LOW TEMPERATURE SEED GERMINATION OF CUCUMBER:
GENETIC BASIS OF THE TOLERANCE TRAIT

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Received: December 2, 2013;   Accepted: December 19, 2013

ABSTRACT

Cucumber (Cucumis sativus L.) germinates in an optimal temperature ranging from 24 to 28 °C. In order to develop cultivars with low temperature germination ability, knowledge regarding its genetic basis is needed. In our earlier study, we identified the accession PI 390953 as chilling tolerant and a good cold germinator. The objective of our present study was to compare cold germinability of cold tolerant breeding line B 5669 with PI 390953, and to measure the inheritance of this trait. At 13 °C, both tested cultigens (B 5669, PI 390953) showed the highest germinability and we found no significant differences between them regarding the rate of germination, days to germination (DTG), or germination index (GI). We also observed differences in the germination ability at 13 °C among seven hybrid populations of cucumber, derived from the cross between good cold germinator B 5669 (P1) and B 6115 (P2) lacking cold-germination ability. The fastest low temperature germination and the highest low temperature germination percentages were observed in B 5669 (P1) with germination of 78 and 100% on the 6th and 10th day of the test, respectively. In addition, the cultigen B 5669 exhibited the fastest germination, reaching on average of DTG = 5.7. B 6115 (P2) and BC1P2 proved unable to germinate at 13 °C even within 21 days. The seed germinability of F2 population fits a three-recessive gene model. Cucumber cultigens B 5669, PI 390953, and PI 246903 showed low temperature tolerance, but of them B 5669 may become the most desirable to breeders since it exhibits cold germinability combined with good fruit quality traits.

Key words: Chilling, inheritance, cold tolerance, Cucumis sativus, genetic factors

INTRODUCTION

Cucumber (Cucumis sativus L.), a warm-season crop, germinates at optimal temperatures of 24 to 28 °C (Staub & Wehner 1996). Fluctuating temperatures encountered in many production areas during the early part of the growing season may negatively influence germination and seedling growth in cucumber. In Poland, cucumbers are often direct-seeded in the middle of May, when the occurrence of cold stress may affect seed germination and seedling establishment, and thus cause significant stand loss and delayed growth. Cultivars with low temperature tolerance are currently unavailable, although differences in response to chilling temperatures were found among the cultivars present on the market (Cabrera et al. 1992). Therefore, growers have the option of protecting their crop through safe seeding date or row covers (Staub & Wehner 1996). Such treatments, however, can be costly and ineffective. Cucumber cultivars with low-temperature tolerance provide an efficient way to protect crops from chilling injuries. Cultivars with improved cold tolerance might also be planted earlier for an earlier harvest; this could provide added benefits in escaping diseases that usually come in to a production region later in the season.

Cucumber cultigens differ in their requirements for the optimal (Roeggen 1987) and minimal (Nienhuis & Lower 1981; Wehner 1981, 1982, 1984) temperature for germination. This might be
associated with the geographic region they originated from. Lower (1975) showed that 11 cucumber cultivars exhibited differences in germination speeds at temperatures between 14 and 17 °C. Cultivars developed in the northern part of the United States (‘SMR 58’, ‘Wisconsin SMR 18’) were better cold germinators than those developed in the southern part of the U.S. (‘Palomar’, ‘Chipper’, ‘Ashley’). The exception was ‘Pixie’, with good cold germination ability despite originating from the South. Similarly, Wehner (1981, 1982) found differences in days to germination at 15 °C among 203 cucumber breeding lines and cultivars (from 3.5 to 17.3), but not at 20 °C. Significant differences were found also among 15 cucumber lines when germinated at 15 °C, but not at 25 °C (Li et al. 1998).

We aimed to determine the genetic basis of cold tolerance in cucumber and later to transfer the trait into breeding lines or cultivars. Recently, we identified two cucumber accessions as promising sources of chilling-tolerance: PI 390953 exhibited both high cold tolerance at seedling stage and high seed germination rate under low temperatures (11 and 13 °C), while PI 246903 showed cold tolerance limited to the seedling stage (Kozik et al. 2007, 2010; Kozik & Wehner 2008). We also found that low temperature seed germination ability and chilling resistance at the seedling stage were distinct traits inherited independently, based on the non-allelic gene interactions in the cold-tolerant accession PI 390953 (Kozik et al. 2012). Those accessions, however, lack the elite horticultural traits (Kozik et al. 2010). Therefore, we developed cucumber lines, among these the B 5669 that also showed cold tolerance. The objective of this study was: (i) to compare the low temperature germinability of the new cold-tolerant breeding line B 5669 with accession PI 390953, and (ii) to analyze the inheritance mode of cold germinability in B 5669.

MATERIALS AND METHODS

Seed and plant material

Six cultigens of cucumber PI 390953, PI 246903, ‘Little John’ (sometimes referred to as ‘AR 79-75’), ‘Chipper’, B 5669, and B 6115 were chosen on the basis of their reaction to low temperatures in our previous study (Kozik & Wehner 2008; Kozik et al. 2007, 2010, 2012). Two monoecious cucumber inbred lines (B 5669 and B 6115, developed in our lab by intensive selection in an incubator in germination tests at 13 °C) were compared with two cold-tolerant accessions: PI 390953 cold-tolerant at both germination and seedling stage; PI 246903 cold-tolerant at seedling stage. Two cold-sensitive (at both stages) control cultivars Chipper and Little John were also tested.

Other plant material tested included hybrid populations F₁, F₁ reciprocal (RF₁), F₂, and backcross populations BC₁₁ and BC₁₂ derived from the cross between lines B 5669 (P₁) and B 6115 (P₂). All crosses were made by hand pollinations. The descendant plants were greenhouse-grown at the Research Institute of Horticulture, Skiermiewice, Poland.

Germination tests

Germination tests were conducted in an unlighted incubator set at 13 or 26 °C (control). Non-imibed seeds of all populations undergoing testing were placed in 150 mm diameter Petri dishes (50 seeds/Petri dish) lined with two layers of absorbent filter paper saturated with 3 mL of distilled water. The experiments were conducted in three replications for six cucumber cultigens including both tested parents, four replications for both F₁ populations, six replications for BC₁₁ and BC₁₂, and eight replications for F₂ (one Petri plate was considered one replication).

Seed germination (radicles ≥ 3 mm long) was recorded daily for three weeks. Data were expressed as following parameters:

- energy of germination (GE) measured at the 4th, 6th, 8th, and 10th day of test using formula \( GE\% = \frac{\sum N_i}{\sum D_i} \times 100 \),
- rate of germination (GR) calculated using the above formula on the 21st day of test,
- germination index (GI) calculated with the formula: \( GI = \frac{\sum (N_i/D_i)}{T} \), where \( N_i \) is the number of seeds germinated on \( i \)th day and \( D_i \) is the number of days after experiment initiation,
- mean number of days to germination (DTG) was calculated with the formula: \( GI = \frac{\sum (N_i/D_i)}{T} \).
where T is total number of seeds germinated, N,

is the number of seeds germinated on ith day and

D, is the number of days after experiment initia-
tion (Smith & Millet 1964).

The results of the tests comparing the germina-
bility of the cultigens and of hybrid populations
were analyzed using the program STATISTICA 8.0
(StatSoft), and the Newman-Keul’s test at p = 0.05.

For the genetic analyses, the hybrid populations
segregating for cold germinability were

grouped into two classes: tolerant (T; seeds germi-
nated within first 10 days of treatment, similarly to
their cold-tolerant parent B 5669), sensitive (S;
seeds did not germinate within 10 days).

In order to investigate the potential of the seg-
regating hybrid populations for seed germination
under low temperature conditions, the analyses
were continued over 21 days. This period was much
longer than the seeds of the cold-tolerant parent
B 5669 required for 100% germination. Plants were
classified as cold-tolerant (T; seeds germinated
within first 10 days of incubation at 13 °C), moder-
ately cold-tolerant (M; seeds germinated between
the 11th and 21st day of the study), or cold-sensitive
(S; seeds failed to germinate within the 21 days of
observation).

Statistical analysis of the genetic studies of
germinability in cold-tolerant line B 5669 were
performed by χ² test on F₂ and BC₁₁ data to determine
goodness-of-fit to the hypothetical segregation
ratios for the T and S classes.

RESULTS AND DISCUSSION

Germination rates of the cucumber cultigens
and hybrid populations tested at control tempera-
ture (26 °C) ranged from 94 to 100%, with no significant
differences (data not shown). At 13 °C, two culti-
gen (B 5669 and PI 390953) showed the highest
germinability (Table 1) and we found no significant
differences between these cultigens regarding the
rate of germination (GR), days to germination
(DTG), or germination index (GI). The only param-
eter differing between the two cultigens was the ener-
gy of seed germination (GE) on the 4th day of the
experiment (Table 1). None of remaining cucumber
cultigens tested (PI 246903, ‘Little John’, ‘Chipp-
per’), showing cold tolerance at the seedling stage
(Smeets & Wehner 1997; Chung et al. 2003; Kozik
et al. 2007), and line B 6115 germinated at 13 °C.

Differences in low temperature (13 °C) germi-
nation ability among the seven populations were
assessed with the following parameters: GE, GR,
DTG, and GI (Table 2). The fastest germination and
the highest germination percentage were observed
in the cold-germinating line B 5669 (P₁) with GE of
78 and 100% on the 6th and 10th day of the test,
respectively. In addition, this line showed the lowest
value of DTG index of 5.7 (Table 2). In contrast, the
lines B 6115 (P₂) and BC₁₁ failed to germinate at
13 °C. Segregating hybrid populations derived from
a cross between the lines B 5669 and B 6115 exhib-
ted varying degrees of cold germinability. None of
these hybrid populations, however, showed the cold
germinability (GE, GR, GI) close to the tolerant par-
ent B 5669 (Table 2). The mean value of the DTG
index (16.5) for F₁ and RF₁ was higher than the mid-
parent value (13.3) indicating the recessive charac-
ter of low temperature germination ability in
B 5669, similarly to cold-tolerant PI 390953 (Kozik
et al. 2012). The F₁ and RF₁ populations showed sig-
nificant differences in their DTG values (15.1 and
17.9, respectively). Additionally, the F₁ showed
6.5% and 30% energy of germination (GE) on the
10th and 14th day of study, respectively, in compari-
son to 2% and 4.5% germination for RF₁ (Table 2
and data not shown). Based on the results of F₁ and
RF₁, we concluded that the cold germinability in
B 5669 is controlled by both nuclear and cytoplas-
mic genes. Maternal effects for cold germinability
have been previously reported in muskmelon (Ner-
son & Staub 1989; Hutton & Loy 1992) and tomato
(De Vos et al. 1981; Ng & Tigchelaar 1973; Foolad
& Lin 1998). Regarding cucumber, our previous
study showed no maternal effects underlying the
low temperature germinability in PI 390953 (Kozik
et al. 2012).
Table 1. Germination ability at low temperature (13 °C) in six cucumber cultigens

<table>
<thead>
<tr>
<th>Cultigens</th>
<th>Energy of germination (GE) in %</th>
<th>Rate of germination (GR) in %</th>
<th>Days to germination (DTG)</th>
<th>Germination index (GI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B 5669</td>
<td>4th day: 38.0 b 6th day: 78.0 ab 8th day: 94.0 a</td>
<td>100.0 a</td>
<td>5.7 c</td>
<td>19.2 ab</td>
</tr>
<tr>
<td>PI 390953</td>
<td>4th day: 63.0 a 6th day: 94.0 a 8th day: 98.0 a</td>
<td>100.0 a</td>
<td>4.8 c</td>
<td>24.0 a</td>
</tr>
<tr>
<td>‘Little John’</td>
<td>4th day: 0.0 c 6th day: 0.0 c 8th day: 0.0 b</td>
<td>1.0 b</td>
<td>14.0 b</td>
<td>0.1 c</td>
</tr>
<tr>
<td>‘Chipper’</td>
<td>4th day: 0.0 c 6th day: 0.0 c 8th day: 0.0 b</td>
<td>0.0 b</td>
<td>21.0 a</td>
<td>0.0 c</td>
</tr>
<tr>
<td>B 6115</td>
<td>4th day: 0.0 c 6th day: 0.0 c 8th day: 0.0 b</td>
<td>0.0 b</td>
<td>21.0 a</td>
<td>0.0 c</td>
</tr>
<tr>
<td>PI 246903</td>
<td>4th day: 0.0 c 6th day: 0.0 c 8th day: 0.0 b</td>
<td>0.0 b</td>
<td>21.0 a</td>
<td>0.0 c</td>
</tr>
</tbody>
</table>

Means followed by the same letter within each column are not significantly different at p = 0.05

Table 2. Low temperature (13 °C) seed germination ability of seven generations from the cross of cold-tolerant B 5669 with cold-sensitive B 6115 cucumber

<table>
<thead>
<tr>
<th>Population</th>
<th>Energy of germination (GE) in %</th>
<th>Rate of germination (GR) in %</th>
<th>Days to germination (DTG)</th>
<th>Germination index (GI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₁ B 5669</td>
<td>6th day: 78.0 a 10th day: 100.0 a</td>
<td>100.0 a</td>
<td>5.7 a</td>
<td>19.2 a</td>
</tr>
<tr>
<td>P₂ B 6115</td>
<td>6th day: 0.0 b 10th day: 0.0 de</td>
<td>0.0 c</td>
<td>21.0 e</td>
<td>0.0 e</td>
</tr>
<tr>
<td>F₁</td>
<td>6th day: 0.0 b 10th day: 6.5 c</td>
<td>89.0 b</td>
<td>15.1 b</td>
<td>6.2 bc</td>
</tr>
<tr>
<td>RF₁</td>
<td>6th day: 0.0 b 10th day: 2.0 d</td>
<td>79.0 b</td>
<td>17.9 cd</td>
<td>3.6 d</td>
</tr>
<tr>
<td>F₂</td>
<td>6th day: 0.0 b 10th day: 1.7 d</td>
<td>82.0 b</td>
<td>16.8 bc</td>
<td>8.5 b</td>
</tr>
<tr>
<td>Bc₁P₁</td>
<td>6th day: 0.0 b 10th day: 10.0 b</td>
<td>80.8 b</td>
<td>16.4 bc</td>
<td>4.2 c</td>
</tr>
<tr>
<td>Bc₁P₂</td>
<td>6th day: 0.0 b 10th day: 0.0 de</td>
<td>0.0 c</td>
<td>21.0 e</td>
<td>0.0 e</td>
</tr>
</tbody>
</table>

Means followed by the same letter within each column are not significantly different at p = 0.05

Table 3. Analysis of the genetic background of low temperature (13 °C) seed germination ability in seven generations derived from cross of B 5669 (tolerant) with B 6106 (sensitive) cucumber

<table>
<thead>
<tr>
<th>Generation</th>
<th>Segregation observed (S : T)</th>
<th>Segregation theoretical (S : T)</th>
<th>χ²</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₁ B 5669</td>
<td>0 : 100</td>
<td>0 : 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P₂ B 6115</td>
<td>100 : 0</td>
<td>1 : 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₁</td>
<td>182 : 18</td>
<td>1 : 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF₁</td>
<td>196 : 4</td>
<td>1 : 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₂</td>
<td>393 : 7</td>
<td>63 : 1</td>
<td>0.091</td>
<td>1</td>
<td>0.763</td>
</tr>
<tr>
<td>Bc₁P₁</td>
<td>225 : 25</td>
<td>7 : 1</td>
<td>1.42</td>
<td>1</td>
<td>0.233</td>
</tr>
<tr>
<td>Bc₁P₂</td>
<td>250 : 0</td>
<td>1 : 0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*S – cold-sensitive – not germinating within 10 days at 13 °C
T – cold-tolerant germinating within first 10 days at 13 °C

Data from the F₂ fitted the Mendelian inheritance models (Table 3). Seeds of F₂ population germinating at 13 °C as rapidly as the cold-tolerant parent (P₁; B 5669) were classified as homozygous recessive. F₂ hybrid population germinability fitted a theoretical segregation of 1 T : 63 S, which is in accordance with a model of three recessive genes (χ² = 0.09, P = 0.76). Segregation ratios in the backcross populations further confirmed this interpreta-
tion. Distribution of phenotypes regarding their exhibited cold tolerance (tolerant : sensitive) in Bc1P1 population fitted the ratio 1:7 ($\chi^2 = 1.42, P = 0.23$). Seeds of Bc1P2 population did not germinate at 13 °C, similarly to the cold-sensitive parent (B 6115; P2) (Tables 2 & 3).

Reports regarding the inheritance mode of cold germination ability in cucumber are limited. Studies focused on the inheritance of chilling tolerance at the seedling stage rather than at the seed germination stage. Our recent report provided information on the recessive character and non-allelic gene interactions (double dominant epistasis) conferring the cold germination ability in cucumber accession PI 390953 (Kozik et al. 2012). Low heritability of this trait in other cucumber accessions was previously reported by Nienhuis and Lower (1981), and Wehner (1982, 1984).

On the basis of low-temperature germination ability within 21 days, we observed some variation in the hybrid populations and classified the plants as (i) T – cold-tolerant, when seeds germinated within first 10 days, (ii) M – moderately cold-tolerant, seeds germinated between 11th and 21st day, (iii) S – cold-sensitive, when seeds failed to germinate within 21 days (Fig. 1). In the F1, RF1, F2, and Bc1P1, three groups of genotypes (T, M, S) were observed, with a small percentage of T genotypes (6.5, 2, 1.7, 10%, respectively), and a large share of M genotypes (82, 77, 80, 70%, respectively). This result demonstrates the potential of the segregating hybrid populations for seed germination under low temperature conditions in a period much longer than 10 days, required by the cold-tolerant parent B 5669 for 100% germination.

Fig. 1. Frequency distribution of cold-tolerant (T), moderately cold-tolerant (M), and cold-sensitive (S) genotypes in seven generations of the cross of cold-tolerant (P1; B 5669) with cold-sensitive (P2; B 6115) cucumber. Sprouts were classified as cold-tolerant (T; seeds germinated within first 10 days), moderately cold-tolerant (M; seeds germinated between 11th and 21st day), or cold-sensitive (S; seeds failed to germinate within 21 days) at 13 °C

Our present research, in agreement with the previous studies (Kozik et al. 2007, 2010, 2012), implies that low temperature seed germination ability and chilling resistance at the seedling stage are inherited as separate traits. Another study of cucumber supported this claim, arguing that average DTG at low temperature (15 °C) and cold tolerance of seedlings were negatively correlated (Li et al. 1998). Likewise, tomato (Solanum lycopersicum) cold tolerance during seed germination was genetically independent from cold tolerance during the vegetative growth (Foolad & Lin 2001).

In conclusion, all three cucumber cultigens B 5669, PI 390953, and PI 246903 offer cold tolerance at different stages of plant development. The line B 5669 may potentially prove more useful than...
PI 390953, because of its higher fruit quality (white spines, cylindrical shape, and uniform green color of fruit).

Acknowledgements
This research was funded by the Polish Ministry of Agriculture and Rural Development, grant No. 90 HOR hn–801–15/13. The authors gratefully acknowledge the technical assistance provided by Ewa Matysiak and Karolina Mrozińska.

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