
Differential Thermal Analysis for Large-scale Evaluation of Pear Cold Hardiness

J.M. Montano, M. Rebhuhn, K. Hummer, and H.B. Lagerstedt
National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97333

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More than 4500 accessions of eight genera including Pyrus at the National Clonal Germplasm Repository, Corvallis, Ore., require testing for cold hardness. Since pear xylem deep supercools (7), differential thermal analysis (DTA) would be a suitable test if large numbers of samples could be examined simultaneously. The object of this study was to produce a method of multichannel DTA for defining cold hardness of pear accessions. Visual browning was also examined to confirm cold hardness values.

Pear samples were collected from mature trees on 16 Dec. 1985, in Corvallis, Ore., and stored in polyethylene bags for 3 weeks at −3°C prior to testing. This storage treatment allowed plant samples to develop maximum cold hardness (8). For DTA, eight stem samples, each 5 mm in diameter and 1.5 cm long, were cut from four branches of each accession. Four of the eight stem samples were oven-dried and stored between two packages of plastic gel refrigerant previously equilibrated at −3°C to prevent sample thawing during preparation. About 30 min elapsed from the time the samples were removed from cold storage until DTA was started. Chromel—constantan thermocouples (36 gauge) were inserted into predrilled holes in the xylem of each sample and reference. The samples and references were wrapped with aluminum foil and lowered into wells drilled in two aluminum blocks. The aluminum blocks served as heat sinks and as temperature stabilizers. Each aluminum block consisted of two paired rows of wells about equidistant to contain the samples and references. Temperatures were recorded for 20 sample/references pairs during each freeze run using a data logger (Campbell Scientific CR-7 Management and Control System). The resolution of differential temperature measurement was 50 nV at a full scale range of ±1.5 mV. Both reference and sample temperature were recorded every 15 sec. The aluminum blocks containing the samples and references were cooled at a rate of 0.6° ± 0.2°/min in a low-temperature cooler.

The data logger converted electrical signals to temperature and calculated differential temperature by subtracting reference temperature from sample temperature. Data from the data logger were recorded on cassette tapes, then transferred to a personal computer equipped with a 20 MB hard disk memory system. The raw data were converted into a series of files containing columns of reference and differential temperature using dBase 2.4. These data columns were graphed using Lotus 1-2-3 and plotted with a line plotter.

A separate group of samples was prepared for visual evaluation of oxidative browning from the same branches that were used to prepare DTA samples. Sample preparation was conducted in a cold room at −3°C. Five branches that were collected from each accession were cut into 10-cm-long pieces. The basal and tip portions of each branch sample were discarded. Each branch piece was sorted into five bundles so that each bundle contained a branch section from each of the five sample branches. The sample bundles were placed in five wire baskets, sprayed with water, then seeded with ice crystals and stored overnight at −3.0°C. The following morning, all baskets were placed in the programmable low-temperature freezer. The baskets were set on top of a perforated aluminum table with a recirculating fan underneath to prevent air stratification. The program was set to lower the temperature in 5°C decrements per hour and hold for 2 hr at each test temperature to allow sample equilibration. The five test temperatures used were: −15°, −20°, −25°, −30°, and −35°. At the end of each 2-hr set point, a basket representing each test temperature was removed. The samples were thawed at 4°C, then incubated for 10 days in a mist chamber. The temperature within the mist chamber was maintained at 21° ± 4°C with a hot water heating system (Engineering Inc., Petaulama, Calif.). After 10 days of incubation, the xylem of each sample was evaluated for browning by using a score of 1 (no discoloration) to 4 (deepest discoloration) (4). Each sample was cut diagonally to score the xylem. Scores from the series of five test temperatures were used to construct survival curves from which incipient injury temperature was interpolated. Incipient injury was defined as the lowest temperature at which the score indicated slight discoloration, implying a trace of injury. These survival curves were superimposed on computer-generated DTA profiles.

Incipient injury temperature and the initiation of the first LT exotherm frequently coincided within 3°C (Table 1), which is in agreement with previous work (1, 2, 5, 6, 9). A rapid increase in the degree of xylem discoloration generally occurred after initiation of the first LT exotherm. The presence of broad LT exotherms may be related to intracellular freezing of water occurring as distinct events in individual cells or group of cells (3).

By using the multiple sample apparatus described above, we conducted four controlled freezes each with 20 sample-references per working day. This apparatus could be of beneficial use to cold hardness evaluation programs that require testing of large numbers of individual selections.

Table 1. Oxidative browning evaluation and DTA evaluation of various pear species from samples collected on December 16, 1985.

<table>
<thead>
<tr>
<th>Species</th>
<th>Incipient damage</th>
<th>LT exotherm</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. communis</td>
<td>31.8 d</td>
<td>34.8 c</td>
</tr>
<tr>
<td>P. pashia</td>
<td>18.8 ab</td>
<td>16.3 a</td>
</tr>
<tr>
<td>P. pyrifolia</td>
<td>18.2 ab</td>
<td>19.7 a</td>
</tr>
<tr>
<td>P. calleryana</td>
<td>18.6 ab</td>
<td>17.0 a</td>
</tr>
<tr>
<td>P. glabra</td>
<td>17.6 ab</td>
<td>17.8 a</td>
</tr>
<tr>
<td>P. caucasica</td>
<td>28.0 c</td>
<td>27.6 b</td>
</tr>
<tr>
<td>P. betulifolia</td>
<td>22.0 b</td>
<td>21.6 ab</td>
</tr>
</tbody>
</table>

*Means of five replicates.
+Means of four replicates.
*Mean separation within columns by Duncan's multiple range test, P = 1%.
Literature Cited


