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

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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 (P_1) and B 6115 (P_2) lacking cold-germination ability. The fastest low temperature germination and the highest low temperature germination percentages were observed in B 5669 (P_1) 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 (P_2) and BC_{1P2} proved unable to germinate at 13 °C even within 21 days. The seed germinability of F_2 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 (Røeggen 1987) and minimal (Nienhuis & Lower 1981; Wehner 1981, 1982, 1984) temperature for germination. This might be

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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 ºC 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 F_1, $F_{1reciprocal}$ (RF_1), F_2, and backcross populations BC_{1P1} and BC_{1P2} derived from the cross between lines B 5669 (P_1) and B 6115 (P_2). All crosses were made by hand pollinations. The descendant plants were greenhouse-grown at the Research Institute of Horticulture, Skierniewice, Poland.

Germination tests

Germination tests were conducted in an unlighted incubator set at 13 or 26 °C (control). Non-imbibed 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 F_1 populations, six replications for BC_{1P1} and BC_{1P2}, and eight replications for F_2 (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 4^{th}, 6^{th}, 8^{th}, and 10^{th} day of test using formula GE% = (total number of germinated seeds/total seed) × 100,
- rate of germination (GR) calculated using the above formula on the 21^{st} day of test,
- germination index (GI) calculated with the formula: $GI = \sum(N_i/D_i)$; where N_i is the number of seeds germinated on ith day and D_i is the number of days after experiment initiation,
- mean number of days to germination (DTG) was calculated with the formula: $GI = \sum(N_i/D_i)/T$;

where T is total number of seeds germinated, N_i is the number of seeds germinated on *i*th day and D_i is the number of days after experiment initiation (Smith & Millet 1964).

The results of the tests comparing the germinability 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 germinated 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 segregating 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 ºC), moderately 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 χ^2 test on F_2 and BC_{1P1} 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 temperature (26 °C) ranged from 94 to 100%, with no significant differences (data not shown). At 13 °C, two cultigens (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 parameter differing between the two cultigens was the energy of seed germination (GE) on the 4th day of the experiment (Table 1). None of remaining cucumber cultigens tested (PI 246903, 'Little John', 'Chipper'), 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. That was similar to our previous results (Kozik et al. 2007, 2010).

Differences in low temperature (13 °C) germination 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 (P_1) 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 (P_2) and BC_{1P2} failed to germinate at 13 °C. Segregating hybrid populations derived from a cross between the lines B 5669 and B 6115 exhibited varying degrees of cold germinability. None of these hybrid populations, however, showed the cold germinability (GE, GR, GI) close to the tolerant parent B 5669 (Table 2). The mean value of the DTG index (16.5) for F_1 and RF_1 was higher than the mid-parent value (13.3) indicating the recessive character of low temperature germination ability in B 5669, similarly to cold-tolerant PI 390953 (Kozik et al. 2012). The F_1 and RF_1 populations showed significant differences in their DTG values (15.1 and 17.9, respectively). Additionally, the F_1 showed 6.5% and 30% energy of germination (GE) on the 10th and 14th day of study, respectively, in comparison to 2% and 4.5% germination for RF_1 (Table 2 and data not shown). Based on the results of F_1 and RF_1, we concluded that the cold germinability in B 5669 is controlled by both nuclear and cytoplasmic genes. Maternal effects for cold germinability have been previously reported in muskmelon (Nerson & 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

Cultigens	Energy of germination (GE) in %			Rate of germination (GR) in %	Days to germination (DTG)*	Germination index (GI)
	4th day	6th day	8th day			
B 5669	38.0 b	78.0 ab	94.0 a	100.0 a	5.7 c	19.2 ab
PI 390953	63.0 a	94.0 a	98.0 a	100.0 a	4.8 c	24.0 a
'Little John'	0.0 c	0.0 c	0.0 b	1.0 b	14.0 b	0.1 c
'Chipper'	0.0 c	0.0 c	0.0 b	0.0 b	21.0 a	0.0 c
B 6115	0.0 c	0.0 c	0.0 b	0.0 b	21.0 a	0.0 c
PI 246903	0.0 c	0.0 c	0.0 b	0.0 b	21.0 a	0.0 c

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

Population	Energy of germination (GE) in %		Rate of germination (GR) in %	Days to germination (DTG)	Germination index (GI)
	6th day	10th day			
P_1 B 5669	78.0 a	100.0 a	100.0 a	5.7 a	19.2 a
P_2 B 6115	0.0 b	0.0 de	0.0 c	21.0 e	0.0 e
F_1	0.0 b	6.5 c	89.0 b	15.1 b	6.2 bc
RF_1	0.0 b	2.0 d	79.0 b	17.9 cd	3.6 d
F_2	0.0 b	1.7 d	82.0 b	16.8 bc	8.5 b
Bc_{1P1}	0.0 b	10.0 b	80.8 b	16.4 bc	4.2 c
Bc_{1P2}	0.0 b	0.0 de	0.0 c	21.0 e	0.0 e

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

Generation	Segregation		χ^2	df	*P*
	observed (S : T)*	theoretical (S : T)*			
P_1 B 5669	0 : 100	0 : 1			
P_2 B 6115	100 : 0	1 : 0			
F_1	182 : 18	1 : 0			
RF_1	196 : 4	1 : 0			
F_2	393 : 7	63 : 1	0.091	1	0.763
Bc_{1P1}	225 : 25	7 : 1	1.42	1	0.233
Bc_{1P2}	250 : 0	1 : 0			

*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 F_2 fitted the Mendelian inheritance models (Table 3). Seeds of F_2 population germinating at 13 °C as rapidly as the cold-tolerant parent (P_1; B 5669) were classified as homozygous recessive. F_2 hybrid population germinability fitted a theoretical segregation of 1 T : 63 S, which is in accordance with a model of three recessive genes (χ^2 = 0.09, *P* = 0.76). Segregation ratios in the backcross populations further confirmed this interpretation. Distribution of phenotypes regarding their exhibited cold tolerance (tolerant : sensitive) in Bc_{1P1} population fitted the ratio 1:7 ($\chi^2 = 1.42$, $P = 0.23$). Seeds of Bc_{1P2} population did not germinate at 13 °C, similarly to the cold-sensitive parent (B 6115; P_2) (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 F_1, RF_1, F_2, and Bc_1P_1, 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.

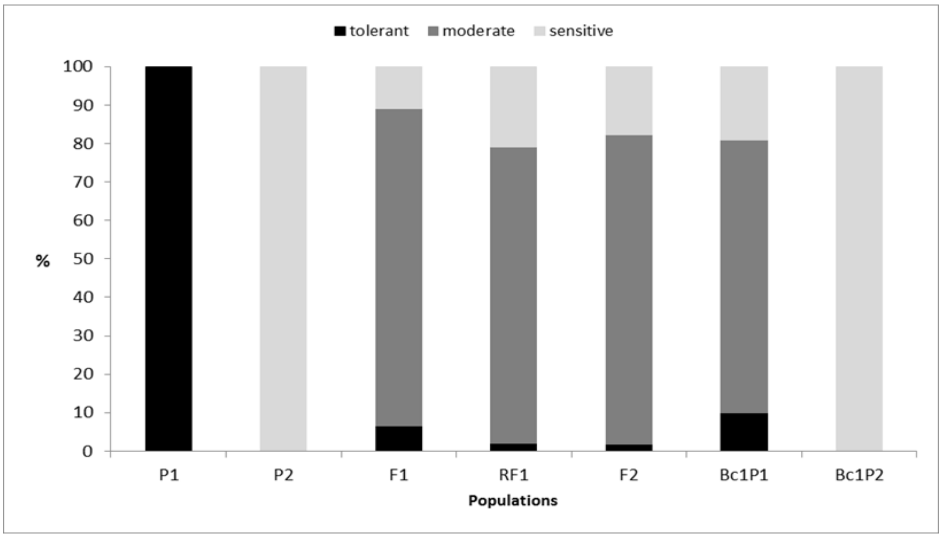

Fig. 1. Frequency distribution of cold-tolerant (T), moderately cold-tolerant (M), and cold-sensitive (S) genotypes in seven generations of the cross of cold-tolerant (P_1; B 5669) with cold-sensitive (P_2; B 6115) cucumber. Sprouts were classified as cold-tolerant (T; seeds germinated within first 10 days), moderately cold-tolerant (M; seeds germinated between 11th and 21th 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).

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REFERENCES

Cabrera R.M., Saltveit Jr. M.E., Owens K. 1992. Cucumber cultivars differ in their response to chilling temperatures. J. Amer. Soc. Hort. Sci. 117: 802-807.

Chung S.M., Staub J.E., Fazio G. 2003. Inheritance of chilling injury: a maternally inherited trait in cucumber. J. Amer. Soc. Hort. Sci. 128: 526-530.

De Vos D.A., Hill R.R. Jr., Hepler R.W., Garwood D.L. 1981. Inheritance of low temperature sprouting ability in F_1 tomato crosses. J. Amer. Soc. Hort. Sci. 106: 352-355.

Foolad M.R., Lin G.Y. 1998. Genetic analysis of low-temperature tolerance during germination in tomato, *Lycopersicon esculentum* Mill. Plant Breeding 117: 171-176. DOI:10.1111/j.1439-0523.1998.tb01473.x.

Foolad M.R., Lin G.Y. 2001. Relationship between cold tolerance during seed germination and vegetative growth in tomato: analysis of response and correlated response to selection. J. Amer. Soc. Hort. Sci. 126: 216-220.

Hutton M.G., Loy B.J. 1992. Inheritance of cold germinability in muskmelon. HortScience 27: 826-829.

Kozik E.U., Kłosińska U., Wehner T.C. 2007. New sources of chilling resistance in cucumber. In: Nowaczyk P. (Ed.), Spontaneous and induced variation for the genetic improvement of horticultural crops. University Press, University of Technology and Life Sciences, Bydgoszcz, Poland, pp. 227-232.

Kozik E.U., Wehner T.C. 2008. A single dominant gene *Ch* for chilling resistance in cucumber seedlings. J. Amer. Soc. Hort. Sci. 133: 225-227.

Kozik E.U., Kłosińska U., Wehner T.C. 2010. Progress in the development of cucumber breeding lines with low-temperature resistance. In: Thies J.A., Kousik S., Levi A. (Eds.), Cucurbitaceae 2010 Proc. Charleston, SC, US, pp. 46-49.

Kozik E.U., Kłosińska U., Wehner T.C. 2012. Inheritance of low temperature seed germination ability in cucumber. In: Proc. of 10th Eucarpia International Meeting on Genetics and Breeding Cucurbitaceae 2012, Antalya, Turkey, pp. 575-578.

Li J., Cui H., Zhang M. 1998. The relationship between low-temperature germination and chilling tolerance in cucumber. Cucurbit Genet. Coop. Rpt. 21: 11-13.

Lower R.L. 1975. Measurement and selection for cold tolerance in cucumber. Pickle Pak. Sci. 4: 8-11.

Nerson H., Staub J.E, 1989. Low temperature germination in muskmelon is dominant. Cucurbit Genet. Coop. Rpt. 12: 50-51.

Nienhuis J., Lower R.L. 1981. An estimate of the heritability of low temperature and seed germination in cucumber. Cucurbit Genet. Coop. Rpt. 4: 12-13.

Ng T.J., Tigchelaar E.C. 1973. Inheritance of low temperature seed sprouting in tomato. J. Amer. Soc. Hort. Sci. 98: 314-316.

Røeggen O. 1987. Variation in minimum germination temperature for cultivars of bean (*Phaseolus vulgaris* L.) cucumber (*Cucumis sativus* L.) and tomato (*Lycopersicum esculentum* Mill.). Sci. Hortic. 33: 7-65. DOI: 10.1016/0304-4238(87)90032-X.

Smeets L., Wehner T.C. 1997. Environmental effects on genetic variation of chilling resistance in cucumber. Euphytica 97: 217-225.

Smith P.G., Millet A.H. 1964. Germinating and sprouting responses of tomato at low temperatures. J. Amer. Soc. Hort. Sci. 84: 480-484.

Staub J.E., Wehner T.C. 1996. Temperature stress. In: Zitter T.A., Hopkins D.L., Thomas C.E. (Eds.), Compendium of cucurbit diseases. APS Press, St. Paul, MN, USA, pp 66-67.

Wehner T.C. 1981. Screening for low-temperature germination ability in cucumber. HortScience 16: 399. (abstr.).

Wehner T.C. 1982. Genetic variation for low-temperature germination ability in cucumber. Cucurbit Genet. Coop. Rpt. 5: 16-17.

Wehner T.C. 1984. Estimates of heritabilities and variance components for low-temperature germination ability in cucumber. J. Amer. Soc. Hort. Sci. 109: 664-667.