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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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 106 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 = \(\sum (\text{disease grade} \times \text{plant number of each grade}) / \text{highest disease grade} \times \text{inoculated plant number}\)
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 membrane 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...
Table 1. Relative electrolyte leakage rate and cell membrane damage rate of Solanum lycopersicoides and Lycopersicon esculentum after low-temperature treatment (72 h).

| Materials          | 6/2 °C | 25/15 °C | Membrane damage |
|--------------------|--------|----------|-----------------|
|                    | $P_{\text{init}}$ | $P_{\text{max}}$ |                |
| **S. lycopersicoides** |        |          |                |
| LA1990             | 10.38  |         |                 |
| LA2386             | 10.09  |         |                 |
| LA2730             | 11.79  |         |                 |
| LA2776             | 9.32   |         |                 |
| LA2951             | 11.00  |         |                 |
| L. esculentum      |        |          |                |
| UC828              | 15.70  |         |                 |

*Each value is average of three replications in this table.

Treatments were a precise, sensitive, and objective predictor of changes or differences in tissue 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$^+$ 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**

**S. lycopersicoides**

was 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*$_{0.05}$ 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. lycopersicoides* also shows high resistance or immunity to CMV and leaf mold.

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