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Investigation of Disease Resistance and Cold Tolerance of *Solanum lycopersicoides* for Tomato Improvement

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***Abstract*. *Solanum lycopersicoides* is a valuable genetic resource for tomato (*Lycopersicon esculentum*) genetic improvement. However, there are few reports on its agronomic traits such as disease resistance and cold tolerance. In this paper, the resistance to cucumber mosaic virus (CMV) and leaf mold (*Cladosporium fulvum* Cooke) and cold tolerance of five lines of *S. lycopersicoides* were studied through investigation of disease inoculation and electrolyte leakage analysis. The results showed that *S. lycopersicoides* was highly resistant or immune to CMV and leaf mold and more tolerant to low temperature than *L. esculentum*. This study is helpful for the genetic improvement of tomato by using *S. lycopersicoides* as breeding materials.**

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Lack of genetic variability is currently a major problem for tomato (*Lycopersicon esculentum*) breeding, which was attributed to two major factors: 1) genetic variability of the tomato's ancestor (*L. esculentum* var. *cerasiforme*) was lost during migration from its originating region (Andean area through northern South America) to Mesoamerica, and 2) self-pollination coupled with long periods of domestication and modern breeding (Chetelat et al., 1997; Rick, 1988; Rick and Chetelat, 1995; Rick and Holle, 1990). Hence, some necessary breeding materials such as those resistant or tolerant to cucumber mosaic virus (CMV) and cold temperatures are lacking. Despite genes resistant to some strains of *Cladosporium fulvum* Cooke exist in *Lycopersicon* such as *L. esculentum, L. peruviamum* Mill., *L. chilens* Dun., and *L.pimpinellifolium* Mill., numerous strains and quick variation of *C. fulvum* could eliminate the resistance of the cultivated varieties (Li, 1995; Rick and Chetelat, 1995; Zhang et al., 2000). Consequently, it is necessary to find much more resistance resources to *C. fulvum* for tomato improvement.

Solanum lycopersicoides Dun. (2n = 2x = 24), originating from the west Andes area along the Chile-Peru border, is a close wild relative of tomato (*Lycopersicon esculentum* Mill.). It is the only species in genus *Solanum* that can cross with *L. esculentum*. Hence, *S. lycopersicoides* has attracted tomato breeders' attention due to its excellent tolerance to abiotic stresses, such as cold and infertility, and its resistance or tolerance to several diseases, such as tomato mosaic virus, phytophthora root rot (*Phytophthora parasitica* Dast.), and gray mold (*Botrytis cinerea* pers. ex Fr.) (Chetelat et al., 1997, 1998; Hossain et al., 1994; Kamps et al., 1987; Rick, 1951; Rick and Chetelat, 1995; Rick et al., 1988; Wolf et al., 1986).

Using *S. lycopersicoides* as breeding material for genetic improvement of tomato, Rick obtained an intergeneric hybrid [F_1LS], but the hybrid could not be backcrossed directly to *L. esculentum* as neither the staminate parent, due to its pollen sterility, nor the pistillate parent, due to its unilateral incompatibility (Rick, 1951). Therefore, during the last half-century, the research on *S. lycopersicoides* has mainly focused on how to obtain fertile intergeneric hybrid between *S. lycopersicoides* and *L.esculentum* (Chetelat and Meglic, 2000; Chetelat et al., 1989, 1997, 1998, 2000; Guri et al., 1991; Handley et al., 1986; Hossain et al., 1994; Matsumoto et al., 1997; Rick, 1986; Rick et al., 1986). Fertile intergeneric hybrids between *S. lycopersicoides* and *L. esculentum* were recently obtained by both sexual and asexual means (Chetelat and Meglic, 2000; Chelelat et al., 1997; Matsumoto et al., 1997). This progress is a milestone in genetic improvement of tomato because it successfully uses *S. lycopersicoides* as breeding material.

Presently, there are few detailed reports on abiotic stress tolerance and disease resistance of *S. lycopersicoides*, though these agronomic traits are very important for tomato genetic improvement. In present study, several lines of *S. lycopersicoides* were evaluated for resistance to CMV and leaf mold, and tolerance to cold stress.

Materials and Methods

Plant materials. Five lines of *S. lycopersicoides* (LA1990, LA2386, LA2730, LA2776, and LA2951) and two lines of *Lycopersicon* (*L.esculentum* 'UC82B' and 'Zaofen No. 2') were used in the present study. The five lines of *S. lycopersicoides* were kindly provided by R.T. Chetelat from the C.M. Rick of Tomato Genetic Resources Center at the University of California–Davis, while *L.esculentum* cultivars UC82B and Zaofen No.2 were obtained from the School of Horticulture, Northeast Agricultural University, China.

Pathogen inoculation. CMV (severe mosaic strain) and leaf mold (*C. fulvum* strain 1.2.3) were obtained from the School of Horticulture, Northeast Agricultural University, China. Inoculation, disease scoring, and statistical analysis of CMV and leaf mold resistances of *S. Lycopersicoides*, including all the five lines, were carried out using the method described by Li (1995).

CMV was maintained in tobacco (*Nicotiana tabacum* 'Samsun') and propagated using sensitive 'Zaofen No. 2' under 28 °C for 10 d. Ten-day-old leaves infected by CMV were collected and homogenized with 8 mL phosphate buffer (pH = 8.0, 0.03 mol·L^{-1}) per gram of tissue, and then centrifuged for 15 min at 3000 rpm/min. Supernatant (containing CMV) was collected, then quartz sands were added to it to increase wounding of leaves. Sterile cotton swabs were used to uniformly apply, with slight pressure, the supernatant-quartz sands mixture onto the whole surface of the tested materials. Inoculations were repeated on the third day. Three- or four-euphylla-old plantlets were used for inoculation. Investigation time was at 21, 28, 35, and 42 d, respectively.

Leaf mold (*C. fulvum* strain 1.2.3) was propagated on potato dextrose agar (PDA) medium, and diluted to 10^6 spores/mL suspension solution with sterile distilled water for inoculation. Leaf mold solution was sprayed uniformly on back of leaves using a sprayer (low flow) under 22 to 25 °C with >90% relative humidity. Five- or six-euphylla-old plantlets were used for inoculation. Investigation time was at 14 and 21 d.

Each disease treatment experiment was repeated three times and six plants were investigated in each replicate, while 'Zaofen No. 2' tomato was used as the susceptible control.

Disease index and infection percentage were calculated using two different formulae:
1) disease index = ∑(disease grade × plant number of each grade)/highest disease grade × inoculated plant number)
and
2) infection percentage = (infected number/total inoculated number) × 100%

Cold tolerance: Experiment 1. To determine a non-damaging temperature and appropriate cold treatment duration on *L. esculentum* 'UC82B', a cold-tolerant tomato line, three levels of low-temperature intensity [(day 6 °C/night 2 °C), (day 14 °C/night 10 °C) and (day 25 °C/night 15 °C)] and six levels of treatment duration (24, 48, 72, 96, 120, and 144 h) were applied. The treatments were repeated three times with each replication using six plants.

Cold tolerance: Experiment 2. To determine cold tolerance of *S. lycopersicoides*, plants with six to seven leaves from the five lines of *S. lycopersicoides* (LA1990, LA2386, LA2730, LA2776, and LA2951) and *L. esculentum* 'UC82B' were treated with day 6 °C/night 2 °C and day 25 °C/night 15 °C for 72 h. These treatments were repeated three times with each replicate using six plants. The whole experiment was carried out in HPG-250 artificial climate chambers under a photoperiod of 12 h light/12 h dark.

Electric conductance was measured with a electric conductivity meter (Mc26; Japan) as described by Zhang (1989). Relative electrolyte leakage rate and cell membrane damage rate were calculated using three different formulae:
1) exosmosis electric conductance (μs·cm^{-1}·g^{-1}·mL^{-1}) = (treatment conductance – background conductance)/fresh weight × solution volume
2) relative electrolyte leakage rate = (treatment conductance)/(boiled total conductance) × 100%
and
3) cell membrance damage rate = (conductance of treatment – conductance of control)/(boiled total conductance of treatment – conductance of control) × 100%

Results and discussion

Resistance of S. lycopersicoides to CMV. Infection percentage of *L. esculentum* 'Zaofen No. 2' was 9%, 37%, 82%, and 100% respectively, with the disease index being 8%, 28%, 56%, and 84% respectively at days 21, 28, 35, and 42 after inoculation. However, there were no infection symptoms on the five lines of *S. lycopersicoides* during the whole investigation period (42 d). This result indicated that *S. lycopersicoides* was highly resistant to CMV.

Resistance of S. lycopersicoides to leaf mold. At day 21 after inoculation, the percentage of infected plants of *L. esculentum* 'Zaofen No. 2' reached 100% with a disease index of

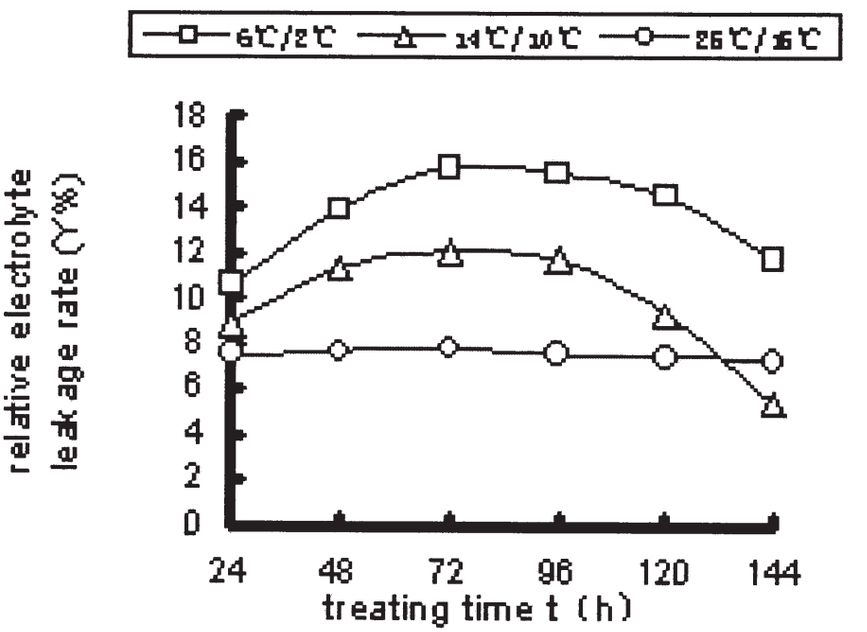

Fig. 1. Changes in relative electrolyte leakage rate by time at different low-termperature levels.

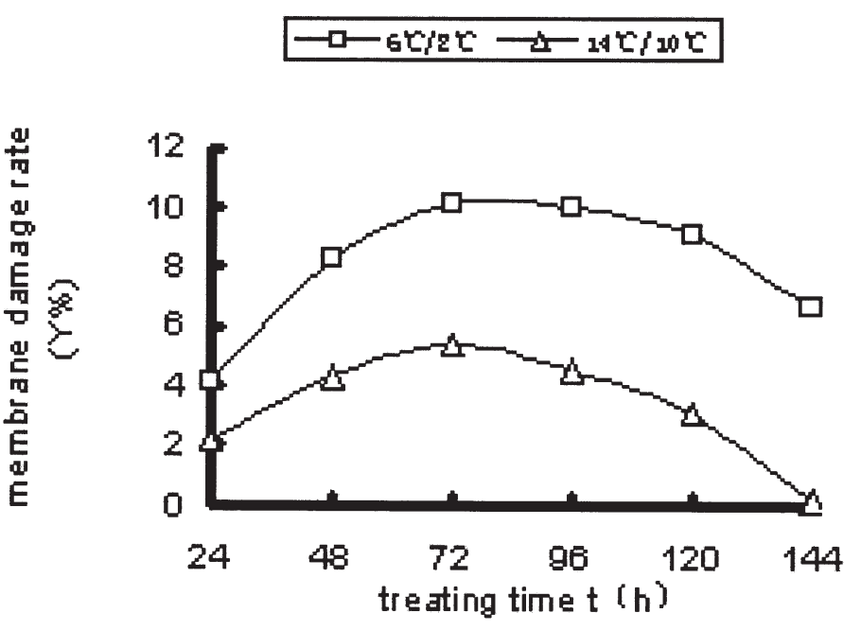

Fig. 2. Changes in membrane damage rate by time at different low-termperature levels.

Table 1. Relative electrolyte leakage rate and cell membrane damage rate of *Solanum lycopersicoides* and *Lycopersicon esculentum* after low-temperature treatment (72 h).[z]

Materials	Relative electrolyte leakage rate						Membrane damage rate		
	6/2 °C[y]	$P_{0.01}$[x]	$P_{0.05}$	25/15 °C	$P_{0.01}$	$P_{0.05}$	6/2 °C	$P_{0.01}$	$P_{0.05}$
S. lycopersicoides									
LA1990	10.38	CD	cde	7.09	BC	c	4.81	D	d
LA2386	10.09	CD	de	8.85	AB	ab	3.61	E	e
LA2730	11.79	BC	c	7.69	B	bc	7.19	B	b
LA2776	9.32	D	e	5.56	C	d	6.06	C	c
LA2951	11.00	CD	cd	8.00	B	bc	7.38	B	b
L. esculentum									
UC82B	15.70	A	a	7.81	B	bc	10.12	A	a

[z]Each value is average of three replications in this table.
[y]Treatment temperature, photoperiod is 12 h light/12 h dark. Left of bias is under light treatment temperature, right of bias is under dark treatment temperature.
[x]Duncan's test (SSR), the same capital or small letter indicated no significance at $p = 0.01$ or 0.05.

89%. No symptoms appeared on the leaves of *S. lycopersicoides* line LA2386, indicating its resistance to leaf mold. The other four *S. lycopersicoides* lines (LA1990, LA2730, LA2776, and LA2951) showed chlorotic spots, not necrotic, with a 1 mm diameter on inoculated leaves, which did not increase in severity. The disease index of these four four lines was about 11%, suggesting a substantially high resistance to leaf mold.

Relative electrolyte leakage rate and cell membrane damage rate of 'UC82B' under low temperature stress. Both relative electrolyte leakage rate and cell membrane damage rate of 'UC82B' increased with a decrease temperature. The electrolyte leakage rates of 'UC82BV' began to rapidly increase from 24 h, peaked at about 84 h, and then reduced gradually under treatment at 6/2 °C. Those of 'UC82B' showed similar variation tendency (low–high–low), peaking at about 72 h under 14/10 °C. Variation tendency of the electrolyte leakage rates during the whole tested period closely followed a roughly straight line treated under 25/15 °C (Fig. 1), indicating that it is not obviously harmful to tomato at 25/15 °C. Therefore, 25/15 °C was used as the nondamaging control for the low-temperature study in tomato. Furthermore, cell membrane damage rate of UC82BV also showed low–high–low variation tendency under treatment at 6/2 °C and 14/10 °C (Fig. 2).

Based on our experimental results, the models for electrolyte leakage rate and cell membrane damage rate under cold treatments were proposed as follows:

$Y_{E6°C/2°C} = 5.9507 + 0.2261t - 1.2963E^{-3}t^2$ ($F = 126.2031^{**}$)

$Y_{E14°C/10°C} = 4.6757 + 0.2045\ t - 1.3861E^{-3}t^2$ ($F = 302.4729^{**}$)

$Y_{C6°C/2°C} = 0.7516 + 0.2137\ t - 1.2028\ E^{-3}t^2$ ($F = 98.2094^{**}$)

$Y_{C14°C/10°C} = 1.1554 + 0.1664\ t - 1.0943\ E^{-3}t^2$ ($F = 79.7454^{**}$)

where Y_E was relative electrolyte leakage rate, Y_C was damage rate of cell membrane and t was treatment duration of low temperature.

According to above simulation equations, the time of relative electrolyte leakage rate and cell membrane damage value reaching maximum levels could be calculated, which were 87 and 89 h respectively in the 6/2 °C treatments, and 74 and 76 h respectively in 14/10 °C treatments.

Total electrolyte leakage rate was determined at any time point under specific cold treatment condition, which included naturally basal electrolyte leakage (with no stress, or baseline leakage) and cold-induced electrolyte leakage. After naturally basal electrolyte leakage was eliminated, the remained electrolyte leakage rates represented cell membrane damage degree, produced only by low temperature stress.

Furthermore, according to the last two simulation equations above, the time duration that 'UC82B' completely recovered from the damage (the dynamic balance between the tomato active response and the low temperature damage) could be calculated. At 6/2 °C and 14/10 °C treatments, values of $t_{6°C/2°C}$ and $t_{14°C/10°C}$ were 181 and 145 h respectively (Fig. 2). These results suggested the greater the low temperature stress intensity, the more time is required for tomato to completely recover from the damage. Burr et al. (1990) reported that freeze-induced electrolyte leakage analysis tests were a precise, sensitive, and objective predictor of changes or differences in tissue cold hardiness. Sutinen et al. (1992) successfully estimated freezing stress resistance in winter-hardy red pine needles by combining the electrolyte leakage analysis method with visual observations. Bigras (1997) assessed root cold tolerance of black spruce seedlings using electrolyte leakage analysis as a viability test in relation to survival and regrowth. Campos et al. (2003) compared five *Coffea* genotypes differing in their sensitivity to low temperatures as well as their ability to recover from cold-induced injury upon rewarming for 6 d, results differing among various genotypes. However, little is known about the time needed for recovery of damaged cell membranes. In the present study, our results provide useful information for studying the relation between cold treatment intensity and recovery time for reversion of cold-induced membrane damage.

As shown in Figs. 1 and 2, the variation tendency of both relative electrolyte leakage rate and cell membrane damage rate varied with the treatment time of 'UC82B'. Both peak values appeared at around day 3 to day 4 (75 to 88 h) after low temperature treatment. This pattern was consistent with Ca^{2+} concentration change, gene expression, and vacuole pinocytosis, reflecting shock response of tomato to low temperature stress (Jian, 1999; Wang et al., 1994). Therefore, the 25/15 °C treatment was used as the nonstress control in low temperature tolerance study of *S. lycopersicoides*. At 6/2 °C, it was appropriate to use a treatment of 72 h duration.

Relative electrolyte leakage rate and cell membrane damage rate of S. lycopersicoides under low temperature stress. Relative electrolyte leakage rates of all five lines of *S. lycopersicoides* ranging from 9% to 12% were significantly lower than that of 'UC82B' (16%) at 6/2 °C at the $P_{0.01}$ level (Table 1), suggesting that all lines of *S. lycopersicoides* were significantly more cold-tolerant than 'UC82B'. The relative electrolyte leakage rates of *S. lycopersicoides*, however, were not significantly different from that of 'UC82B' at 25/15 °C at the $P_{0.01}$ level, except for LA2776 (Table 1).

In order to further investigate cold tolerance of *S. lycopersicoides,* cell membrane damage rates of all five lines of *S. lycopersicoides* were measured, and the values ranged from 5% to 8%, which were significantly lower than that of 'UC82B' (10%) at the $P_{0.01}$ level (Table 1). These results were consistent with those from the relative electrolyte leakage rate analysis. Therefore, it can be concluded that *S. lycopersicoides* is more cold-tolerant than *L. esculentum*, implying its potential value as breeding material for improving tomato's cold tolerance. Cold tolerance of *S. lycopersicoides* differed among lines (Table 1), suggesting that much attention should be given in choosing resistant lines of *S. lycopersicoides* to improve cold tolerance of tomato.

In addition to cold tolerance, *S. lycopersicodes* also shows high resistance or immunity to CMV and leaf mold.

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