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Cold Temperature Tolerance of Wild Tomato Species

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Abstract. **Accessions of several wild tomato species [*Lycopersicon hirsutum* H. and B. (LA 1363 and LA 1777), *L. chilense* Dun. (LA 1969 and LA 1971), and *Solanum lycopersicoides* Dun. (LA 1964)] were examined for cold tolerance and compared to the fast germination of *L. esculentum* Mill. PI 341988 and to the normal germination of 'UC82B'. The wild accessions were collected above 3000 m and presumed to be cold-tolerant because of natural habitat. A number of characteristics, including germination, emergence, chlorophyll fluorescence, electrolyte leakage, and plastochron index were used to evaluate chilling resistance. PI 341988 germinated faster than the other genotypes at temperatures above 10°C, but germination of this accession virtually ceased below 10°. The high-altitude accessions continued to germinate, albeit at a reduced rate, below 10°. Growth rates at 12°/6° (day/night) were compared to growth at 24°/18° and were found to be greater in the high-altitude accessions than 'UC82B'. The reduction in chlorophyll fluorescence when leaf disks were exposed to 1° was less in the high-altitude accessions than in 'UC82B', indicating less effect of this temperature on photosynthesis in the wild species. Electrolyte leakage was greater in 'UC82B' and LA1777 (*L. hirsutum*) than high-altitude accessions of *L. chilense* and *S. lycopersicoides*, but evidence is presented that this method is not reliable in screening for cold tolerance. Crosses were made between 'UC82B' and the wild species, and segregating populations were screened using the methods mentioned above. In each population, there were plants that showed cold resistance similar to the wild parent, suggesting the possibility of developing cold-tolerant cultivars.**

Temperatures lower than 10°C injure many crops of tropical origin, including tomatoes (9). This injury, which occurs at temperatures higher than 0°, has been called chilling injury and was reviewed by Lyons (6). There have been several reports on the temperature response of tomato (8, 17). Chilling injury occurs at 10° to 12°, and extended exposure to temperatures <6° can kill tomato plants (2).

Tolerance of tomatoes to temperatures <10°C is of considerable practical importance. Low temperature-tolerant genotypes might show improved earliness, adaptability, water use, and yields of high-quality fruits when grown at suboptimal temperatures. Also, energy costs for greenhouse production could be reduced for a cold-tolerant cultivar. For several reasons, earliness is a focus for tomato breeding programs. It extends the harvest season, allowing for efficient use of available processing facilities. It results in water savings in areas with a Mediterranean climate, because early plant growth makes better use of late-season rains and water available in the root zone. Finally, production is usually improved in the early harvest period because early plantings avoid high temperature problems, which can reduce fruit set during mid-summer.

A cold-tolerant processing cultivar for California and Israel requires: a) a high level of germination and emergence at temperatures <10°C, and b) good seedling vigor and growth under low temperature conditions. Germplasm for cold tolerance exists in several cultivated and wild species accessions (8, 17). The potential of this material has not been realized because of limited effort to introgress this character into cultivars and because of a poor understanding of the physiological differences of cold-tolerant genotypes needed to develop valid selection criteria.

The time required for germination and emergence of tomato seeds is influenced by soil temperature. Time to germination progressively increases as the temperature is decreased from 15° to 10°C; below 10° germination is inhibited. There are differences among *L. esculentum* genotypes for rate of germination at temperatures >10°, and rapid germination rate is heritable (4). The rapid germination of PI 341988 appears to be controlled by a single gene (3, 4, 7).

An increase in temperatures from 12° to 24°C has a positive effect on plant growth. In many tomato genotypes, photosynthesis is maximum at about 25° (1). Brief exposure to low night temperatures (10° to 16°) during the vegetative stage, after full cotyledon expansion, slowed vegetative growth and advanced

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reproductive growth by early initiation of the first inflorescences (18, 19). Exposure to temperatures <10° inhibits plant growth and flowering.

When photosynthetic organisms absorb photons via chlorophyll, a portion of the acquired energy is dissipated by emission. It has recently been found that the rate of increase of variable fluorescence in dark-adapted leaves decreased in chilling temperature-sensitive plants but not in chilling temperature-tolerant plants (14). The variable part of chlorophyll a fluorescence is correlated with the concentration of reduced election acceptor (q) of Ps II and is a method of studying photosynthetic mechanisms. Currently, in vivo chlorophyll fluorescence also is being used to assess the effects of genotype and stress on plant cells (15).

The purpose of this research was to characterize the cold-tolerance status of several genotypes with proven or presumed (because of natural habitat) cold tolerance (11).

Materials and Methods

Seven lines from 4 different species were used in this study: 'UC82B' and PI 341988 (*Lycopersicon esculentum*), LA1363 and LA1777 (*L. hirsutum*), LA1969 and LA1971 (*L. chilense*), and LA1964 (*Solanum lycopersicoides*). Seeds of the wild species accessions were provided by C.M. Rick, Tomato Genetics Stock Center, Univ. of California, Davis.

Crosses. The crosses between the *L. hirsutum* accessions and 'UC82B' were obtained using normal hybridization procedures. The crosses with *L. chilense* were obtained using embryo rescue techniques (16). Backcrosses to 'UC82B' with the *L. hirsutum* hybrids presented no problem—the BC_1 with the *L. chilense* hybrids also was made using embryo rescue.

The F_1 hybrids between *L. esculentum* and *S. lycopersicoides* were obtained with considerable difficulty. *L. esculentum* was used as the pistillate parent, and, following repeated crosses, a few fruits were set containing very small seeds. Since the seeds did not germinate, fruits were opened under sterile conditions, the ovules dissected, and the endosperm–embryo contents placed in sterile culture (16).

Germination. Seeds of all genotypes were extracted and processed using a standard procedure involving treatment with 6% hydrochloric acid for 4 hr. Seeds were incubated in petri dishes on moist filter paper at 12°/6°C (12 hr at each temperature) in the dark. Germinated seeds were counted when the radicle was 1 mm long. For germination on a thermogradient plate, filter paper was placed directly on the plate.

Emergence. Seeds were planted in a sterile soil–peatmoss mix at a depth of 15 mm, and maintained at a constant soil temperature of 9°C in the dark. Emergence was measured at the time required for appearance of cotyledons.

Plastochron index. The plastochron is defined as "the time interval between initiation of two successive leaves" (5). During that period of growth when successive plastochrons are equal in duration, the plastochron index is a useful indicator of the effect of temperature on growth rate. Plants were grown for 20 days at 24°/18°C (day/night) with a 15-hr photoperiod, followed by a growth period of 20 days at 12°/6° (day/night) with the same photoperiod. The growth rate in each temperature regime was measured using the plastochron index (5). The plastochron index at low temperature relative to that at high temperature was calculated, and a high relative rate was assumed to be related to increased resistance to cold temperatures.

Chlorophyll fluorescence. To measure leaf chlorophyll fluorescence, disks 7 mm in diameter were cut from the center of the left of the first fully expanded leaf. The disks were placed, abaxial surface up, on moist filter paper and the disk was covered with a 2nd filter paper having a hole in its center. After dark adaptation for 1 hr, chlorophyll fluorescence of the leaf disks was induced and measured with a portable fluorometer (Model SF-10 Richard Brancher Research, Ltd., Ottawa, Ontario, Canada) and recorded with a strip-chart recorder.

An experimental apparatus was constructed in which chlorophyll a fluorescence could be measured from 104 leaf disks uniformly kept at the desired temperature (1°C).

Electrolyte leakage. Leaflets from young fully expanded leaves were cut with a razor blade and washed 3 times with deionized water. The leaflets were suspended in 10 ml of deionized water in a test tube and chilled for the duration of the experiment. The conductivity of the bathing solution was measured with a Selectro Mark Analyzer conductivity meter (Markson Science). The tissue samples were kept in the dark, except for brief daily exposure to room fluorescent lighting during the readings of conductivity. At the end of the chilling period, the tubes were capped and autoclaved for 20 min. After a further 4 hr the conductivity of the bathing solution was measured. This measurement was assumed to represent the total potential leakage of the samples and the measurements taken during the chilling period were expressed as a percentage of the conductivity of the autoclaved samples.

Results

Germination. After 30 days at 12°/6°C (12 hr at each temperature), percentage of germination was greatest in PI 341988. LA 1777 (*L. hirsutum*), LA 1969 (*L. chilense*), and LA 1964 (*S. lycopersicoides*) also germinated at these low temperatures (Table 1). The germination of LA1964 at 12°/6° was more than 80% of the germination at 25°; however, the germination of this line was poor at all temperatures. Studies with the thermogradient plate showed that the germination of PI 341988 was rapid at temperatures >10° but was totally inhibited at 10° or lower. There was a gradual decrease in the germination rate of LA 1969 and LA 1777, indicating that these lines continue to germinate at temperatures <10° (Fig. 1).

Emergence. Emergence of the wild accessions was delayed and variable at both 25° and 9°C. Progenies of the early backcrosses also emerged slowly and variably at 25°, making emergence a poor criterion for selection for low temperature tolerance in the wild species and early backcrosses. However, after the 3rd backcross, uniformity of emergence was improved, and selection of progeny that emerge rapidly under low temperatures became practical. BC_3 and BC_4 'UC82B' x ('UC82B' x LA1777) and BC_3 and BC_4 'UC82B' x ('UC82B' x LA1969) were examined for emergence, and in each population there were individual plants that emerged faster at 9° than the high-altitude parent.

Six plants were chosen from the BC_3 F_1 population of 'UC82B' x ('UC82B' x LA 1777), and F_2 progeny were evaluated for emergence (Table 2). With one exception, the emergence of the BC_3 F_2 progeny corresponded to the emergence of the selected BC_3 F_1 lines, which indicates that selection was effective. The BC_3 F_2 progeny that emerged rapidly tended to have a high relative plastochron index (Fig. 2), indicating that emergence at 9°C may be an indicator of low-temperature growth potential.

Plastochron index. The accessions used had successive plastochrons that were equal in duration; the plastochron index was linear with time between the 2nd and the 12th leaf. In later

Table 1. Percentage of germination of seeds of UC82B. PI 341988, and several high-altitude accessions of *Lycopersicon* and *Solanum* at 12°/6°C (day/night) after 15 and 30 days and at 25° after 10 days.

	Germination (%)		
	12°/6° (day/night)		25°
Cultivar/accession	15 days	30 days	(10 days)
UC82B (*L esculentum*)	0	0	84
PI 341988 (*L. esculentum*)	6	87	96
LA1777 (*L. hirsutum*)	18	21	94
LA1964 (*S. lycopersicoides*)	0	8	11
LA1969 (*L. chilense*)	8	9	100
LA1971 (*L. chilense*)	0	2	96
LA1363 (*L. hirsutum*)	0	0	90

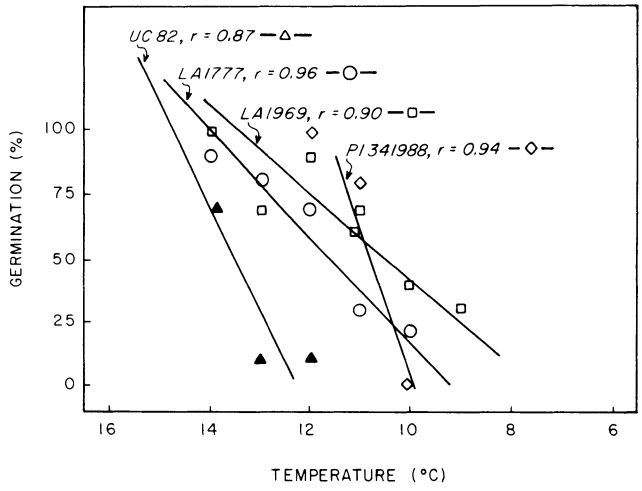

Fig. 1. Percentage of germination of 'UC82B', PI 341988 (*L. esculentum*), LA1777 (*L. hirsutum*), and LA1969 (*L. chilense*) after 15 days at temperature from 8° to 15°C.

stages, irregularities in growth rate increased, possibly due to variation in initiation of the reproductive stage.

The relative plastochron index of the high-altitude accessions were significantly less affected by low temperature than that of 'UC82B', while PI 341988 had an intermediate relative plastochron index (Fig. 3).

Chlorophyll fluorescence. During the first day of chilling there was a rapid reduction in the variable fluorescence in leaf disks of 'UC82B'; after 3 days there was almost no variable fluorescence. There was very little reduction in the variable fluorescence of the high-altitude accessions in 24 hr (Fig. 4). To ascertain whether the differences between 'UC82B' and the wild accessions were due to the cold treatment, variable fluorescence at 16°C was measured. There was very little reduction in variable fluorescence of samples of any of the lines, even after 3 days at 16°, and there was no significant difference among the lines (Fig. 5).

Electrolyte leakage. Electrolyte leakage at 0°C did not differentiate between 'UC82B' and the other *Lycopersicon* accessions; however, LA1964 (*S. lycopersicoides*) showed less leakage than the other lines. Exposure of the leaves to 1° resulted in more severe electrolyte leakage from LA1777 (*L. hirsutum*) and 'UC82B' than from LA1969 (*L. chilense*) and LA1964 (*S. lycopersicoides*). There was more leakage from LA1777 leaves than from other accessions.

Interspecific crosses. One of the most promising sources of chilling resistance is *S. lycopersicoides*, a perennial native to the southern coast of Peru. It shows a unique resemblence to the tomatoes in its yellow flowers, leaf dissection, and lack of tubers. LA1964 was found at a higher altitude and at a more southern latitude than the other wild species, indicating that it withstood low temperatures. *S. lycopersicoides* may have some degree of freezing resistance and, therefore, is considered extremely important plant material for cold tolerance (13).

The cultured embryos of 'UC82B' x LA1964 grew very slowly at first, many being deformed, but after the initial period of slow growth they developed very rapidly and eventually were much more vigorous than either parent. Chlorophyll fluorescence measurements indicated that the cold-tolerance character is present in the hybrid. The fertility of the hybrids were extremely low, and efforts to produce backcross progeny were not

Table 2. Emergence of several BC_3 F_1 ['UC82B' x ('UC82B' x LA 1777)] plants and their F_2 progeny at 9°C.

Plant No.	Days for emergence of BC_3 F_1	Percentage of emergence in F_2 progeny after 42 days
21-6-1	24	2
23-3-6	42[z]	0
28-6-10	43[z]	0
37-5-1	21	15
37-5-3	25	20
92-1-8	42[z]	0
LA1777	---	28
UC82B	---	0

[z]Emergence occurred after termination of the cold treatment (40 days).

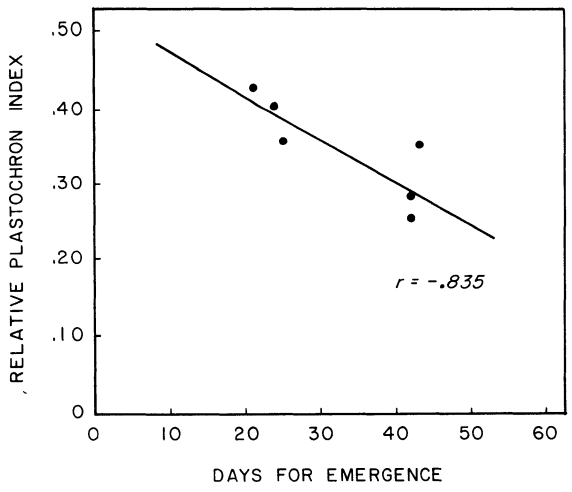

Fig. 2. Mean relative plastochron index of BC_3 F_2 progeny relative to the number of days to emergence at 9°C of their BC_3 F_1 ['UC82B' x ('UC82B' x LA 1777)] parents. Each number is a mean relative plastochron index of 10 F_2 plants. *Regression significant at the 5% level.

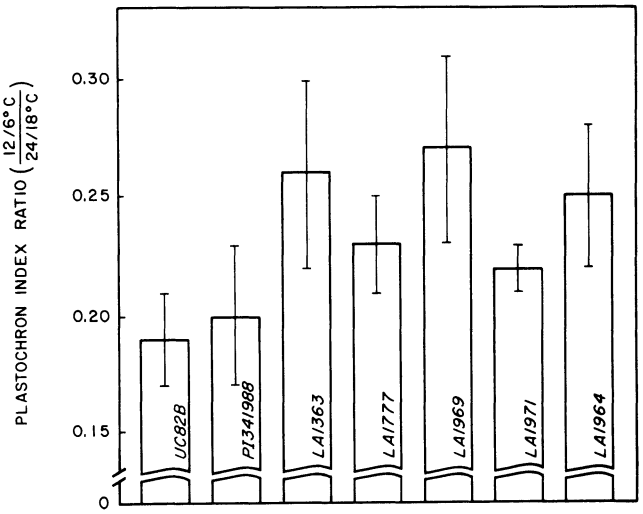

Fig. 3. The ratio of the plastochron index at 12°/6°C (day/night) to plastochron index at 24°/18° (day/night) in plants of 'UC82B' and PI 341988 (*L. esculentum*), LA1363 and LA1777 (*L. hirsutum*), LA1969 and LA1971 (*L. chilense*), and LA1964 (*S. lycopersicoides*). SD of the mean indicated by vertical bars (n = 8).

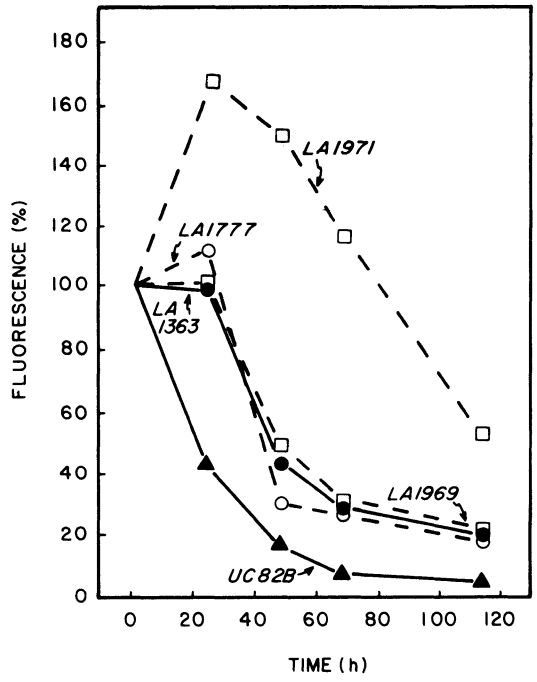

Fig. 4. The effect of chilling at 0.5°C on the variable chlorophyll fluorescence of leaf disks of plants of 'UC82B', LA1363 and LA1777 (*L. hirsutum*), and LA1969 and LA1971 (*L. chilense*).

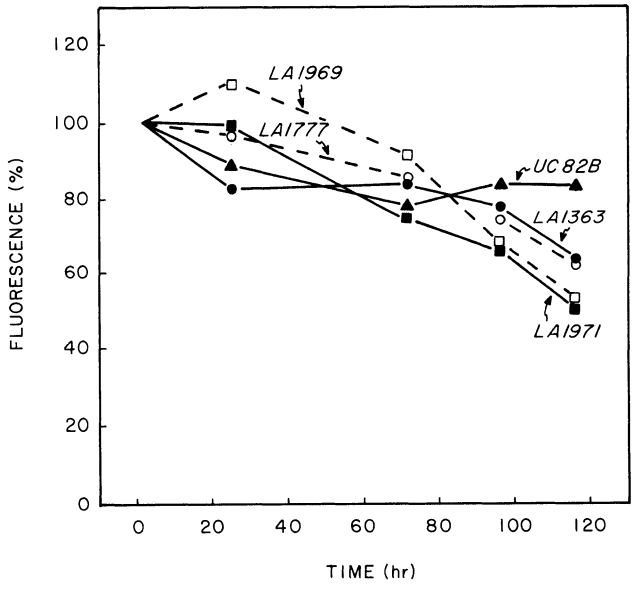

Fig. 5. Variable chlorophyll fluorescence of leaf disks of 'UC82B', LA1363 and LA1777 (*L. hirsutum*), and LA1969 and LA1971 (*L. chilense*) at 16°C. The start of the experiment was used as 100% variable fluorescence.

successful. The relatively low degree of chromosome pairing in the hybrid suggests that some degree of fertility might be restored in amphidiploids (10). The F_1 plants were treated with colchicine and a BC_1 population was obtained using 4n 'UC82B' x 4n ('UC82B' x LA1964). Sesquidiploid hybrids have been obtained from this cross (12) and it is hoped that alien addition lines will eventually be generated.

Discussion

High-altitude accessions of *L. hirsutum* (LA1363 and LA1777) and *L. chilense* (LA1969 and LA1971) are more resistant to low temperature than 'UC82B' as measured by germination rate, growth rate, chlorophyll fluorescence, and electrolyte leakage. The genetic potential for cold tolerance of these wild species was demonstrated by these several methods.

PI 341988 (*L. esculentum*) can germinate considerably faster than cultivars at a constant temperature of 10°C (7). These results show no germination of PI 341988 below 10° after 15 days. However, LA1777 (*L. hirsutum*) and LA1969 (*L. chilense*) germinated below 10°. Combining of the rapid germination characteristic of PI 341988 with the cold tolerance of the wild species could result in increased earliness for early planted fields.

The greater growth of the high-altitude accessions at low temperature was shown with a plastochron index. The ratio between plastochron index at low temperatures (12°/6°C) and at normal

temperatures (24°/18°) was significantly increased for the high-altitude accessions. To be useful in a breeding program, an assay should be rapid and nondestructive. The relative plastochron index is simple and accurate and appears to be useful for screening segregating populations. This method becomes useful because it eliminates variation among plants of the same chronological age but different stage of development, as long as the plants are between the 2nd and 12th leaf stages.

Chlorophyll fluorescence was found to be a simple, rapid method for screening large populations, which is important for a practical procedure. With the fluorescence apparatus, 104 plants can be tested in 20 min at any desired temperature. Forty-eight hours of low temperature treatment was adequate to distinguish between the genotypes. Chlorophyll fluorescence measures only one physiological response (electron transport in photosynthesis) of plants to chilling temperatures. The importance of this response is not presently known.

Electrolyte leakage is of limited value. Differences between the plants were seen only after long exposure to low temperature. After a long treatment, factors other than chilling injury (i.e., senescence and microbial growth) may become important making it a questionable tool for selection.

In our breeding program, several backcrosses have been made to 'UC82B' and the segregating population after each backcross has been examined by the methods mentioned. In each population there were plants that had similar cold resistance to the wild parent according to the criteria used. Evidence of cold tolerance according to these parameters was found following BC_5.

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