Further Evidence for the Break on the Arrhenius Plot of Germination Activity in Rice Seeds

Iwao Nishiyama

(Hokkaido National Agricultural Experiment Station, Hitsujigaoka, Sapporo 061-01)

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The present author reported that a break occurred on the Arrhenius plot for germination activity in rice seeds\(^{13}\). This paper describes the results of experiments on this phenomenon with more precise temperature intervals, and the thermal characteristics of some physiological activities during the germination.

Materials and Methods

The seeds of a japonica rice variety “Shiokari” were used throughout all experiments.

For the germination tests, seeds were sterilized in a 1:10 dilution of antiformin for 5 minutes, washed with water 5 times and soaked in water for 24 hours before use. The seeds were germinated in a rectangular stainless steel dish \((5 \times 34 \text{ cm})\) with 3 sheets of wet filter papers, which were supplied with distilled water during the experiment. This dish was placed on an aluminium alloy block which had a temperature gradient from approximately \(8\,^\circ\text{C}\) in one side to approximately \(33\,^\circ\text{C}\) in the other side.

Germination of the seeds was estimated by a 5 mm elongation of the radicle or the plumule. \(E_{30}\) is the number of days during which the radicle or the plumule elongates by 5 mm for 50 percent of the tested seeds. Thus, \(E_{30}\) means the reciprocal of germination activity.

For the estimation of respiration and respiratory quotient, the seeds were hulled, then sterilized, washed and presoaked in the same way as for the germination test, after which they were germinated at \(20\,^\circ\text{C}\) for 2 or 3 days. The germinating grains having the plumule length of 0.1–0.5 mm were used.

The activity and quotient of respiration were estimated by measuring the oxygen uptake manometrically. The different number of grains were used according to the estimation temperature (Table 1). The grains were placed on a filter paper strip in the main compartment of a Warburg vessel. The filter paper strip was moistened with 0.3 ml of 0.2 M phosphate-0.1 M citrate buffer (pH 4.9). The center well contained 0.2 ml of 20 percent KOH or of the phosphate-citrate buffer.

For the estimation of acid phosphatase activity, the seeds were sterilized, washed and presoaked in the same way as for the germination test, after which they were germinated at \(20\,^\circ\text{C}\) for 2 or 3 days. Twenty embryos were excised from the seeds having

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Table 1. The number of grains used at different temperatures.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Number of grains</th>
</tr>
</thead>
<tbody>
<tr>
<td>6°C</td>
<td>80</td>
</tr>
<tr>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>12</td>
<td>40, 60 or 80</td>
</tr>
<tr>
<td>15</td>
<td>60 or 80</td>
</tr>
<tr>
<td>18</td>
<td>40</td>
</tr>
<tr>
<td>21</td>
<td>30</td>
</tr>
<tr>
<td>24</td>
<td>20 or 30</td>
</tr>
<tr>
<td>27</td>
<td>20</td>
</tr>
<tr>
<td>30</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 2. The length of reaction time at different temperatures.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>6°C</td>
<td>30, 60min.</td>
</tr>
<tr>
<td>10</td>
<td>20, 40</td>
</tr>
<tr>
<td>15</td>
<td>15, 30</td>
</tr>
<tr>
<td>21</td>
<td>10, 20</td>
</tr>
<tr>
<td>30</td>
<td>5, 10</td>
</tr>
</tbody>
</table>

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the plumule length of 0.1–0.5 mm, and homogenized in a mortar with 0.1 M citrate buffer (pH 6.0). The homogenate was diluted to 22 ml with 0.05 M citrate buffer (pH 6.0). After 5 minutes of temperature equilibration at each of 5 reaction temperatures, reaction was started by adding 1.0 ml of 2.0 mM disodium p-nitrophenyl phosphate to 4.0 ml of the diluted homogenate. The 1.0 ml of the reaction mixture was pipetted out and added to 1.0 ml of 10 percent trichloroacetic acid (TCA) solution immediately after the start of the reaction, and this pipetting-out into the TCA solution was repeated twice after the definite durations of time according to the reaction temperature (Table 2). To each of these mixtures of the reaction solution and the TCA solution, 2.0 ml of a saturated sodium carbonate solution was added. The produced yellow colour of p-nitrophenol was measured electrophotometrically at 420 nm. The activity of acid phosphatase was calculated from the time required to a definite increase in the absorbance irrespective of the reaction temperature.

**Results**

Figs. 1–4 show Arrhenius plots for germination activity in rice seeds. The ordinate is the logarithm of the activity (1/E sub 50), the abscissa the reciprocal of the absolute temperature, and the slope shows the activation energy of the germination. The values obtained from estimations at precise temperature intervals well agree with 2 straight lines (or 3 straight lines in the case of Fig. 3). The temperature coefficient of germination is larger at lower temperatures. The temperatures where breaks occurred on the Arrhenius plots are summarized in Table 3. A break occurred in the vicinity of 15°C throughout these 4 figures, and another break near 30°C was observed in Fig. 3.

In the germination on wet filter papers, the radicle reaches the length of 5 mm earlier than the plumule at higher temperatures, while the plumule reaches 5 mm earlier at lower temperatures. Table 3 shows the relation between temperature and

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**Fig. 1.** Effect of temperature on germination activity (1/E sub 50) in rice seeds (1).

**Fig. 2.** Effect of temperature on germination activity (1/E sub 50) in rice seeds (2).

- △: Radicle only
- ●: Radicle>Plumule
- ○: Plumule>Radicle
- ×: Plumule only
the elongation of the plumule and the radicle. Below 12–13°C the plumule exclusively attained 5 mm at the 50 percent germination \( E_{50} \). The number of plumule was larger than the number of radicle between 12–13°C and 17–18°C, and the number of radicle became larger above 17–18°C. The temperature above which the radicle exclusively attained the length of 5 mm changed over a wide range from 20 to 30°C in different experiments.

Fig. 5 shows an Arrhenius plot for respiratory \( O_2 \) uptake by germinating rice seeds. The shape of this figure is very close to that for the germination activity (Figs. 1–4), and a break was observed at 15°C.

Fig. 6 shows the effect of temperature on the respiratory quotient of germinating rice seeds.

![Graphs showing Arrhenius plots for germination activity and respiratory quotient](image)

**Table 3.** A summary of 4 experiments on the germination of rice seeds.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Break temperature</td>
<td>1 15.2°C</td>
<td>15.4°C</td>
<td>14.7°C</td>
<td>13.5°C</td>
</tr>
<tr>
<td>Break temperature</td>
<td>2 30.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plumule only</td>
<td>} 13.1°C</td>
<td>11.5°C</td>
<td>12.8°C</td>
<td></td>
</tr>
<tr>
<td>Plumule &gt; Radicle</td>
<td>} 17.2</td>
<td>17.7</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td>Radicle &gt; Plumule</td>
<td>} 20.6</td>
<td>30.4</td>
<td></td>
<td>28.8</td>
</tr>
</tbody>
</table>
Fig. 5. Effect of temperature on respiratory 
O₂ uptake by germinating rice seeds. 
Each dot is the average of 2 or 3 estimations.

Fig. 6. Effect of temperature on the respiration 
quotient of germinating rice seeds. 
Each dot is the average of 2 or 3 estimations.

seeds. The quotient was approximately 1.0 
at 30°C, decreased with decreasing temperature and was approximately 0.76 below 15°C. Thus, a break occurred, again, at 15°C.

Arrhenius plots for acid phosphatase activity in the embryos of germinating rice seeds were shown in Figs. 7-9 (These 3 figures represent the average of 8 repeated experiments). These figures are similar to those for the germination, but the break occurred near 10°C.

Discussion

It was shown in the preceding paper that a break occurred on the Arrhenius plot for germination activity in rice seeds and that the temperature of the break was approximately 17°C irrespective of varietal difference in the germination activity. The
occurrence of the break was further confirmed in the present paper by the experiments with more precise temperature intervals (approximately 2.8 estimations per degree) over a range from 8 to 33°C (Figs. 1–4). The break temperature was approximately 15°C, slightly lower than that in the preceding paper, and another break was found at 30°C.

In the germination in air, the radicle grows faster and reaches the length of 5 mm than the plumule at higher temperatures, while the plumule grows faster at lower temperatures. For the elongation of the plumule and the radicle, the following 4 temperature ranges (consequently 3 critical temperatures) are distinguished:

1. A range where plumules exclusively attain 5 mm elongation at the time of 50 percent germination (E₅₀).
2. A range where both plumules and radicles reach 5 mm, but the percentage of the plumule is larger than that of the radicles.
3. A range where both plumules and the radicles reach 5 mm and the percentage of the radicles is larger.
4. A range where radicles exclusively attain 5 mm elongation.

None of these critical temperatures estimated agreed with the break temperature of the germination (Table 3), though the critical temperature between ranges 2 and 3 coincided with the break temperature in the preceding paper.

Figs. 5 and 6 showed the occurrence of breaks at 15°C for the respiration and respiratory quotient in germinating rice seeds. This temperature agrees with the break temperature for the germination, and thus the results suggest that the break in the germination is related to respiration.

On the other hand, a break observed for the activity of acid phosphatase was near 10°C, much lower than that for the germination (Figs. 7–9). Therefore the participation of acid phosphatase in the thermal anomaly in the germination is unlikely.

The present paper described only a few characters concerning the germination of rice seeds, but it showed at least a possibility that the cause of the break in the germination is attributed to some enzymes or enzyme systems of respiration.

Summary

Experiments with precise temperature intervals confirmed the occurrence of a break near 15°C on the Arrhenius plot for the germination of rice seeds (Figs. 1–4), and showed another break at 30°C (Fig. 3). The break near 15°C showed no clear relation with the elongation characteristics of the plumule and the radicle of seeds (Table 3).

Thermal anomalies were observed at 15°C for respiration and respiratory quotient in germinating seeds (Figs. 5 and 6), while a break for the activity of acid phosphatase in the embryos of germinating seeds occurred near 10°C (Figs. 7–9). These facts suggest that the thermal anomaly in the germination is related to the anomaly in the respiration.

Reference

イネ種子の発芽における温度異常

西山 岩男
（農林省北海道農業試験場）

イネ種子の発芽活性を約 8°C から約 33°C までの範囲についておよそ 0.36°C 間隔で測定し、アレニウス作図において 15°C 付近に温度異常がみられることを確認した（第 1～4 図）。この温度異常は発芽において幼芽がきさきにのびるか幼根がきさきにのびるかにはかならない関係がないようであった（第 3 表）。

発芽中の種子における呼吸活性および呼吸商についても 15°C において温度異常が観察された（第 5, 6 図）。発芽中の中の胚の酸性フォスファターゼ活性については温度異常は約 10°C においておこり、発芽活性にみられる温度異常とは一致していなかった（第 7～9 図）。これらの事実はイネ種子の発芽における温度異常が関与していることを示唆している。