosambi, 1944). QTLs were conducted for the F₂:3 families, and the M₂₂ and M₁₄ populations using WinQTLCart 2.5 (Zeng, 1993; Christopher et al., 2002).

Data analysis method

The genetic analysis was carried out using the major gene and polygene models (Zhang et al., 2000). Least-square means and a column diagram of frequencies for traits were calculated according to the SPSS16.0 software.

RESULTS

Distribution of the low-light tolerance index in cucumber

The degree of damage to most plants was lower, higher, and advisable in the low-light tolerant line M₂₂ (P₁), low-light sensitive line M₁₄ (P₂), and F₁ group, respectively (Table 1). The variation in the degree of damage (i.e., grade) in the cross P₁ x P₂ was 0-4 and exceeded the range of its parents, which showed that favorable genes and leaky genes had a scattered distribution and could form transgressive segregation lines via genetic recombination.

\[
\text{Low-light tolerant index} = \frac{1}{\text{Low-light inhibition index}} \quad \text{(Equation 2)}
\]
Table 1. Frequency of low-light tolerance index for 6 generations.

<table>
<thead>
<tr>
<th>Generation</th>
<th>0 grade</th>
<th>1st grade</th>
<th>2nd grade</th>
<th>3rd grade</th>
<th>4th grade</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>8</td>
<td>8</td>
<td>10</td>
<td>4</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>P1</td>
<td>2</td>
<td>6</td>
<td>12</td>
<td>10</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td>4</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>F2</td>
<td>13</td>
<td>20</td>
<td>25</td>
<td>48</td>
<td>14</td>
<td>120</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>11</td>
<td>19</td>
<td>16</td>
<td>7</td>
<td>60</td>
</tr>
<tr>
<td>B1</td>
<td>2</td>
<td>8</td>
<td>17</td>
<td>28</td>
<td>5</td>
<td>60</td>
</tr>
</tbody>
</table>

Phenotypic variation in the low-light tolerance index in the F_{2:3} lines

In the F_{2:3} lines, the total mean for the low-light tolerance index in the S was greater than that in the A (Table 2). The low-light tolerance index of the lines and seasons significantly or highly significantly differed.

The mean value for the low-light tolerance index in the F_{2:3} lines (Table 3) was intermediate between that of its parents, and the coefficients of variation were 30.9 and 32.1% in the S and A, respectively. The standard deviations for the S and A were <1.0.

Table 2. Variance in low-light tolerance index of the F_{2:3} lines across 2 seasons.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>MS</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F_{2:3} family</td>
<td>122</td>
<td>1335.137</td>
<td>6.808*</td>
</tr>
<tr>
<td>Season</td>
<td>1</td>
<td>2226.215</td>
<td>11.352**</td>
</tr>
<tr>
<td>Error</td>
<td>122</td>
<td>196.0992</td>
<td></td>
</tr>
<tr>
<td>Total variance</td>
<td>245</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference at P < 0.05 level. **Significant difference at P < 0.01 level.

Table 3. Variance analysis of low-light tolerance index (LLTI) in the F_{2:3} lines.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Ranges</th>
<th>Means</th>
<th>Variance</th>
<th>Standard deviation</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLTI_spring</td>
<td>1.43-3.88</td>
<td>2.17</td>
<td>0.449</td>
<td>0.670</td>
<td>30.9</td>
</tr>
<tr>
<td>LLTI_autumn</td>
<td>1.00-4.05</td>
<td>2.00</td>
<td>0.412</td>
<td>0.642</td>
<td>32.1</td>
</tr>
</tbody>
</table>

Normal sex distribution test for the low-light tolerance index in the F_{2:3} lines

Results of the normal sex distribution test (Figure 1) showed that the low-light tolerance index for the S and A conformed to the normal and skewed normal distributions, respectively. Thus, segregation traits may be multi-gene distribution characteristics, and the low-light tolerance index distribution in the S and A conformed to the QTL mapping requirements.

Genetic analysis of low-light tolerance for cucumber seedlings

The ABC scale genetic testing was adopted for the genetic analysis of low-light tolerance in cucumber seedlings, and the P values of 3 models were >0.05, indicating that the inheritance of low-light tolerance is in accordance with the additive-dominant model (Table 4).

Gene effects of low-light tolerance are listed in Table 5, and the total mean values,
additive effects, and dominant effects were 1.973, 0.385, and -1.917, respectively, which indicates that heredity of low-light tolerance exhibits positive additive and negative dominant effects. Moreover, the mean dominance degree \([H/D]^{1/2}\) was 2.230, suggesting that the inheritance of low-light tolerance exhibits negative super-dominant and mainly dominant effects, with the effect of additive interactions.

Furthermore, the main gene and polygenic inheritance analyses were adopted in this study. The results showed that low-light tolerance is controlled by an additive-dominant poly-gene, and there were epistasis in these genes. Estimated values of the genetic parameters for the low-light intensity tolerance index in Table 6 show that the polygenic inheritance rate of the separate generations were >30%; the highest rate was observed in the F₂ generation (i.e., 39.3%). The additive effect of the polygenes was 0.517, and the dominant effect value was -1.267. Each of these values were small, which indicates that there are no significant differences in the gene interactions. The environmental variance, accounting for the phenotypic variance ratio of the F₂ generation, returned the lowest value (i.e., 30.36%), suggesting that inheritance of low-light tolerance is rarely impacted by the environment.

Figure 1. Normality test of the low-light tolerance index.

<table>
<thead>
<tr>
<th>Model</th>
<th>Mean</th>
<th>SE</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-0.980</td>
<td>0.802</td>
<td>1.222</td>
<td>0.275</td>
</tr>
<tr>
<td>B</td>
<td>0.470</td>
<td>0.975</td>
<td>0.482</td>
<td>0.634</td>
</tr>
<tr>
<td>C</td>
<td>0.210</td>
<td>2.375</td>
<td>0.088</td>
<td>0.892</td>
</tr>
</tbody>
</table>

Table 4. ABC scale test for low-light tolerance.

<table>
<thead>
<tr>
<th>Genetic effect</th>
<th>Estimated value</th>
<th>Standard error</th>
<th>Mean dominance degree ([H/D]^{1/2})</th>
<th>Broad-sense heritability (h^2) (%)</th>
<th>Narrow-sense heritability (h^2) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total mean</td>
<td>1.973</td>
<td>2.148</td>
<td>2.230</td>
<td>73.71</td>
<td>28.83</td>
</tr>
<tr>
<td>Additive effect</td>
<td>0.385</td>
<td>1.865</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant effect</td>
<td>-1.917</td>
<td>4.763</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Genetic effects and estimated values of low-light tolerance in cucumber.
**Table 6.** Estimated values of genetic parameters of low-light tolerance.

<table>
<thead>
<tr>
<th>1st parameter</th>
<th>Estimated value</th>
<th>2nd parameter</th>
<th>Estimated values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2nd parameter</td>
<td>Estimated values</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B₁</td>
</tr>
<tr>
<td>Total mean</td>
<td>2.08</td>
<td>σ² p₁</td>
<td>29.18</td>
</tr>
<tr>
<td>Additive effect</td>
<td>0.517</td>
<td>σ² p₂</td>
<td>17.25</td>
</tr>
<tr>
<td>Dominant effect</td>
<td>-1.267</td>
<td>σ² p₂</td>
<td>11.93</td>
</tr>
<tr>
<td>h² (%)⁴</td>
<td>59.12</td>
<td>1- h² p₁ (%)⁴</td>
<td>47.27</td>
</tr>
<tr>
<td>1- h² p₁ (%)⁴</td>
<td>40.88</td>
<td></td>
<td>52.73</td>
</tr>
</tbody>
</table>

¹σ² p, phenotypic variance. ²σ² ng, major gene variance. ³σ² e, environmental variance. ⁴h² p, polygene heritability value.

**QTL analysis of low-light tolerance for cucumber seedlings**

Five QTLs related to the low-light tolerance index were detected in the S and A, including 2 QTLs (i.e., Llti1.1 and Llti1.2) on the first linkage group, with variances of 6.0 and 9.5%, positive additive effect values of 10.5 and 9.5, and negative dominant effect values of -0.09 and -0.07, respectively. Three QTLs (Llti2.1, Llti2.1, and Llti2.1) were mapped on the second linkage group, with variances of 10.1-14.0%; additive effect values of 8.07, -0.06, and -2.1; and dominant effect values of 0.06, 0.09, and 0.45, respectively (Figure 2; Table 7).

![Figure 2. Quantitative trait loci of low-light tolerance in cucumber.](image-url)
Table 7. Positions, intervals, effects, and varieties of quantitative trait loci (QTLs) for low-light tolerance.

<table>
<thead>
<tr>
<th>Trait/QTL</th>
<th>Linkage group</th>
<th>Flanking locus</th>
<th>Position (cM)</th>
<th>LOD</th>
<th>Variety R (%)</th>
<th>Additive effect</th>
<th>Dominant effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Llt1.1</td>
<td>1</td>
<td>ME23EM2-SSR13109</td>
<td>1.1</td>
<td>13.2</td>
<td>6.0</td>
<td>10.5</td>
<td>-0.09</td>
</tr>
<tr>
<td>Llt1.2</td>
<td>1</td>
<td>SSR13109-ME1SA18</td>
<td>1.8</td>
<td>12.7</td>
<td>9.6</td>
<td>9.5</td>
<td>-0.07</td>
</tr>
<tr>
<td>Llt2.1</td>
<td>2</td>
<td>ME23GA2-ME4EM4</td>
<td>1.3</td>
<td>8.9</td>
<td>14.0</td>
<td>8.07</td>
<td>0.06</td>
</tr>
<tr>
<td>Llt2.2</td>
<td>2</td>
<td>ME1EM8-ME2EM8</td>
<td>1.9</td>
<td>10.1</td>
<td>13.6</td>
<td>-0.06</td>
<td>0.09</td>
</tr>
<tr>
<td>Llt2.3</td>
<td>2</td>
<td>ME1EM6-ME2EM3</td>
<td>12.3</td>
<td>7.9</td>
<td>10.1</td>
<td>-2.1</td>
<td>0.45</td>
</tr>
</tbody>
</table>

1Number + QTL serial number. 2Position of the LOD peak value. 3Positive value is the add-effect derived from the parental line M_{22} and the negative value is the add-effect derived from the parental line M_{14}. 4Positive value is the trait controlled by gene likely to one of parental line M_{22} and the negative value is the trait controlled by gene likely to one of parental line M_{14}.

DISCUSSION

Low-light tolerance in cucumber plants is a quantitative trait, and the low-light tolerance index is a comprehensive and effective evaluation of low-light tolerance (Li et al., 2009a). Quantitative traits of the plant are controlled by a few major genes or a large polygene, and the effects of those genes differ (Wang et al., 2008).

An in-depth and comprehensive elaboration on the genetic characteristics of low-light tolerance for cucumber is given in this paper. The results of 2 genetic methods showed that the heredity of low-light tolerance is controlled by major genes and a mini-polygene, and they fit to an additive-dominance or additive-dominance-epistasis polygene model. The genetic model results are similar to those of a prior study investigating traits related to low-light tolerance (Li et al., 2009c). In addition, 5 QTLs were related to the low-light tolerance index in both the S and A, supporting the hypothesis that low-light tolerance is controlled by a major gene and a mini-polygene.

In this study, among the additive effect values of the 5 QTLs, 3 and 2 QTLs were positive and negative, respectively, indicating the additive and subtractive effects of the genes (Gong et al., 2001). Moreover, gene cluster regions were identified, such as QTL Llti2.2 in the interval ME1EM8-ME2EM8, since Chla2.1 for ch1.a content and Chla+b2.2 for ch1.a+b content were also controlled by this interval. QTL Llti2.3 was detected in the interval ME1EM6-ME2EM3; 2 QTLs controlling ch1.a content and hypocotyl length were identified in the interval ME1EM6-ME2EM3 in each season. This result is similar to that of a prior report on traits of low-light tolerance in cucumber (Li et al., 2010). Gene cluster regions have been reported in many studies (Moynihan et al., 2009; Sorice et al., 2011). QTLs gathered in an identical region but controlled different traits, which may indicate close linkage, and pleiotropism or physiological associations. Thus, 2 closely adjacent regions on the 2nd linkage group may have a more significant role in the gene expression of low-light tolerance or related traits. Therefore, we presumed that the QTLs detected in the 2 seasons would be the genes controlling low-light tolerance.

Several factors for cucumber low-light tolerance were analyzed, and QTL mapping was performed. First, the SRAP markers can now be closely linked to the 5 QTLs and transformed into SCAR markers to facilitate breeding of plants with low-light resistance (Ren et al., 2012). Second, molecular markers constitute an efficient tool for indirect selection in plant breeding (Lecomte et al., 2004). Marker-assisted selection (MAS) could aggregate QTLs of low-light tolerance from different cucumber lines into a single line. Different environments affected this trait, and the selection of this trait by phenotypic grade was not feasible. Using...
MAS, seedlings were selected based on molecular markers linked to low-light tolerance. They have been widely used for following the introgression of monogenic traits, such as disease resistance (Yu et al., 2000; Singh et al., 2001). This action can shorten the breeding process and increase breeding efficiency. Further studies can contribute to an increase in the efficiency of cucumber breeding in the future.

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