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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 $F_{2:3}$ 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_{22} was crossed with the low-light sensitive line M_{14}. M_{22} grew normally and exhibited acclimation when exposed to low-light stress. In contrast, M_{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_1, B_1, and B_2 were obtained from the parents. The F_1 generation obtained from the crossing of M_{22} and M_{14} was self-pollinated to produce 152 F_2 progeny, which were then self-pollinated by single-seed descent to obtain 123 $F_{2:3}$ families.

Field evaluation and plant characteristics

The 2 parental lines (i.e., M_{22} and M_{14}), their F_1 generation, and the $F_{2:3}$ families were evaluated in a greenhouse at the Horticulture School, BaYi Agricultural University, Daqing, China. The $F_{2:3}$ 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_1, P_2, F_1, B_1, B_2, and F_2, and the $F_{2:3}$ families were arranged in a randomized complete block design with 3 replications. Each replicate of P_1, P_2, and F_1 had 10 plants; there were 20 plants for B_1 and B_2 and 40 plants for F_2 and the $F_{2:3}$ 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 μmol·m^{-2}·s^{-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.

$$\text{Low-light inhibition index} = \sum Xa/(n\sum x) \text{ or}$$
$$\text{Low-light inhibition index} = (X_1 \times a_1 + X_2 \times a_2 + X_3 \times a_3 + \ldots X_N \times a_n)/(n\sum x) \quad \text{(Equation 1)}$$

where $X_1, X_2, X_3, \ldots X_N$ 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.

Low-light tolerant index = 1 / Low-light inhibition index (Equation 2)

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 F_2 (123), F_1, 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 $MgCl_2$, 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 $MgCl_2$, 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 F_2 data, with a log-likelihood threshold ≥3.0 and the Kosambi mapping function (Kosambi, 1944). QTLs were conducted for the $F_{2:3}$ families, and the M_{22} and M_{14} 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 M_{22} (P_1), low-light sensitive line M_{14} (P_2), and F_1 group, respectively (Table 1). The variation in the degree of damage (i.e., grade) in the cross P_1 x P_2 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.

Table 1. Frequency of low-light tolerance index for 6 generations.

Generation	0 grade	1st grade	2nd grade	3rd grade	4th grade	N
P_1	8	8	10	4		30
P_2		2	6	12	10	30
F_1	4	8	7	7	4	30
F_2	13	20	25	48	14	120
B_1	7	11	19	16	7	60
B_2	2	8	17	28	5	60

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.

Source	DF	MS	F value
$F_{2:3}$ family	122	1335.137	6.808*
Season	1	2226.215	11.352**
Error	122	196.0992	
Total variance	245		

*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.

Trait	Ranges	Means	Variance	Standard deviation	CV (%)
LLTI_spring	1.43-3.88	2.17	0.449	0.670	30.9
LLTI_autumn	1.00-4.05	2.00	0.412	0.642	32.1

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 addictive 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 polygene, 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 F_2 generation (i.e., 39.3%). The addictive 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 F_2 generation, returned the lowest value (i.e., 30.36%), suggesting that inheritance of low-light tolerance is rarely impacted by the environment.

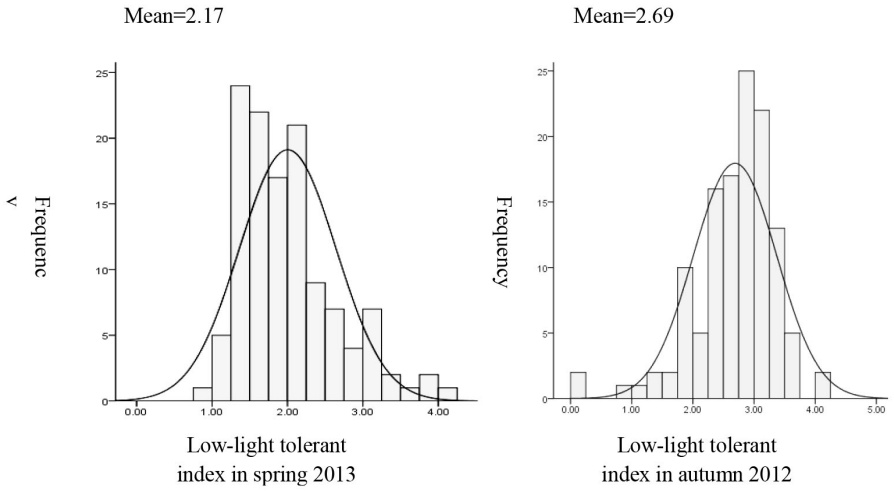

Figure 1. Normality test of the low-light tolerance index.

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

Model	Mean	SE	*t* value	P value
A	-0.980	0.802	1.222	0.275
B	0.470	0.975	0.482	0.634
C	0.210	2.375	0.088	0.892

Table 5. Genetic effects and estimated values of low-light tolerance in cucumber.

Genetic effect	Estimated value	Standard error	Mean dominance degree [$(H/D)^{1/2}$]	Broad-sense heritability h_B^2 (%)	Narrow-sense heritability h_N^2 (%)
Total mean	1.973	2.148	2.230	73.71	28.83
Additive effect	0.385	1.865			
Dominant effect	-1.917	4.763			

Table 6. Estimated values of genetic parameters of low-light tolerance.

1st parameter	Estimated value	2nd parameter	Estimated values		
			B_1	B_2	F_2
Total mean	2.08	σ^2_{P} [1]	29.18	22.62	39.30
Additive effect	0.517	σ^2_{mg} [2]	17.25	10.70	27.37
Dominant effect	-1.267	σ^2_{e} [3]	11.93	11.93	11.93
		h^2_{pg} (%)[4]	59.12	47.27	69.64
		$1-h^2_{pg}$ (%)	40.88	52.73	30.36

[1]σ^2_p, phenotypic variance. [2]σ^2_{mg}, major gene variance. [3]σ^2_e, environmental variance. [4]h^2_{pg}, 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 addictive 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%; addictive 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).

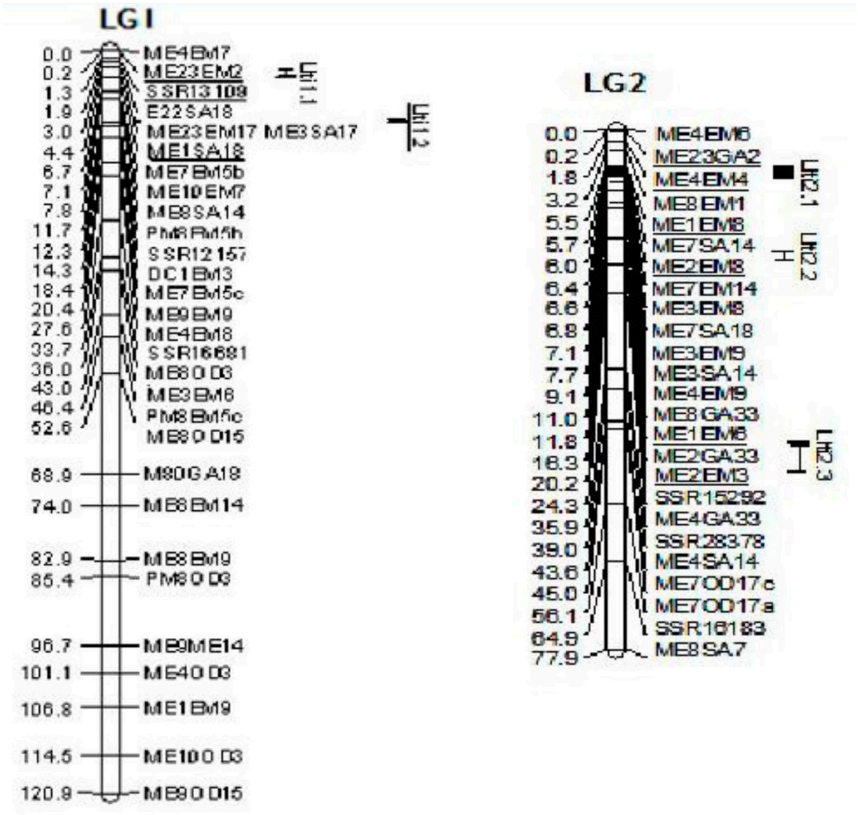

Figure 2. Quantitative trait loci of low-light tolerance in cucumber.

Table 7. Positions, intervals, effects, and varieties of quantitative trait loci (QTLs) for low-light tolerance.

Trait/QTL[1]	Linkage group	Flanking locus	Position (cM)[2]	LOD	Variety R^2 (%)	Addictive effect[3]	Dominant effect[4]
Llti1.1	1	ME23EM2-SSR13109	1.1	13.2	6.0	10.5	-0.09
Llti1.2	1	SSR13109-ME1SA18	1.8	12.7	9.6	9.5	-0.07
Llti2.1	2	ME23GA2-ME4EM4	1.3	8.9	14.0	8.07	0.06
Llti2.2	2	ME1EM8-ME2EM8	1.9	10.1	13.6	-0.06	0.09
Llti2.3	2	ME1EM6-ME2EM3	12.3	7.9	10.1	-2.1	0.45

[1]Number + QTL serial number. [2]Position of the LOD peak value. [3]Positive 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}. [4]Positive 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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