Metabolic Changes in After-Ripening Seed of Prunus cerasus

L. J. LaCroix and A. S. Jaswal

Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada

Received October 31, 1966.

Summary. Metabolic changes were studied in embryonic axes and cotyledons isolated from after-ripening seeds of Prunus cerasus. During the seventh week of after-ripening, a striking increase in the respiration rate at 25°C of embryonic axes occurred along with a sharp change from the dormant to non-dormant state of the seed. On the basis of C-6/C-1 ratio determinations this change may be related to an increased activity of the pentose phosphate cycle.

Seed of woody plant species in general possess an intensive dormancy frequently termed embryo dormancy. Incubation of seed in a moist medium at a temperature of 0°C to 5°C for a period of several weeks (after-ripening) is the common method of obtaining germination of such seed.

Pollock and Olney (3) studied changes in respiration and levels of nitrogen and phosphorus during the after-ripening of seed of Prunus cerasus. Our study was undertaken to extend the knowledge of the metabolic changes occurring during the after-ripening of this seed. A preliminary report has been presented (2).

Materials and Methods

Seed of sour cherry was obtained from the commercial crop in British Columbia. After-ripening was carried out in a moist sand-peat mixture (1:1) at 1°C. The same lot of seed was used in all the experiments reported.

Embryonic axes or cotyledons were incubated in 1.0 ml of 0.05 M potassium phosphate buffer, pH 5.5. For metabolic studies, 5 μmole (0.1 μg) glucose-1-14C or glucose-6-14C was added to the incubation mixture. Potassium hydroxide was used to trap 14CO2. The 14CO2 was converted to Ba14CO3, plated on microporous porcelain discs, dried and the radioactivity determined by a continuous gas flow Geiger counter.

Results

Assay of Dormancy. Seeds at various stages of after-ripening were removed from the sand-peat mixture and tested for germination. Germination tests carried out on moist filter paper indicated that dormancy terminated after approximately 7 to 8 weeks of after-ripening. The germination percentage at 6 weeks was 16, at 8 weeks 59 and thereafter approximately 70. Tests carried out in soil verified these findings.

---

1 Contribution No. 118, Department of Plant Science, University of Manitoba.
2 Present address: New Brunswick Research and Productivity Council, Fredericton, New Brunswick, Canada.

**Fig. 1.** Changes in respiration rate and C-6/C-1 ratio of after-ripening embryonic axes and cotyledons of cherry seed. O—O, Oxygen uptake of embryonic axes; □—□, carbon dioxide release of embryonic axes; △—△, oxygen uptake of cotyledons; ▲—▲, carbon dioxide release of cotyledons; ●—●, C-6/C-1 ratio of embryonic axes $\times 10^{-2}$. The maximum standard error of these values was ± 0.015. Standard error refers only to C+6/C-1 values.
Table I. Effect of DNP on Oxygen Uptake (μl/hr/Sample) of Embryonic Axes and Cotyledons Isolated from After-Ripening Seeds of Prunus cerasus

Embryonic axes or cotyledons were incubated in 2 ml 0.05 M potassium phosphate buffer, pH 5.5. After 2 hours, 0.5 ml of 0.5 mM DNP was tipped into the main compartment to yield a final concentration of 0.1 mM. Values are the means of 2 determinations.

<table>
<thead>
<tr>
<th>After-ripening time (weeks)</th>
<th>Embryonic axes</th>
<th>Cotyledons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−DNP μl/hr</td>
<td>+DNP μl/hr</td>
</tr>
<tr>
<td>0</td>
<td>20.0</td>
<td>38.2</td>
</tr>
<tr>
<td>6</td>
<td>21.3</td>
<td>36.3</td>
</tr>
<tr>
<td>8</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>11</td>
<td>61.0</td>
<td>86.7</td>
</tr>
<tr>
<td>16</td>
<td>66.7</td>
<td>79.0</td>
</tr>
</tbody>
</table>

Respiration and Metabolic Studies. After-ripening was carried out at 5° and seeds were removed from the medium at various intervals of time for respiration and pathway studies. These studies were conducted at 25°. As would be expected the respiration rate at 25° was far greater in the embryonic axes than in the cotyledons. The oxygen uptake and carbon dioxide release for embryonic axes remained relatively constant during the first 6 weeks of after-ripening, then increased abruptly during the seventh week. Increases were gradual during the remainder of the after-ripening period. Cotyledons showed a gradual but small increase in respiration rate at 25°, during the 16 week after-ripening period.

The relative contribution of the pentose phosphate and Embden-Meyerhof-Parnas pathways, as estimated by the C-6/C-1 ratio method (1), was studied in both embryonic axes and cotyledons. Trapped 14CO2 was collected at 1, 2, 4 and 8 hours after the start of the experiment. The C-6/C-1 ratio was found to be near constant over the 8 hour period and values presented in figure 1 are means of the 4 determinations, on duplicate samples.

An initial C-6/C-1 ratio of approximately 0.95 was observed with embryonic axes. This value was maintained for the first 6 weeks of after-ripening, then decreased abruptly during the seventh week, and continued to decrease gradually during the remainder of the after-ripening period. The C-6/C-1 ratio of cotyledons varied from 0.82 to 0.89, but showed no obvious relationship to after-ripening time.

Effect of 2,4-Dinitrophenol on Respiration. As after-ripening time progressed, a marked reduction in the DNP stimulated respiration of embryonic axes occurred (table 1). With cotyledons, on the other hand, no such effect was observed. These observations pointed to the fact that embryonic axes were the site of metabolic changes which did not occur in cotyledons. Such changes are also indicated by the data on C-6/C-1 ratio presented in figure 1.

Acknowledgments

This research was supported by a grant from the National Research Council of Canada. The seed was supplied by the Research Station of the Canada Department of Agriculture, Summerland, British Columbia. The assistance of Mr. D. Murray in the preparation of the manuscript is acknowledged.

Literature Cited